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FATTY ACID COMPOSITIONS IN THAI MARINE SPONGES (ORDER HADROMERIDA).

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ABSTRACT

Seven specimens of marine sponges (Order *Hadromerida;* Families *Spirastrellidae, Tethyidae,* and *Clionaidae*) were analyzed for their fatty acid compositions. The predominant compositions were 16:0, 20:5n3 and 22:6n3. Most of them were saturated (22.53-36.41%) and polyunsaturated (19.38-55.54%) fatty acids. The essential fatty acids, eicosapentaenoic acid or EPA (20:5n3) and docosahexaenoic acid or DHA (22:6n3), were found with the highest concentrations, KODA-13; 11.85±0.35% and LSNG-07; 24.36±0.52%, in non-polar lipid of *Spira strella sp.* and *Spirastrella solida,* respectively.

Keywords: Fatty acid, EPA, DHA, marine sponges, *Spirastrellidae, Tethyidae,* and *Clionaidae.*

INTRODUCTION

Fatty acids (FAs) in marine sponges are currently interesting because marine sponges are rich sources of natural compounds. Several of them have shown to contain a variety of compounds with biological activities (Litchfield and Morales, 1976). Sponges lipids differ markedly in their chemical and biochemical properties from lipids obtained from other living organisms. They are a rich source of polyunsaturated fatty acids (PUFAs) of the n-3 series, such as eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA), which are known to prevent and cure thrombosis, atherosclerosis and subsequent blood circulation disease (Kelly, 1991). These fatty acids are also known to have a high nutritional value in the diet of many economically important mariculture species, increasing the overall health and growth as well as the disease resistance in juvenile. Consequently, the investigations of fatty acids from marine sponges are of interesting because they can be developed as nutritional supplements, pharmaceuticals or other applications.

Marine sponges are the primitive multi-cellular organisms, and they have been able to adapt to their environments with special structural features in cell membranes. It has been known that these membranes contain high proportion of unusual long chain fatty acids in their phospholipids, C16-C22 atoms (Litchfield et al., 1976; Lawson et al., 1988). The lipids in marine organisms often possess a very complex composition, probably connected with the specific membrane requirements of sponges in the marine environment. The relative proportion and composition of fatty acids in marine organisms are characteristic for every species and genus and also depend on environmental condition (Christie, 2003). Lipids are major sources of metabolic energy and

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essential materials for the formation of cell and tissue membranes; therefore, they are very important in the physiology and reproductive processes of marine animals (Pazos et al., 1997; Sargent, 1995; Sargent et al., 1995). The interest of researcher in lipids and fatty acids from marine animals has been stimulated, in particular, by the recognition that PUFAs are important to human health and nutrition. Due to the fact that detailed quantitative studies of lipids in marine invertebrates are lacking, the objective of this research is to generate the data available on fatty acids and lipids of marine sponges.

MATERIALS AND METHODS

Collection of marine sponges

All sponge specimens were collected from the coastal area of Chonburi and Trat Provinces in February - June 2005 using scuba diving team. The examples of sponges' photo of *Spheciospongia* sp. (Family *Clionaidae*), *Tethya seychellensis* (Family *Tethyidae*), *Spirastrella solida* and *Spirastrella* sp. (Family *Spirastrellidae*) are shown in Figure 1. The species names and habitats of the sample are shown in Table 1. These sponges are the members of order Hadromerida. Several sponges in the families Demospongiae, Tethyidae, and Spirastrellidae are frequently massive growth form with megascleres radially arranged throughout the entire skeleton, but sponges in the family *Clionaidae* are typical excavating growth forms. They have uniform spiculum of monaxonic megascleres (tylostyles or subtylostyles) and microscleres. Megascleres usually consist of more than one size class, with ectosomal spicules often smaller than choanosomal spicules while microscleres consist of euasters, sterrasters, streptasters. In general, sponging fibers are poorly developed. Dr. Sumaitt Putchakarn identified the sponge materials and the records of the specimens were deposited at the Institute of Marine Science, Burapha University, Chonburi, Thailand. The materials were frozen immediately at -20°C prior to extraction.

Table 1. Lists of sample codes, classification and locations of the sample collection of marine sponges.

Field code	BIMS code	Scientific name	Family	Location	Province	Depth (m)
RRIN-01	BIMS-I -2387	*Spheciospongia* sp.	*Clionaidae*	Ko Rin	Chonburi	17
CKRAM-03	BIMS-I -2421	*Tethya seychellensis*	*Tethyidae*	Ko Kram	Chonburi	25
RADE-06	BIMS-I -2456	*Spirastrella solida*	*Spirastrellidae*	Ko Rad	Chonburi	10
LSNG-07	BIMS-I -2350	*Spirastrella solida*	*Spirastrellidae*	Ko Lan	Chonburi	20
CHANG-I-07	BIMS-I -2792	*Spirastrella solida*	*Spirastrellidae*	Ko Chang	Trat	10
KODA-10	BIMS-I -2868	*Spirastrella solida*	*Spirastrellidae*	Ko Kut	Trat	5
KODA-13	BIMS-I -2871	*Spirastrella* sp.	*Spirastrellidae*	Ko Kut	Trat	6

Total lipids and lipid class analysis

Total lipids were extracted from 50-100 g of wet sample according to Bligh and Dyer (1959). All samples were extracted by chloroform (contained BHT 0.1 ppm): methanol (contained BHT 0.1 ppm) in a ratio of 2:1 for 3 times. The combined extracts were then extracted with 0.88% KCl solution to remove non-lipid materials. The sample was dried with anhydrous Na_2SO_4 and excess solvent was evaporated under reduced pressure to yield the crude total lipid, and the remainder (yellow oil) was further dried by nitrogen gas then stored at -20°C for lipid class and fatty acid analysis.

The neutral lipids and phospholipids were separated from crude total lipids by silica gel column chromatography (2.5 x 30.0 cm inner diameter) with chloroform (neutral lipids) and methanol (phospholipids). Each lipid fraction was

concentrated with rotary evaporator and the weight was determined gravimetrically.

Fatty acids methyl esters were prepared by acid-catalyzed transmethylation of each lipid fractions by adding 10 ml of 2% sulfuric acid in methanol and keeping in oven at 80°C for 4 hours, then added the mixture of 5 ml of 5% sodium chloride, 5 ml of hexane and 40 ml of 2% potassium bicarbonate. The fatty acid methyl esters were then filtered through anhydrous sodium sulfate and dried under nitrogen gas.

Fatty acid analysis

Separation and identification of fatty acids were carried out. They were analyzed in Hewlett Packard, HP 5890 series II equipped with a FAME WAX, USA fused silica capillary column (30 m x 0.25 mm inner diameter, 0.25 µm film thickness). The carrier was helium gas at a constant flow rate of 1.3 ml/min. The column temperature was programmed initially at 120°C holding for 0.5 min and then thermal gradient to 195°C at 18°C/min holding for 5 min, 195 to 205°C at 3°C/min holding for 7 min, 205 to 220°C at 8°C/min holding for 10 min. The temperature of injector and flame ionization detector were at 250°C. Fatty acids methyl esters were identified by comparing with standard mixtures (PUFA No.3 Cat. 47085-U, Supelco, USA) and quantified by area percent of total fatty acids.

Data control and data recovery

The reference material, PUFA No.3 (Menhaden oil), was identified and calculated to compare the results of known reference material with our method. The external standard (cis-5, 8, 11, 14, 17-eicosapentaenoic acid) was analyzed to find out the percent recovery, which was 89.19 % (n = 3).

RESULTS AND DISCUSSION

1. Total lipid and lipid classes

Total lipids accounted for 0.28-1.06 % crude extracted sponges in all samples were studied. Table 2 showed the lipid class compositions of marine sponges: *S. solida*, *Spirastrella* sp., *Spheciospongia* sp., and *T. seychellensis*. Neutral lipids (NL) were the major lipid class (26.26-83.36 %), whereas phospholipids (PL) were the minor lipid class (11.36-31.71 %). There are few studies in lipid class separation from marine sponges; however, Barnathan et al. (2003) reported about the lipid composition of several *Cinachyrella* sponges that neutral lipids were predominant.

2. Fatty acid compositions of neutral and phospholipid

Eighteen fatty acids were identified in all samples of sponges, and fatty acid compositions for each sponge are presented in Table 3 and Figure 2. The quantitative of fatty acids was different between family and location. The most abundant fatty acids in sponges were polyunsaturated fatty acids (PUFAs), accounting for 19.38-55.54 % of total FA, except in polar lipid of *Spirastrella* sp. from Trat (KODA-13).

Saturated fatty acids (SFAs) constituted 22.53-36.41% of total FA from all samples. The predominant saturated acids were palmitic acid (16:0), which existed in all species. The major of palmitic acid content in NL was more important than PL, which the high contents were obtained from the marine sponges of *T. seychellensis* (CKRAM-03; 15.64±0.96%), *Spirastrella* sp. (KODA-13; 15.52± 0.73%), and *Spheciospongia* sp. (RRIN-01; 15.25± 0.57%). The prominent presence of palmitic acid in marine sponges was previously reported from *Tedania dirhaphis*, *Forcepia uschakowi* and two calcareous sponges that are typical for sponges (Rod'kina, 2005, 2007; Schreiber et al., 2006).

Polyunsaturated fatty acids constituted 28.89-55.54% of total FA. The most abundant ones were 22:6n3: DHA (docosahexaenoic acid; 0.57-24.36%), while 20:5n3: EPA (eicosapentaenoic acid; 1.01-11.85%) occurred in minor concentrations. The highest concentration of 22:6n3 and 20:5n3 acids was found in neutral lipids (NL) of *S. solida* (LSNG-07; 24.36±0.52%) and *Spirastrella* sp. (KODA-13; 11.85±0.35%), respectively. Other observed PUFAs included linoleic (18:2n6), linolenic (18:3n3) and arachidonic (20:4n6) acid. The fatty acid compositions

of sponge, *S. solida*, in different locations (Table 3) were not similar which possibly depended on habitat and reflected the adaptation of sponges to the environmental condition (Christie, 2003). According to the literatures, C20 and C22 acids have also been reported from marine sponges, *Halichondria panacea*, and *Tedania dirhaphis* and calcareous sponges (Rod'kina et al., 2003, 2005; Schreiber et al., 2006).

The results of the essential fatty acids, EPA and DHA, are shown in Figure 3 and Table 2. The result showed a similar tendency in *Spheciospongia* sp. and *T. seychellensis*, whereas it displayed different result in *Spirastrella* sp. and *S. solida*. A high percentage of EPA was found in NL of *Spirastrella* sp. (KODA-13) from Ko Kut, Trat province (11.85%), whereas DHA was found in NL of *S. solida* (LSNG-07) from Ko Lan, Chonburi province (24.36%).

The difference in EPA and DHA of *Spirastrella* sp. and *S. solida* might be influenced by the phytoplankton, ecological conditions and geographical location. The concentration of PUFAs in marine organisms is known to vary with phytoplankton composition in water column because the majority of naturally occurring PUFAs, especially 20:5n3 and 22:6n3, can only be synthesized by phytoplankton and bacteria (Cho et al., 1999). Noiraksar and Taleb (2005) reported the distribution and abundance of phytoplankton along the river mouth of the Eastern Coast of Thailand that consisted of five genera of blue-green algae, 11 genera of green algae, 47 genera of diatom, 10 genera of dinoflagellate, etc. The phytoplankton community was mostly influenced by salinity, DO, turbidity, pH and temperature. The high content of EPA and DHA from sponges may be explained by the incorporation of phytoplankton in sponge diets. Thus, it is interesting that the source of PUFAs actually derived from the sponge cells or algae or bacteria.

A comparison of EPA and DHA contents of the studied sponges with seawater fishes and freshwater fishes from Pattani Province, Thailand and cultured microalgae at Institute of Marine Science, Burapha University (Table 4) indicated that the studied sponges showed higher DHA contents than microalgae, whereas they exhibited higher EPA contents than seawater fishes and freshwater fishes.

In summary, our results showed the high PUFAs content in *S. solida*, *Spheciospongia* sp., and *T. seychellensis* that may suggest their development for PUFA preparation in nutraceutical and / or functional-food formulations. Research into fatty acids biosynthesis pathways and structure determination of fatty acids from sponges can help to explain some phenomena related to the life of sponges. The unique adaptation abilities of sponges, enabling them to prosper so long after the Precambrian in the most varied water ecosystems, in all geographical ranges at various depths and in wide ranges of water salinity and temperature, may receive a new interpretation.

Table 2. Total lipid content and lipid class composition in sponges, order *Hadromerida*.

Sponges	Total lipids (%)	Neutral lipids (%)	Phospholipids (%)
RRIN-01 *(Spheciospongia* sp.*)*	0.68	36.80	18.86
CKRAM-03 *(T. seychellensis)*	1.06	26.26	23.25
RADE-06 *(S. solida)*	0.40	79.90	12.62
LSNG-07 *(S. solida)*	0.28	63.45	31.22
CHANG-I-07 *(S. solida)*	0.41	51.22	31.71
KODA-10 *(S. solida)*	0.56	67.55	17.78
KODA-13 *(Spirastrella* sp.*)*	0.88	83.36	11.36

Table 3. The compositions of fatty acids values (mean±SD) of sponges, order *Hadromerida*. The compositions were expressed as per cent total fatty acids.

Species	***Spheciospongia* sp.**		***T. seychellensis***		***Spirastrella* sp.**	
Location	**Chonburi**		**Chonburi**		**Trat**	
	RRIN-01		**CKRAM-03**		**KODA-13**	
Fatty acid	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**
C14:0	4.20±0.47	2.26±0.30	3.32±0.33	2.64±0.48	0.98±0.02	3.44±0.15
C16:0	15.25±0.57	14.04±2.69	15.64±0.96	9.10±0.76	8.14±0.05	15.52±0.73
C18:0	0.66±0.05	nd	nd	nd	0.87±0.02	nd
SFAs	**20.11**	**16.3**	**18.96**	**11.74**	**9.99**	**18.96**
C16:1n7	4.23±0.20	4.09±0.35	3.19±0.25	7.11±0.76	2.62±0.02	4.29±0.25
C18:1n9	4.10±0.08	5.45±1.74	5.55±0.07	6.20±0.29	2.61±0.02	2.87±0.12
C18:1n7	2.07±0.04	1.17±0.46	1.85±0.04	0.35±0.01	1.60±0.03	nd
C20:1n9	0.68±0.02	0.52±0.11	1.38±0.01	0.57±0.02	0.46±0.02	nd
MUFAs	**11.08**	**11.23**	**11.97**	**14.23**	**7.29**	**7.16**
C16:2n4	nd	nd	nd	nd	nd	nd
C16:3n4	1.03±0.00	1.60±0.41	1.11±0.01	0.42±0.41	0.68±0.02	1.26±0.07
C18:2n6	2.04±0.06	5.85±0.52	2.04±0.03	3.34±0.05	2.84±0.06	3.87±0.39
C18:3n4	1.25±0.05	1.10±0.16	1.42±0.01	1.29±0.08	1.67±0.04	nd
C18:3n3	0.37±0.02	0.62±0.22	0.75±0.03	0.74±0.07	0.49±0.01	nd
C18:4n3	0.45±0.01	0.53±0.01	1.30±0.01	1.49±0.00	0.48±0.0	nd
C20:4n6	nd	nd	0.83±0.03	0.43±0.05	nd	nd
C20:4n3	nd	0.94±0.07	nd	0.28±0.00	0.93±0.0	nd
C20:5n3	6.52±0.52	4.39±0.82	6.81±0.23	5.15±0.25	11.85±0.35	4.25±0.30
C22:5n3	nd	nd	nd	nd	nd	nd
C22:6n3	5.44±0.10	5.32±0.55	6.76±0.43	7.60±1.28	0.57±0.03	nd
PUFAs	**17.1**	**20.35**	**21.02**	**20.74**	**19.51**	**9.38**

Table 3. (Continued).

Species	***S. solida***							
Location	**Chonburi**				**Trat**			
	RADE-06		**LSNG -07**		**CHANG-I-07**		**KODA-10**	
Fatty acid	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**
C14:0	7.83±0.01	2.45±0.06	2.14±0.14	2.13±0.13	2.23±0.43	2.28 ±0.07	1.98±0.19	1.81±0.22
C16:0	13.26±0.06	10.37±0.42	9.02±0.24	9.24±0.08	9.80±1.24	11.88 ±.22	13.44±0.77	6.95±0.77
C18:0	nd	nd	nd	nd	nd	nd	nd	nd
SFAs	**21.09**	**12.82**	**11.16**	**11.37**	**12.03**	**14.16**	**15.42**	**8.76**
C16:1n7	6.20±0.02	2.23±0.10	1.66±0.07	1.73±0.07	1.14±0.12	2.41±0.05	2.85±0.12	1.72±0.23
C18:1n9	3.22±0.03	5.25±0.06	2.59±0.03	4.03±0.33	1.75±0.12	6.16±0.23	2.94±0.16	1.61±0.27
C18:1n7	1.40±0.01	2.17±0.00	1.22±0.01	2.02±0.01	0.85±0.03	1.53±0.13	1.86±0.0	nd

Species	*S. solida*							
Location	Chonburi				Trat			
	RADE-06		LSNG -07		CHANG-I-07		KODA-10	
Fatty acid	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**	**NL**	**PL**
C20:1n9	0.40±0.00	nd	nd	0.32±0.02	nd	nd	0.22±0.01	nd
MUFAs	**11.22**	**9.65**	**5.47**	**8.1**	**3.74**	**10.1**	**7.87**	**3.33**
C16:2n4	0.28±0.02	nd	nd	nd	nd	nd	1.21±0.13	2.07±0.32
C16:3n4	1.38±0.01	0.32±0.01	nd	0.25±0.02	0.50±0.34	0.87±0.18	0.29±0.01	nd
C18:2n6	1.85±0.02	3.04±0.04	1.75±0.01	3.97±0.01	1.88 ± 0.02	3.85 ± 0.19	3.41±0.01	3.12±0.40
C18:3n4	1.44±0.01	3.03±0.03	1.72± 0.01	1.56± 0.01	1.48 ± 0.03	1.54 ± 0.02	1.67±0.03	1.79±0.21
C18:3n3	0.58±0.00	0.71±0.01	0.61±0.06	0.74±0.09	0.44±0.03	0.59±0.01	0.90±0.0	nd
C18:4n3	0.48±0.02	0.71±0.00	0.79± 0.00	1.67± 0.06	nd	0.87±0.05	1.00±0.06	nd
C20:4n6	0.15±0.00	nd	nd	0.23±0.01	nd	nd	nd	nd
C20:4n3	0.45±0.00	0.81±0.02	1.25±0.02	3.31±0.27	0.90±0.03	1.59±0.05	1.11±0.06	2.42±0.43
C20:5n3	4.51±0.04	2.30±0.04	2.05±0.01	6.27±0.51	1.50±0.41	3.37±0.00	3.54±0.17	1.01±0.15
C22:5n3	nd	nd	nd	nd	nd	nd	nd	nd
C22:6n3	16.36±0.05	11.64±0.75	24.36±0.52	3.44±0.55	nd	nd	12.37±0.72	19.63±1.77
PUFAs	**27.48**	**22.56**	**32.53**	**21.44**	**6.7**	**12.68**	**25.5**	**30.04**

SFAs = saturated fatty acids, MUFAs = mono-unsaturated fatty acids,
PUFAs = poly-unsaturated fatty acids, nd = not detected.

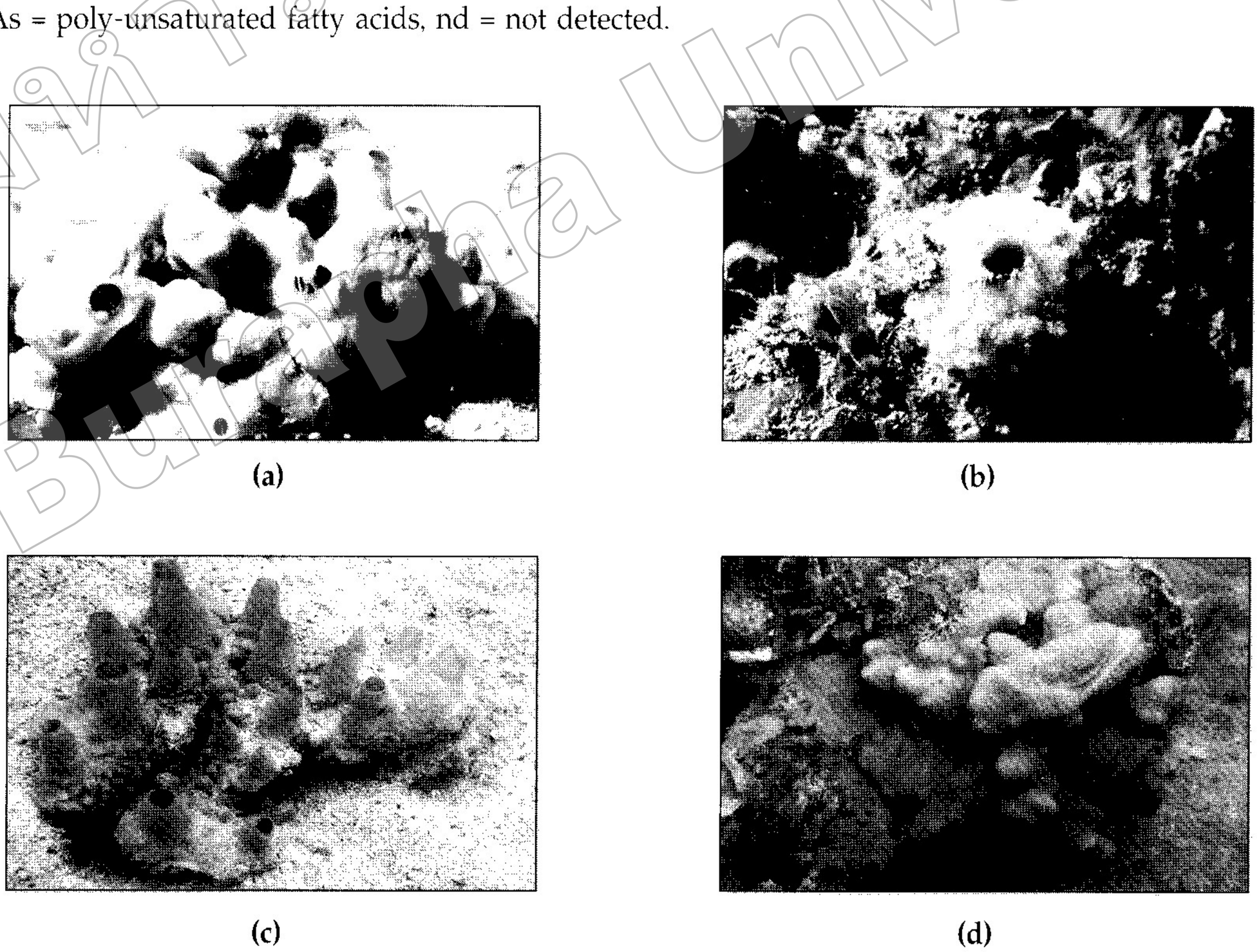

Figure 1. Pictures of marine sponges collected from Chonburi and Trat provinces: (a) *Spheciospongia* sp. (RRIN-01), (b) *T. seychellensis* (Wright, 1881) (CKRAM-03), (c) *S. solida* (Ridley and Dendy, 1886) (LSNG-07, RADE-06, CHANG-I-07, KODA-10), (d) *Spirastrella* sp. (KODA-13).

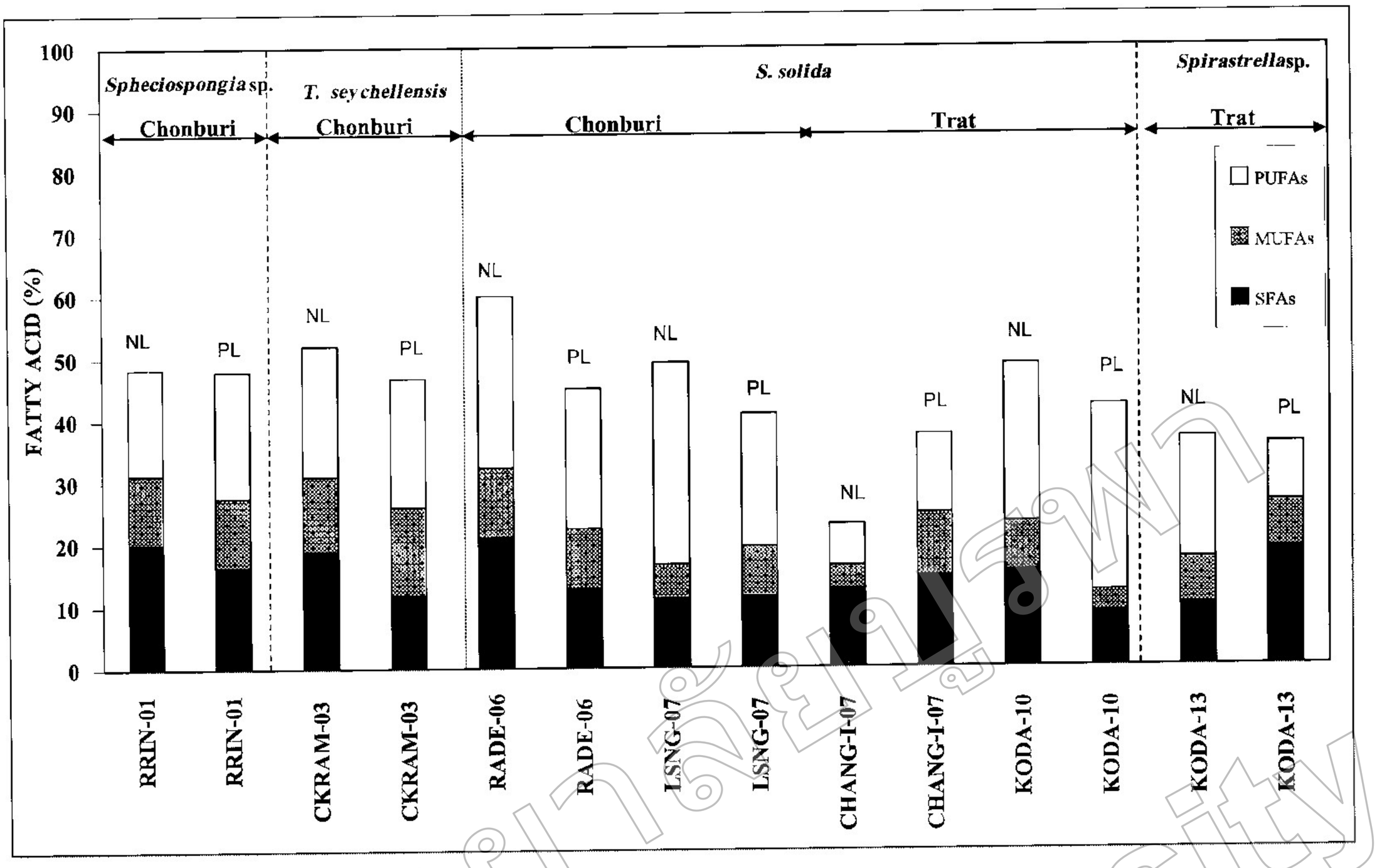

Figure 2. Diagram showing the characteristic of fatty acids in marine sponges, *Hadromerida.*

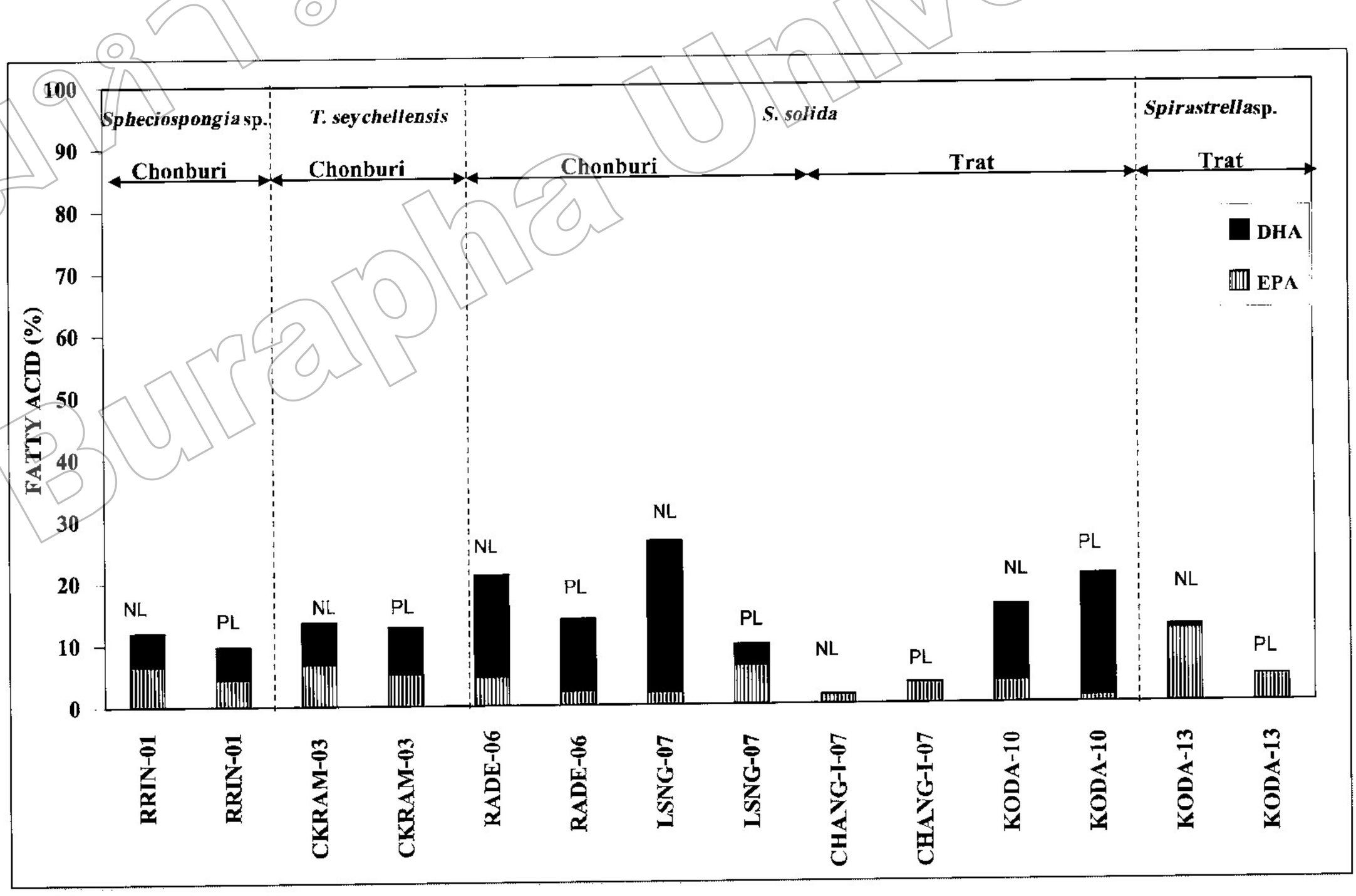

Figure 3. Diagram showing the essential fatty acids (EPA, DHA) in marine sponges, *Hadromerida.*

Table 4. EPA and DHA contents of seawater fish, freshwater fish (Chedoloh et al., 2007) and cultured microalgae (% total fatty acid) (Pratoomyot et al., 2005).

Scientific name	EPA	DHA
Seawater fish		
Caesio crythrogaster	0.00	0.00
Nemipterus spp.	0.23	0.18
Argyrops spinifer	0.16	0.52
Rastrelliger brachysoma	0.31	0.58
Euthynnus affinis	0.629	0.59
Pampus argenteus	0.26	0.54
Megalaspis cordyla	0.59	0.16
Sardinella gibbosa	0.49	0.68
Plectorhynchus pictus	0.00	6.55
Parupeneus cinnabarins	1.09	5.11
Priacanthus spp.	0.44	6.75
Anodontostoma chacunda	0.23	9.01
Lutjanus sanguineus	0.54	12.88
Eleutheronema tetradactylum	0.65	15.40
Caesio sp.	0.17	6.84
Freshwater fish		
Otolithes spp.	0.00	0.00
Hemibagrus spp.	0.00	0.00
Clarias spp.	0.00	0.00
Helostomi temmincki	0.67	1.86
Chonna striata	0.62	1.055
Microalgae		
Nitzschia cf. ovali	26.67	4.20
Thalassiosira sp.	16.65	1.33
Tetraselmis sp.	4.70	0.00
Marine sponges (this study)		
Spheciospongia sp.		
RRIN-01	10.91	10.76
T. seychellensis		
CKRAM-03	11.96	14.36
S. solida		
RADE-06	6.81	28.0
LSNG-07	8.32	27.8
CHANG-I-07	4.87	0.00
KODA-10	3.53	32.0
Spirastrella sp.		
KODA-13	16.10	0.57

EPA: eicosapentaenoic acid; (C20:5n3)

DHA: docosahexaenoic acid; (C22:6n3)

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