BIOCHEMICAL EVALUATION OF JELLYFISH IN KOTA KINABALU WATERS

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ABSTRAK

Tiga spesies obor-obor (*Rhizostoma* sp.1, *Rhizostoma* sp.2 dan *Chiropsalmus quadrigatus*) telah disampel dari Teluk Sepangar dan pantai Tanjung Aru, Sabah untuk analisis ciri-ciri biokimia iaitu kandungan protein, kandungan lipid, kandungan asid lemak, kandungan air and kandungan abu. Spesies-species tersebut telah dibanding untuk mengenalpastikan samada terwujud perbezaan yang ketara dalam ciri-ciri biokimia. Keputusan kajian digunakan untuk menentukan sifat biokimia yang spesifik dalam obor-obor yang menjadikan ia sebagai makanan yang lebih disukai penyu. Didapati perbezaan yang ketara wujud dalam kandungan protein di antara *Rhizostoma* sp.1 dan *Rhizostoma* sp.2 manakala tiada perbezaan yang ketara di kandungan lipid antara semua spesies obor-obor yang dikaji. Kandungan air di antara *Rhizostoma* sp.1 dan *Rhizostoma* sp.2 menunjukkan perbezaan yang ketara manakala kandungan abu analisis menunjukkan perbezaan ketara di antara *Rhizostoma* sp.1 dan *C. quadrigatus*. Analisis asid lemak menunkjukkan profil asid lemak *Rhizostoma* sp.1 dan *Rhizostoma* sp.2 adalah serupa dari segi masa perendungan dan pecahan acid lemak manakala profil *C. quadrigatus* menunjukkan perbezaan yang besar berbanding dengan *Rhizostoma* sp.1 dan *Rhizostoma* sp.2 dengan mengandungi lebih banyak asid lemak tidak tepu. Hasil analisis menunjukkan bahawa *Rhizostoma* sp.1 dan *Rhizostoma* sp.2 merupakan spesis yang berbeza tetapi genus yang sama. Perbezaan ciri-ciri biokimia di antara *Rhizostoma* sp.1 dan *Rhizostoma* sp.2 dengan *C. quadrigatus* terutamanya kandungan asid lemak mungkin menjadikan *C. quadrigatus* sebagai makanan yang lebih disukai penyu.

ABSTRACT

Three species of jellyfish (*Rhizostoma* sp.1, *Rhizostoma* sp.2 and *Chiropsalmus quadrigatus*) were sampled from Sepangar Bay and Tanjung Aru beach, Sabah for analysis of biochemical properties which are protein content, lipid content, fatty acid content, moisture content and ash content. These species were examined to distinguish whether significant differences of biochemical properties exist between comparisons of species. The results of analyses were also used to determine specific biochemical characteristic of jellyfish that qualifies them as preferred food for sea turtles. There was a significant difference in protein content between *Rhizostoma* sp.1 and *Rhizostoma* sp.2, whereas no significant difference was found in lipid content between all analysed species of jellyfish. The moisture contents between *Rhizostoma* sp.1 and *Rhizostoma* sp.2 showed a significant difference while the ash content analysis showed significant differences between *Rhizostoma* sp.1 and *C. quadrigatus*. The fatty acid analysis showed that the profile of *Rhizostoma* sp.1 and *Rhizostoma* sp.2 were very similar in the Retention Time and fatty acid fractions whereas profile of *C. quadrigatus* showed vast differences with *Rhizostoma* sp.1 and *Rhizostoma* sp.2 with more poly-unsaturated fatty acid fractions. The results show that *Rhizostoma* sp.1 and *Rhizostoma* sp.2 are jellyfish of different species but in same genus. The differences in biochemical properties between *Rhizostoma* sp.1 and *Rhizostoma* sp.2 with *C. quadrigatus* especially fatty acid content might make *C. quadrigatus* as preferred food for sea turtles.

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LIST OF ABBREVIATIONS & SYMBOLS

sp.	species
C. quadrigatus	*Chiropsalmus quadrigatus*
C. fleckeri	*Chironex fleckeri*
%	percentage
m	meter
g	gram
kg	kilogram
mg	milligram
ml	milliliter
nm	nanometer
μm	micrometer
mm	millimetre
μl	microliter
°C	degree Celsius
BMRI	Borneo Marine Research Institute
UMS	Universiti Malaysia Sabah
ODEC	Outdoor Development Centre
AOAC	Association of Analytical Communities
SPSS	Statistical Package for the Social Sciences
ANOVA	analysis of variance
CM	Chloroform: Methanol
FAME	fatty acid methyl esters
TLC	Thin Layer Chromatography
HE	Hexene: Ethyl Acetate
Tol	Toluene
CMW	Chloroform: Methanol: Water
Rf	Retardation factor
GCMS	Gas Chromatography Mass Spectrometry
RT	Retention Time
Na^+	Sodium ion
K^+	Potassium ion

COOH	carboxyl functional group
DNA	Deoxyribonucleic acid
dH_2O	distilled water
NaOH	Sodium Hydroxide
KOH	Potassium Hydroxide
MeOH	Methanol
$C_4H_{10}O$	diethyl-ether
$NaSO_4$	Sodium Sulphate Anhydrous
H_2SO_4	Sulphuric Acid
$K_2S_2O_8$	Potassium Persulphate
HgI_2	Mercury (II) Iodide
KI	Potassium Iodide
$(NH_4)_2SO_4$	Ammonium Sulphate
$NaOCH_3$	Sodium Methylate
$C_{17}H_{36}O$	9 – Heptadecanol
$C_{11}H_{13}BrO_2$	2-(1-Benzyloxy-2-bromoethyl)oxirane
$C_{17}H_{36}O$	1-Hexadecanol, 2-methyl
$C_{10}H_{15}Cl_3O$	Cyclohexanone, 2-(4,4,4-trichlorobutyl)
$C_{16}H_{16}O_3$	4-Benzyloxy-3-methoxyacetophenone
$C_{14}H_{24}O_4$	2,2-Dimethyl-6-methylidene-1-(3,5-dihydroxy-1-pentenyl) cyclohexan-1-perhydrol
$C_{19}H_{28}$	10,13-Dimethyl-4,5,6,7,8,9,10,11,12,13,14,15-dodecahydro-1H-cyclopenta(a)phenanthrene

CHAPTER 1

INTRODUCTION

1.1 General Introduction of Jellyfish

Jellyfish is a freely swimming medusa which is jelly-like and usually in a bell or umbrella shape with tentacles. They are found in all oceans of the world. The tentacles have stinging characteristics and they are usually used to sting and entangle prey. When they sting their prey, toxin is injected into prey's body by cnidoblasts, a unique cell found on jellyfish tentacles. Humans are also often stung by jellyfish during sea activities. The symptoms of being stung range from itchy (from smaller sized jellyfish), pain with a burning sensation and can even lead to death. Most species of jellyfish can swim actively by contracting muscles and exploiting the mechanical properties of the mesoglea (also known as ectoplasma, is the clear, inert, jellylike substance that makes up most of the bodies of jellyfish and certain primitive sea creatures in the phylum cnidaria). When the muscle fibers of the swimming bell contract, the water under swimming bell is forced out and the jellyfish is propelled in the opposite direction.

1.2 Biochemical Compounds in Jellyfish

The body of jellyfish is estimated to consist of 95% of water, and the rest is cells and organs (Souza *et al.*, 2007). As with other living organisms, the cells of jellyfish are believed to consist of several essential chemical compounds. These essential chemical compounds which are related to nutritional value that can be extracted: proteins, lipid and fatty acid. These chemical compounds act differently but have significant roles in cells of living organisms, including jellyfish, and these compounds also hold significant characteristics and functions when they are consumed as nutrients by other organisms in the food chain.

1.2.1 Protein

The term protein was first used by the Dutch chemist Gerardus Mulder in 1838 to name a specific group of substances abundant in all plants and animals. The importance of proteins was correctly predicted by Mulder, who derived the name from the Greek word *proteios*, meaning primary or first rank. Proteins, biopolymers constructed from amino acids, display a wide range of structures and functions. The amino acid building blocks are selected from a pool of 20 different molecules, each having a distinct chemical structure. The unique amino acid composition and sequence in each type of protein allow it to fold into the precise three-dimensional arrangement necessary for carrying out its designated biochemical function. The great variety in biological function is the direct result of many variations in amino acid composition and sequence that are possible for a protein (Boyer, 1999).

1.2.2 Lipid

Lipids are organic compounds that exist in living organisms and that are soluble in non-polar solvents. Because compounds are classified as lipids on the basis of a physical property, which is their solubility in a non-polar solvent, rather than on the basis of their structures even though lipids have a variety of structures and functions. The solubility of lipids in non-polar solvents results from their significant hydrocarbon component which is the part of the molecule that is responsible for how oily and how fat the lipid is. In fact, the word *lipid* comes from the Greek *lipos*, which means "fat" (Bruice, 2006).

1.2.3 Fatty Acid

Fatty acids are biomolecules containing a polar carboxyl functional group (- COOH) connected to an unbranched aliphatic chain. These structural features give them a split behaviour: One end is polar and sometimes ionic, which is the carboxyl group, whereas the opposite end, which is the hydrocarbon chain, has non-polar properties. This molecular characteristic is called amphiphilic (Boyer, 1999). The melting point of fatty acids increase with increasing molecular weight because of increased van der Waals interactions between the molecules. This is also true for unsaturated fatty acids as well, but boiling points are lower than those of saturated fatty acids with comparable molecular weights. Fatty acids are called unsaturated if carbon – carbon double bonds exist in hydrocarbon chain. Fatty acids are called polyunsaturated fatty acids when there is more than one carbon – carbon double bonds (Bruice, 2006).

1.3 Significance of Study

The significance of this study is to better understand the nutritional value of jellyfish as potential food source for sea turtles. In general, jellyfish is known as the primary food source for the Leatherback turtle (*Dermochelys coriacea*). However, there is evidence that shows that jellyfish is also consumed by other species of sea turtles such as the Green turtle (*Chelonia mydas*) and the Hawksbill turtle (*Eretmochelys imbricata*) (Mortimer, 1981, as cited in Lutz & Musick, 1997). Since these two species of sea turtles are often found in Kota Kinabalu waters, and reports of Leatherback turtle sighting are not uncommon, understanding the nutritional value of certain species of jellyfish can help figure out the feeding behaviour of sea turtles especially when a jellyfish bloom occurs (e.g., sea turtles' preference of certain species of jellyfish). Other than that, understanding the differences of nutritional value in different species of jellyfish can also help in comprehending the life cycles of sea turtles which consume them, whether sea turtles will have better growth rates if they consume certain species of jellyfish.

1.4 Objectives of Study

The objectives of this study are to determine the biochemical properties of jellyfish and to establish specific biochemical characteristic of jellyfish that qualifies them as preferred food for sea turtles.

CHAPTER 2

LITERATURE REVIEW

2.1 Classification of Cnidarian

Jellyfish is a class of animals (class Scyphozoa) in the phylum Cnidaria of Animalia kingdom which is characterized by its soft-body with cells called cnidoblasts and radial symmetric bodies.

The phylum Cnidaria consists of more than 10,000 species of soft-body animals, including sea anemones, corals, freshwater *Hydra*, Portuguese man-of-war, and jellyfish. The two noticeably different body forms of cnidarians include medusa form, which resembles a gelatinous saucer or upside-down cup, generally swimming in pulsation motion; and polyp form, which has a tubular body and is generally stationary. In different species, each body form is usually present in a different part of the life cycles, and in some species, both are represented at the same time in one individual (Pechenik, 2005).

2.2 Uniqueness of Cnidarians – Cnidoblasts

Cnidarians are unique with remarkable organelles secreted within cells called cnidoblasts (or nematoblasts) which discharged with explosive force, mainly for the purposes of self-defense or capturing prey. These cnidoblasts are actually a rounded proteinaceous capsule with an opening which shoots out a hollow tube when triggered. Within a given individual, discharged hollow tubes may be specialized in wrapping around small objects, sticking to surfaces, penetrating surfaces, or secreting proteinaceous toxins, some of which are among the most deadly toxins known (Pechenik, 2005). Cnidoblasts are especially abundant on the tentacles but can be found on surface of the bell for some species (e.g.: *Pelagia noctiluca*). With this characteristic, cnidaria or jellyfish are considered as one of the most venomous animals in the world.

2.3 Classes and Orders of Cnidarian

Four major classes can be further divided in phylum cnidaria, which are class Anthozoa (corals), class Scyphozoa (jellyfish), class Cubozoa (box jellyfish), and class Hydrozoa. The complete classification of these four classes is shown in Figure 2.1. Among all known species in the world, class Anthozoa consist about 6,000 species, including sea anemones and corals such as Scleractinia (stony star corals). The medusa stage is not known among this class. Class Scyphozoa contains about 200 species, which mostly appear as medusae (Fullerlove, 2002). Class Cubozoa (box jellyfish) encompasses about 20 species, which only appear as medusae. Among them are the species *Chironex fleckerii* and *Chiropsalmus quadrigatus*, known as sea wasps,

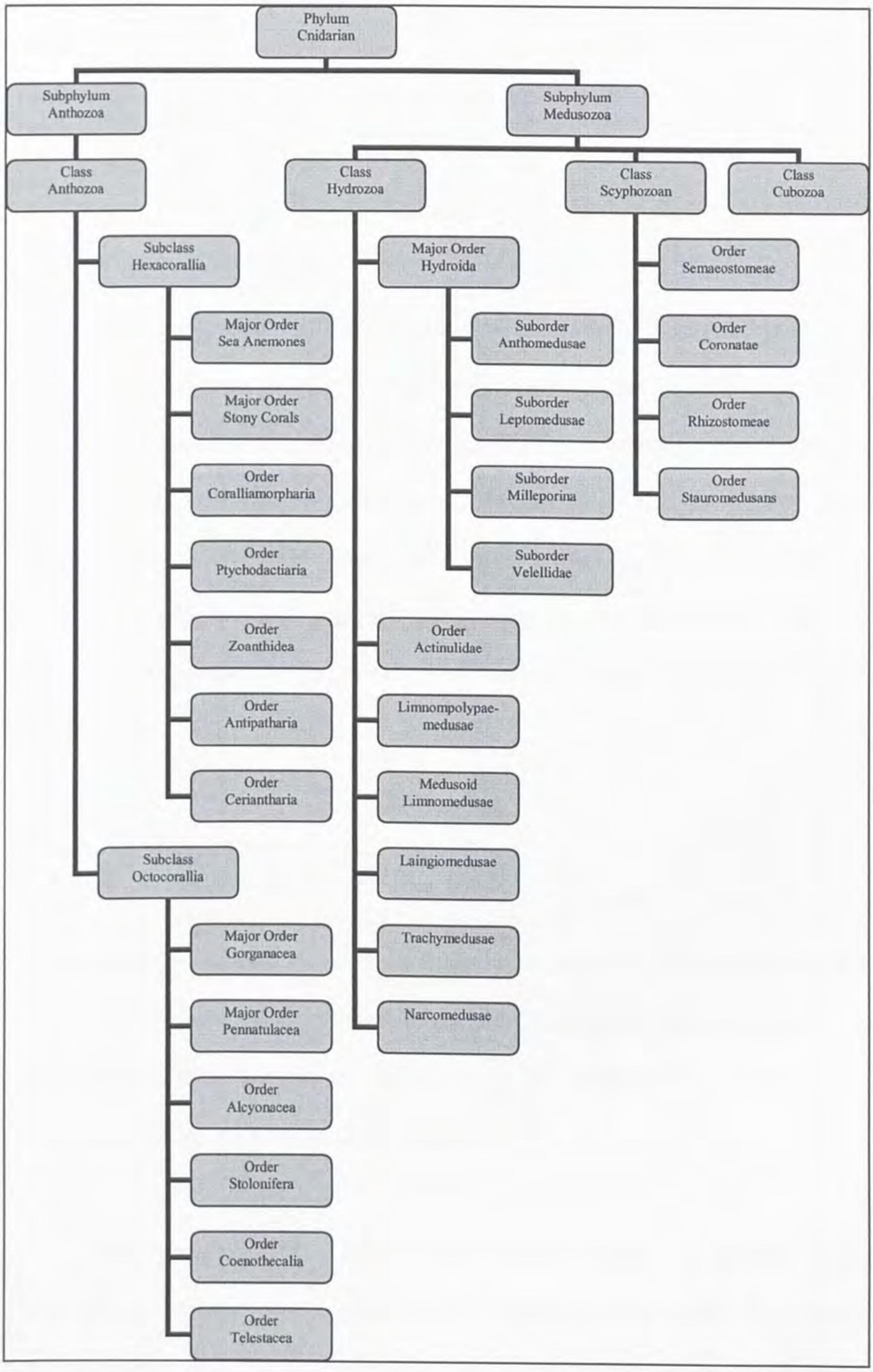

Figure 2.1 Complete classifications of four major classes in phylum Cnidaria

which possess a highly potent toxin. Class Hydrozoa contains about 3,000 species, and is a broad spectrum stretching from the tropical fire corals (Milleporidae) to the hydroids (Sertularia), some of which appear in the North Sea. Hydrozoa often display alternation of generations between medusa and polyp forms (Ruppert *et al.*, 2004).

However, jellyfish that are found in Kota Kinabalu waters are in the order Semaeostomeae, order Coronatae, order Rhizostomeae, and order Stauromedusans from class Scyphozoa (jellyfish) and class Cubozoa (box jellyfish). Eleven species of jellyfish in class Scyphozoa and a species of box-jellyfish (class Cubozoa) were spotted in a study of jellyfish biodiversity in Kota Kinabalu water (Ramachandram, 2006) and another species suspected to be *Pelagia noctiluca* (Forskål 1775) in the order Semaeostomeae was found in bloom in waters off Police Beach, Gaya Island (6° 01' 48.13" N, 116° 01' 11.69" E) on 18 July 2007.

2.3.1 Class Scyphozoa

The scyphozoan contains only about 200 species, all of which are marine and many of which are quite large (might reach up to about 2m in diameter). The mesoglea layer of scyphozoan is thick and has the uniformity of firm gelatin; thus, scyphozoans are known collectively as jellyfish (Pechenik, 2005).

Jellyfish morphology is described as medusoid (Figure 2.2). The body is in the form of an inverted bowl, with tentacles which full of cnidoblasts, extending downward from the bowl/bell. The mouth is found below the inverted bowl, at the end

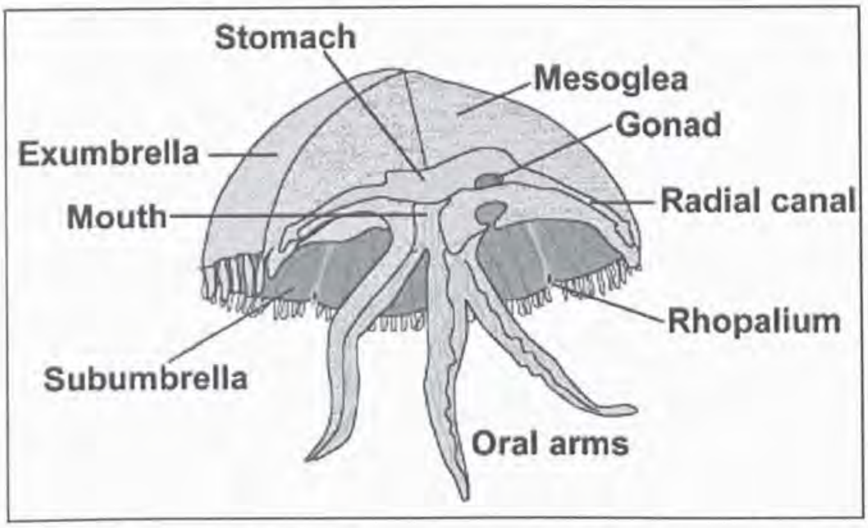

Figure 2.2 Anatomy of Scyphozoa, cutaway view of an adult. (Redrawn from Barnes, 1980)

of a muscular cylinder and known as manubrium. They are also characterized by a well-developed system of fluid-filled gastrovascular canals, connecting to the mouth through the manubrium. Food particles captured by cnidoblasts on the tentacles and/or by oral arms are ingested at the mouth and conveyed to the stomach through the manubrium. Food is then distributed among four gastric pouches, which contain short, cnidoblast-bearing tentacles (gastric filaments) that secrete an array of digestive enzymes (Pechenik, 2005).

As a mobile organism, scyphozoan medusae are equipped with fairly complex sensory receptor, implying that the nervous system is able to process and integrate a variety of sensory input. Sensory systems include balance organs (statocysts), simple light receptors (ocelli) and touch receptors (sensory lappets). The statocysts and ocelli are contained within club-shaped structures called rhopalia, which are distributed along the margins of the swimming bell. An ocellus is simple light-sensitive pigment in ocelli (Ruppert *et al.,* 2004). It is use to detect and sense the strong or weak of light but will not form image or functioning like eyes.

The life cycle of scyphozoan involve the polyp stage and medusa stage (Figure 2.3). In the medusa stage, gonads develop within gastrodermal tissue and are closely associated with the gastric pouches (Pechenik, 2005). Usually the gonads are different in colour with gastrodermal tissue and are obviously visible. Hence this feature helps to identify a species through the shape of gonads, gastric pouches and even the number of gastric pouches. Most individual medusae are separate sexes and the species is said to be gonochoristic (*gono* = reproductive organs; *chorist* = separate, in Greek) or dioecious (Pechenik, 2005). This is another characteristic to distinguish the phylum cnidaria from most of the other invertebrate phylums.

A planula larva forms from fertilization of egg and sperm released by male and female medusae. The non-feeding planula larva soon attaches to a substrate and develops into a polyp. As the polyp grows, they produce additional polyps on it asexually by budding. Eventually, a process called strobilation takes place in most species, with the body column of a polyp subdividing diagonally, forming numerous modules that are stacked on top of each other. Each module eventually breaks away from the stack as a swimming ephyra. As it swims, each ephyra regularly grows and changes in physical appearance, becoming an adult scyphozoan (Pechenik, 2005).

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