THE BIOCHEMICAL COMPOSITION OF SEAWEEDS FROM THE PERSIAN GULF AND THE EFFECT OF SEAWEED EXTRACT ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF MICROALGAE CULTURED AS LIVE FOOD FOR *Penaeus indicus* LARVAE

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by

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LIST OF ABBREVIATIONS

AA	arachidonic acid
BHT	butylated hydroxytoluene
CHA	*C. muelleri*
CNS	central nervous system
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acid
FAME	fatty acid methyl esters
FID	flame ionization detector
GR	grazing rate
ISO	*I. galbana*
LDL	low-density lipoprotein
M_I	mysis I
M_{II}	mysis II
Mixed	*I. galbana* + *C. muelleri*
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
PZ_I	protozoa I
PZ_{II}	protozoa II
PZ_{III}	protozoa III
SFA	saturated fatty acid
SWE	seaweed extract
SWE-Alt	seaweed extract as an alternative to f/2 medium
SWE-Sup	seaweed extract as a supplement to f/2 medium
TL	total length
USDA	United States Department of Agriculture

KOMPOSISI BIOKIMIA RUMPAI LAUT DARI TELUK PARSI DAN KESAN EKSTRAK RUMPAI LAUT TERHADAP PERTUMBUHAN DAN KOMPOSISI BIOKIMIA MIKROALGA YANG DIKULTUR SEBAGAI MAKANAN HIDUP UNTUK LARVA *Penaeus indicus*

ABSTRAK

Komposisi biokimia tiga kumpulan rumpai laut; hijau (*Ulva lactuca* dan *Enthromorpha intestinalis*), perang (*Sargassum illicifolium* dan *Colpomenia sinuosa*) dan merah (*Hypnea valentiea* dan *Gracilaria corticata*) dari Teluk Parsi dan kesan ekstrak rumpai laut (SWE) tersebut sebagai makanan tambahan atau media gantian bagi media f/2 terhadap tumbesaran dan komposisi untuk dua jenis mikroalga, *Isochrysis galbana* dan *Chaetoceros muelleri* yang dikultur sebagai makanan hidup untuk larva *Penaeus indicus* telah diselidik. Keputusan kajian menunjukkan bahawa rumpai laut secara relatifnya mengandungi karbohidrat dan abu yang tinggi, tetapi kandungan lipidnya adalah rendah. Kandungan lipid dalam rumpai laut hijau (*U. lactuca* dan *E. intestinalis*) secara signifikan lebih tinggi daripada kedua-dua rumpai laut merah dan perang ($P < 0.05$). Kandungan protein bagi rumpai laut merah (*G. corticata*) dan hijau (*U. lactuca* dan *E. intestinalis*) adalah jelas lebih tinggi daripada rumpai laut perang ($P < 0.05$). Rumpai laut hijau (*U. lactuca*) dan merah (*G. corticata*) mempunyai perkadaran asid lemak tepu yang paling tinggi, malahan rumpai laut perang (*S. illicifolium* dan *C. sinuosa*) dan merah (*G. corticata*) masing-masing mempunyai perkadaran asid lemak mono-tak tepu dan poli-tak tepu yang tertinggi. Komposisi mineral dalam rumpai laut didapati mengikut turutan K> Mg> Fe> Zn> Mn> Cu> Co.

Kajian ini jelas menunjukkan bahawa *I. galbana* dan *C. muelleri* boleh dikulturkan dengan berjayanya dengan menggunakan pelbagai SWE sama ada sebagai makanan tambahan atau alternatif untuk media f/2. Oleh sebab tiada

perubahan major ditemui dalam kebanyakan parameter tumbesaran yang diukur, komposisi proksimat biokimia, asid lemak poli-tak tepu yang penting dan kandungan mineral melalui pengkulturan dua mikroalga tersebut dengan menggunakan SWE sebagai media alternatif, khususnya ekstrak *U. lactuna, E. intestinalis* dan *G. corticata*, maka dapat disimpulkan bahawa SWE yang dinilaikan dalam kajian ini dapat membekal nutrisi yang diperlukan untuk tumbesaran mikroalga dan mungkin boleh digunakan sebagai gantian untuk mengurangkan kos pengeluaran mikroalga, sekurang-kurangnya dua kali ganda daripada konvesional f/2 media, dalam pembentukan populasi mikroalga untuk penggunaan operasi akuakultur.

Kajian ini menunjukkan bahawa proses penyalinan kulit ke peringkat mysis 2 (M_{II}) bagi larva udang *P. indicus* yang diberi mikroalga (eksklusif atau campuran) yang dikulturkan dengan SWE sebagai media tambahan adalah lebih cepat jika dibandingkan dengan larva yang diberi mikroalga yang dikulturkan dengan media konvensional f/2 (kawalan tanpa SWE). Larva udang yang diberikan diet mikroalga yang mengandungi SWE sebagai makanan tambahan dalam media kultur mencatatkan nilai maksimum untuk jumlah panjang akhir, kadar kemandirian dan kadar tumbesaran speksifik jika dibandingkan dengan larva udang yang diberikan diet kawalan. Keseluruhannya, prestasi tumbesaran jelas menunjukkan bahawa larva *P. indicus* berjaya dipelihara dengan menggunakan diet mikroalga yang diuji, bahkan larva udang yang diberi mikroalga campuran (*I. galbana* + *C. muelleri*) menunjukkan tumbesaran, kemandirian dan pembentukan larva yang lebih baik jika dibandingkan dengan larva yang diberi diet mikroalga tunggal. Tambahan pula, tumbesaran, kemandirian, dan pembentukan larva yang serupa juga diperolehi apabila larva udang diberi mikroalga yang dikulturkan dengan SWE sebagai

pengganti media untuk media f/2. Kesimpulannya, mikroalga yang dikulturkan dengan SWE berpotensi digunakan sebagai kaedah alternatif berkos rendah dalam penghasilan makanan hidup untuk larva udang.

THE BIOCHEMICAL COMPOSITION OF SEAWEEDS FROM THE PERSIAN GULF AND THE EFFECT OF SEAWEED EXTRACT ON THE GROWTH AND COMPOSITION OF MICROALGAE CULTURED AS LIVE FOOD FOR *Penaeus indicus* LARVAE

ABSTRACT

The biochemical composition of three groups of seaweeds; green (*Ulva lactuca* and *Enthromorpha intestinalis*), brown (*Sargassum illicifolium* and *Colpomenia sinuosa*) and red (*Hypnea valentiea* and *Gracilaria corticata*) from the Persian Gulf and the effects of seaweed extracts (SWE) either as a supplement or as a substitute media to the f/2 medium on the growth and composition of two microalgae *Isochrysis galbana* and *Chaetoceros muelleri* cultured as live food for *Penaeus indicus* larvae were investigated.

Results showed that seaweeds were relatively high in carbohydrate and ash, but low in lipid. Lipid content in green (*U. lactuca* and *E. intestinalis*) seaweed was significantly higher than both the red and brown seaweed ($P < 0.05$). Protein content of red (*G. corticata*) and green (*U. lactuca* and *E. intestinalis*) seaweeds was notably higher than brown seaweed ($P < 0.05$). The green (*U. lactuca*) and red (*H. valentiea*) seaweeds had the highest proportion of saturated fatty acids, while the brown (*S. illicifolium* and *C. sinuosa*) and red (*G. corticata*) seaweeds had the highest proportion of monounsaturated and polyunsaturated fatty acids, respectively. The mineral compositions in seaweeds were found in the sequence of K>Mg>Fe>Zn>Mn>Cu>Co.

The study clearly showed that the two microalgae *I. galbana* and *C. muelleri* could be successfully cultured using the various SWE either as a supplement or as an

alternative to the f/2 medium. Since no major changes were found in most of the measured growth parameters, proximate biochemical composition, important polyunsaturated fatty acids and minerals content following culture of the microalgae with SWE as an alternative media, particularly extracts of *U. lactuca*, *E. intestinalis* and *G. corticata*, it was concluded that these SWE are able to provide the necessary nutrients for microalgae growth and could be used as a possible substitute to reduce microalgae production costs, at least two times lower than conventional f/2 medium, in establishing microalgal populations to use in aquaculture operations.

The present study found that when *P. indicus* larvae were fed on microalgae *I. galbana* and *C. muelleri* (exclusively or mixed) that had been cultured with SWE as a supplement media, they molted faster to mysis 2 (M_{II}) stage compared to the larvae fed on microalgae cultured with conventional f/2 media (control without any SWE). Maximum final total length, survival rate and specific growth rate were recorded for shrimp larvae fed on microalgae diets that included SWE as a supplement in the culture media compared to shrimp larvae fed the control diet. The overall growth performance clearly showed that *P. indicus* larvae were successfully reared using microalgae diets tested, and the shrimp larvae fed on mixed microalgae (*I. galbana* + *C. muelleri*) showed better larval growth, survival and development than those that were fed on single microalgal diet. Furthermore, similar larval growth, survival and development were obtained when shrimp larvae were fed with microalgae cultured with SWE as a substitute media to f/2 medium. In conclusion, microalgae cultured with SWE could potentially be used as a low-cost alternative method in producing live food for the hatchery production of shrimp larvae.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Seaweeds

Seaweed or sometimes called macroalga is the common name for benthic macroscopic and multicellular marine algae that almost exclusively grow in the shallow coastal waters from intertidal to the sub-tidal zones, where light is available for photosynthesis. Similar to the higher plants, seaweeds contain principal photosynthetic pigment (chlorophyll *a*) and accessory pigments (chlorophyll *b*, *c*, carotenoids, phycocyanin and phycoerytrin). The accessory pigments assist chlorophyll *a* in absorption of light and consequently in photosynthesis reaction.

1.1.1. Taxonomy

Seaweeds are primitive forms of multicellular photosynthetic organisms in the freshwater and marine environments belonging to the Protista group. They may be sometimes confused with sea grasses that are taxonomically considered as higher plants. Seaweeds are classified into three major groups according to their photosynthetic pigments: Chlorophyta (green seaweed), Phaeophyta (brown seaweed) and Rhodophyta (red seaweed).

1.1.2. Structure

The entire body of the seaweed from top to bottom is known as a thallus which, includes the blade, stipe and holdfast. The pseudo-leaves in seaweeds are known as blades. In comparison to higher plants, seaweeds absorb nutrients from their blade as well as sunlight. Blades in some seaweed are similar to higher plants with a midrib

that is oriented on the stipe. The stipe or stalk (pseudo-stem) in some seaweed bears several smooth cylindrical erect primary branches, and *Sargassum* sp. is one of the best examples of this type of seaweed. Most seaweed is attached to the substrate by a root- like structure, holdfast, which fulfills an anchoring function for seaweeds against currents and tides.

1.1.3. Ecology

In intertidal zones, because of low depth and tide activities, seaweeds suffer many physiological stresses due to drying out, exposure to direct sunlight, seawater currents, and rapidly changing temperature and salinity. Therefore, they must have mechanisms for overcoming these environmental changes. One of these mechanisms is the types of photosynthetic pigments it contains. The green seaweeds are more abundant in the intertidal zones and shallow waters. Brown seaweeds grow commonly in lower parts of intertidal to shallow subtidal zones and red seaweeds can grow in deeper waters than the green and brown seaweeds (White and Keleshian, 1994).

1.1.4. Reproduction

In comparison to higher plants, seaweeds have no generative organs like flower, fruit and seed; instead they can reproduce sexually (alternation of generation) or asexually (fragmentation).

1.1.5. Biochemical composition

Proteins, lipids, carbohydrates and minerals are the most important biochemical components in seaweeds (Manivannan *et al.,* 2009).

1.1.5(a). Proteins

The protein content of brown seaweeds is generally small (average: 5-15 % of the dry weight), whereas higher protein contents are recorded in green and red seaweeds (on average 10-30 % of the dry weight). In some red seaweed, such as *Palmaria palmata* (47% of the dry weight) and *Porphyra tenera* (47% of the dry weight), proteins contents are comparable to those found in high-protein vegetables such as soybeans (in which proteins represents 35 % of the dry weight). The protein levels of *Ulva* spp. are in the range 15-20 % of the dry weight (Burtin, 2003).

1.1.5(b). Lipid and fatty acids

Seaweeds commonly are low in lipids and include only 1-5 % of dry weight (Polat and Ozogul, 2008), but show an interesting polyunsaturated fatty acid (PUFA) composition particularly regarding with omega 3 and omega 6 acids (Burtin, 2003). Of these, the green algae show notable contents of linolenic acid. The red and brown algae are particularly rich in eicosapentanoïc acid (EPA) and arachidonic acid (AA).

1.1.5(c). Carbohydrates

In comparison to proteins and lipids, seaweeds contain large amounts of carbohydrates and is the most important components for metabolism and it supplies the energy needed for respiration and other metabolic processes (Shanmugam and Palpandi, 2008). Alginate from brown, carrageenans and agar from red seaweeds are water-soluble carbohydrates (Jiménez-Escrig and Sánchez-Muniz, 2000). The content of total dietary fibre ranges from 33–50 % of dry weight of seaweeds (Jiménez-Escrig and Cambrodon, 1999) and represents their major component is primarily soluble fibre (Jiménez-Escrig and Sánchez-Muniz, 2000). The typical seaweed carbohydrates are not digestible by the human gastrointestinal tract and,

therefore, they are dietary fibres. The types and abundance of carbohydrates vary strongly between algae species (Dawczynski *et al.,* 2007).

1.1.5(c). Minerals

Seaweeds are a rich source of minerals, especially macro and micronutrients (Mabeau and Fleurence, 1993). The most common minerals found in seaweeds are iodine, magnesium, sodium, calcium, phosphorus, iron, potassium, copper and fluorine. The mineral fraction of some seaweed even accounts for up to 40% of dry weight (Ortega-Calvo *et al.,* 1993); however, in some cases the mineral content of the seaweeds is recorded even higher than that of land plants and animal products (Ito and Hori, 1989). The mineral composition of seaweed varies and is affected by species (Devi *et al.,* 2009), geographic area, season of the year and temperature of water (Rao *et al.,* 2007).

1.2. Microalgae

Microalgae include a great variety of photosynthetic organisms, which have a remarkable potential for cultivation and application from human and animal nutrition to cosmetics, and production of high-content products (e.g., pigments, antioxidants and fatty acids) as well as serving as the base of the aquatic food web. They are microscopic algae, which particularly inhabit the illuminated surface waters of fresh and marine environments. Microalgae are capable to absorb CO_2 to perform photosynthesis and produce approximately half of the atmospheric oxygen. It is claimed that microalgae play an important role in the development of future renewable energy scenarios (Avagyan, 2008). Furthermore, microalgae display better photosynthetic efficiency, using light nearly three times more efficiently than higher plants (Aaronson and Dubinsky, 1982).

1.2.1. Conditions affecting microalgae growth

The most important parameters regulating algal growth are nutrient supply, light, pH, aeration, salinity and temperature.

1.2.1(a). Nutrients

Whereas the concentrations of cells in microalgae cultures are generally higher than those found in nature, the algal cultures must be enriched with nutrients to compensate for the deficiencies in nutrients. Needed macronutrients include nitrate, phosphate and silicate and micronutrients consist of various trace metals and the vitamins thiamin (B_1), cyanocobalamin (B_{12}) and for some species, biotin. Silicate is specifically used for the growth of diatoms, which utilize this element in their external shell (Paasche, 1973).

Several media formulations have been used extensively for microalgae cultivation and are commercially available in laboratory-grade form. There are two media which are suitable for the growth of most algae the Walne medium (Appendix A) and the Guillard's f/2 medium (Appendix B). In large-scale extensive systems, generally aquaculture use alternative enrichment media that are suitable for mass production of microalgae that contain only the most essential nutrients and are composed of agriculture-grade rather than laboratory-grade fertilizers (Appendix C).

Although trace elements are usually found in sufficient quantities, macronutrients are in short supply (usually phosphorus in freshwater and nitrate in saltwater). Several nutrient enrichment media containing soil extract, nitrates, phosphorus, trace elements, and vitamins have been described for freshwater and saltwater (Creswell, 1993). Of the nutrient media formulations, Guillard and Ryther's f/2 media is the most widely used to culture marine microalgae in phycolabs and hatcheries. Guillard's f/2 nutrients are usually used at the rate of 2 mL for each liter of microalgae culture (Baptist *et al.,* 1993).

1.2.1(b). Light

As with the case of land plants, microalgae carry out photosynthesis and assimilate carbon dioxide (CO_2) and water (H_2O) into algal biomass. Light is the source of energy, which drives this reaction. Light may be natural or artificial (supplied by fluorescent bulbs) and is often employed for culture maintenance and experimental purposes. Light intensity of 2,500 to 5,000 lux is optimal for microalgae photosynthesis (Escobal, 1993). Guillard (1973) recommended 3,500 and 4,500 lux for culture of *Thalassiosira pseudonana* under continuous and 14 hours per day illumination, respectively. Too high light intensity may result in photo-inhibition

in microalgae, while too low light may be inadequate for growth. On the other hand, many microalgae species do not grow well under constant illumination, and hence a light/dark (LD) cycle is used (highest 16:8 h LD, typically 14:10 or 12:12 h) (Barsanti and Gualtieri, 2006). Artificial light is usually preferred over sunlight because can be controlled with a simple timer or light monitor (Creswell, 2010). Although artificial lighting can be precisely controlled in terms of quality and quantity, it is costly and accounts for almost 95 percent of the cost to culture microalgae (Muller-Feuga *et al.*, 2003).

1.2.1(c). pH

Most microalgae species are capable of growing at an acceptable pH range between 7 and 9, with the preferable range at 8.2-8.7. High fluctuation of pH can result in complete culture collapse due to the disruption of many cellular processes. In the case of high-density algal culture, however, aerating the culture and addition of carbon dioxide help to correct for increased pH (FAO, 1996).

1.2.1(d). Aeration

Mechanical aeration is crucial for healthy microalgae culture through keeping them in suspension in order to grow, to equally expose them to light and nutrients, to avoid thermal stratification and to increase gas exchange between the culture medium and the air. The latter is of importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. It should be noted that in very dense cultures, pure carbon dioxide might be supplemented to the air supply (at a rate of 1% of the volume of air) instead of the CO_2 originating from the air (containing 0.03% CO_2) (FAO, 1996).

1.2.1(e). Temperature

Temperature normally affects rate of metabolism of an organism. The temperature at which microalgae are maintained should ideally be as close as possible to the temperature of their native habitat. The optimal temperature for microalgae cultures is generally between 20 and 24°C, although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of microalgae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are lethal for a number of species (FAO, 1996).

1.2.1(f). Salinity

Marine microalgae are able to tolerate changes in salinity. They typically grow best at a salinity that is slightly lower than at where they were collected. Salinities of 20-24 ppt have been found to be optimal.

1.2.1(g). Starter or Inoculums

Culturing microalgae usually begins with a pure stock (strains) or starter culture of the desired algal species; therefore, the quality of the starter should be regularly checked for the presence of contaminants. The amount of inoculum to be used depends on the microalgal cell density in the starter culture and the total volume of culture. For large-scale algal production, to faster harvest the cultures, more starters with high densities are required. On the other hand, renewal of cultures is necessary to maintain a continuous supply of strains of microalgae for the hatcheries.

1.2.2. Growth dynamics

The growth dynamic of microalga is shown in Figure 1-1

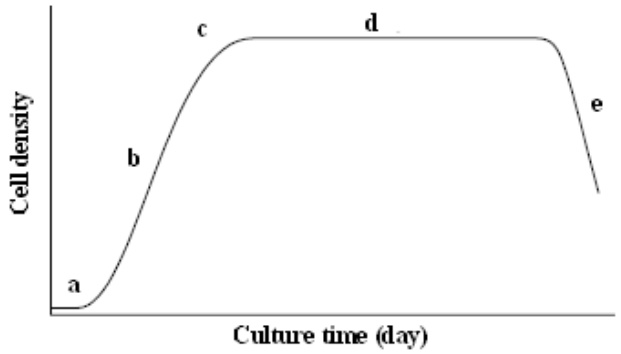

Figure 1.1.The growth dynamic pattern of microalga culture[1]

1.2.2(a). Lag or induction phase

During the adaption or lag phase (a, Figure 1.1), the algal culture adjusts itself to the given cultivation conditions; therefore little increase in cell density occurs. High physiological activity is found during this phase, the cell being much more sensitive to temperature or other environmental changes than cells in a more mature stage of development. The lag in growth is attributed to the physiological adaptation of the cell metabolism to growth, such as increase of the contents of enzymes and metabolites involved in cell division and carbon fixation.

1.2.2(b). Exponential phase

During the second phase (b, Figure 1.1), the cell density increases as a function of time t according to a logarithmic function:

$$C_t = C_0.e^{\mu t}$$

where C_t and C_0 are the cell concentrations at time t and 0, respectively, and μ= specific growth rate.

[1] a, b, c, d and e indicated lag, exponential, decline, stationary and death phases, respectively.

During this phase, the light intensity is not limiting and changes in the nutrient concentration for uptake by the microalga are so small that this has negligible effects on growth rate. The specific growth rate is mainly dependent on algal species, light intensity and temperature. Maintaining of microalgae in the exponential phase of growth is the key to the success of algal production (FAO, 1996).

1.2.2(c). Phase of declining growth rate

During this phase (c, Figure 1.1), microalgal cell division gradually begin to decline due to limitation of nutrients, light, pH, carbon dioxide or other physical and chemical factors as well as increasing accumulation of toxic wastes in culture, which consequently reduces the specific growth rate.

1.2.2(d). Stationary phase

In the stationary phase (d, Figure 1.1), the limiting factor and the growth rate are balanced, which results in a relatively constant cell density. This phase is not so distinct from the previous one but is reached as a slowly increasing phase of the culture.

1.2.4(e). Death phase

During death phase (e, Figure 1.1), due to depletion of a nutrient, oxygen deficiency, overheating, pH disturbance and increasing accumulation of toxic wastes in culture, the water quality deteriorates and cultures are unable to keep further growth therefore, cell density decreases rapidly and the culture finally collapses (FAO, 1996).

1.3. Shrimp *Penaeus indicus*

Penaeus indicus, also called white prawn, banana prawn or Indian white shrimp, is one of the major commercial prawn species of the world. *Penaeus* is a genus within the Penaeidae family with many species of great economic importance in fisheries and aquaculture including the Indian white shrimp (*P. indicus*), giant tiger prawn (*P. monodon),* green tiger prawn (*P. semisulcatus*) and Pacific white shrimp (*P. vannamei*).

Scientific classification

The following taxonomy is based on the latest scientific consensus available, and is provided as a general reference source for classification of the Penaeidae family (Farfante and Kensley, 1997).

Phyllum	Arthropoda
Subphyllum	Crustacea
Class	Malacostraca
Order	Decapoda
Family	Penaeidae
Genus	Penaeus (Fenneropenaeus)
Species	*Penaeus indicus* (H. Milne Edwards, 1837)

1.3.1. Bio-ecological characteristics

Penaeid shrimp include many of the commercially important marine species of the tropics and subtropics and are found naturally in shallow, inshore, tropical, and subtropical waters in the Indo-West Pacific from eastern and south-eastern Africa, through Malaysia and Indonesia to southern China, northern Australia and all across Asia.

The Indian prawn is exclusively found in marine and brackish waters and prefers mud or sandy mud substrates. Whereas adults' penaeus is normally found in marine habitats at depths less than 30 m, younger penaeus mostly spend, their juvenile stages in estuarine habitats, and remain there until they attain 110–120 mm total length (TL). These sub-adults then return to the sea and get recruited into the fishery. The Indian prawn has a life span of 18 months.

Highest body length is 23 cm (for females) and 18.4 cm (for males), but is usually less than 17 cm. The highest carapace length is 56 mm. The body is semi-translucent, somewhat yellowish white (small specimens) or greyish green and covered with numerous minute dark brown dots.

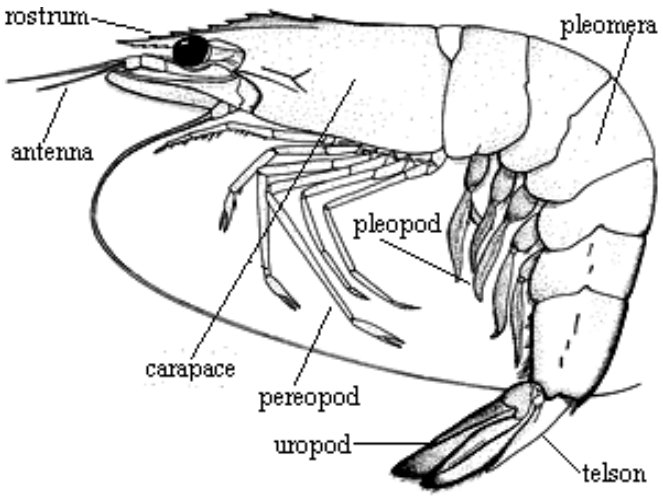

Figure 1.2. Penaeid shrimp anatomy (modified from Fox, 2001).

1.4. Objectives

To our knowledge, seaweeds growing in the Persian Gulf with potential economic importance or benifit have not been previously studied in Iran. Seaweeds have a wide range of applications and functions. The current expansion of production of marine species in Iranian aquaculture, particularly shrimp and pearl oyster, is producing a need for microalgae as live food at various stages in their life cycle. Therefore, in the present study seaweed extracts (SWE) will be evaluated as an organic fertilizer as a cost-effective alternative to the conventional f/2 medium in an attempt to further develop seaweed utilization in the Iranian fisheries industries.

Therefore, the three major objectives of this study were:

1) To determine the proximate biochemical, fatty acid and mineral composition of seaweeds collected from the Persian Gulf of Iran as potential food and feed resources,

2) To investigate the growth rate and biochemical composition of microalgae *I. galbana* and *C. muelleri* cultured with different seaweed extracts as an organic fertilizer in an attempt to further developing of seaweed utilization in the Iranian fisheries industries, and

3) To quantify the performance of *P. indicus* larvae fed with microalgae *I. galbana* and/or *C. muelleri* cultured with locally sourced seaweed extract as a supplement or as an alternative media to f/2 culture media for the Iranian mariculture industries.

CHAPTER 2

LITERATURE REVIEW

2.1. Seaweeds

2.1.1. Uses

Customary use of seaweed as food, forage, fertilizer and also as sources of traditional drugs dates back thousands of years (at least 1500 years ago) in East Asian countries, mainly Japan, China and Korea, and is part of their daily diet (Guiry, 2010). China is the largest seaweed producer (76.7%) followed by the Philippines (8.6%), South Korea (3.6%) and Japan (3.5%) (Wu and Pang, 2006). Nowadays, seaweeds are used mostly for industrial applications. Total annual production of seaweeds is estimated at 7.5-8 million metric tonnes in the world, which generated approximately US$ 5.5-6 billion for the seaweed industry (Adams *et al.,* 2009). Approximately 1 million tonnes of wet red and brown seaweeds were extracted to produce 55,000 tonnes of phycocolloids— like agar, carrageenan and alginate —valued at almost US$ 600 million (McHugh, 2003). Seaweeds are low in protein and lipid, but high in carbohydrates and mineral content (Burtin, 2003).

2.1.1(a). Food

Seaweeds can be eaten directly or used in the preparation of food. As mentioned above, use of seaweed in food diets dates back to maritime people in Asian countries, but demand for seaweed as food is increasing in North and South America and Europe. The main sources for production of hydrocolloids like agar, alginate and carrageenan are red and brown seaweeds, which are used in food industries.

2.1.1(b). Medicine and cosmetics

Seaweeds have a wide range of medicinal applications and functions on human health and healing. Amongst these, seaweeds have been described to have curative effects for arthritis, colds and influenza, worm infestations, maintain acid/base homeostasis in circulation and lymphatic system, control blood sugar content and insulin contents in the blood, inhibit cardiovascular, bone and mental ailments, and also have anti-cancer, tumor and viral properties (Fitton, 2003; Guiry, 2010). Seaweeds and their extracts have wide application in the cosmetic products industries such as hair care, body cream and lotions, moisturizers and emollients, and in anti-ageing and wrinkle and skin products.

2.1.1(c). Agriculture

Early application of seaweeds by maritime people date back at least to the nineteenth century, when people collected drift seaweeds for use as fertilizer. Seaweeds contain useful sources of minerals and trace elements, and therefore could have wide range applications in modern agriculture and horticulture. Furthermore, because of the high content of carbohydrates, seaweeds can operate as a soil conditioner and assist in moisture retention. A wide range of beneficial effects of SWE have been reported by Blunden (1991), which included increased seed germination, crop yields and reductions in storage losses of fruit, and improvement of inorganic nutrient uptake from the soil, more resistance to frost, pest, diseases and environmental stress conditions.

2.1.1(d). Other uses

Besides the above mentioned applications of seaweeds, there are potential uses for seaweed in wastewater treatment in industry and in mariculture in the removal of unwanted nutrients. The effluent water from fish and shrimp farms usually contains high contents of nutrients that can cause problems to other organisms in adjacent waters; in these cases, seaweeds are able to absorb nutrients and heavy metals to improve water quality prior to discharge into receiving water.

2.1.2. Research on seaweeds

For the last few decades, comprehensive studies have been done throughout the world for suitable and nutritional, healthy and easily available supplement for food industries. Seaweeds are considered potentially good sources of nutrients and contain high amounts of protein, carbohydrate, and significant amounts of vitamins A, B, C and B12, lipids, essential fatty and amino acids.

A study on the distribution and density of seaweeds in the Persian Gulf coastal waters (intertidal zone) off the south coast of Iran was performed from September 2001 to August 2002 by Rohani *et al.* (2004). In total, 78 species of seaweeds were identified in the study area. Of these, red seaweed ranked highest with 39 identified species, followed by green seaweed with 21 species and brown seaweed with 18 species (Table 2.1). Additionally, the seasonal biomass survey revealed a highest biomass content of 1.16 kg fresh wt per m^2 (brown seaweed), 0.78 kg fresh wt per m^2 (red seaweed) and 0.52 kg fresh wt per m^2 (green seaweed) in the spring, summer and summer seasons, respectively (Table 2.2). There is currently no reported information on the biochemical composition of seaweeds from the Persian Gulf of Iran.

Table 2.1. Species composition of seaweeds along the Persian Gulf coast of Iran from September 2001 to August 2003 [1].

Seaweeds groups		
Green	Red	Brown
Acetabularia mobii	*Acanthophora muscoides*	*Cystoseira myrica*
Avrainvillea erecta	*A. spicifera*	*C. trinoides*
Bryopsis plumosa	*Chonderia cornata*	*Colpomenia sinosa*
B. pennata	*C. nidifica*	*Dictyota dichotoma*
Caulerpa racemosa	*C. oppositiclada*	*D. divaricata*
C. racmosa var. peletata	*Ceramium manrensis*	*D. linnusa*
C. sertularioides	*C. flaccidium*	*Iyngaria stellata*
C. taxifolia	*Centraceras clavulatum*	*Padina australis*
C. sp.	*Champia compressa*	*P. boergesinii*
Codium papilatum	*C. parvula*	*P. pavonica*
Chaetomorpha antinina	*Dassya* sp.	*P. tenuis*
C. californica	*Gelidiella acerosa*	*P. tetrastromatica*
C. gracilis	*Gelidium pusillum*	*Rosenvingea* sp.
Cladophora fascicularis	*Galaxaura rubusta*	*Sargassum illicifolium*
Cladophoropsis membraanacea	*Gracillaria arcuata*	*S.* sp.
Dictyosphaeria cavernosa	*G. corticata*	*Spatoglossum variable*
Entromorpha compressa	*G. foliifera*	*Stoechospermum marginatam*
E. flexuosa	*G. salicornia*	*Turbinaria conoides*
E. intestinalis	*Hypnea cervicornis*	
Ulva lactuca	*H. cornata*	
U. fasciata	*H. pannosa*	
	H. valentiae	
	Jania adhaerens	
	J. rubens	
	Laurencia papilosa	
	L. pedicularioides	
	L. snyderiae	
	L. sp.	
	Leveillea jungermannioides	
	Lomentaria sp.	
	Polysiphonia sp.	
	Pogonophorella sp.	
	Rhodomenia sp.	
	Sarconema filiforma	
	Scinaia sp.	
	Solieria filiformis	
	S. robusta	
	Spyridia filamentosa	

[1]Data extracted from Rohani *et al*. (2004).

Table 2.2. Seasonal distribution and density (g m^{-2}) of seaweeds along the Persian Gulf and Islands coastal waters from September 2001 to August 2003[1].

	Season	Bandar Lengeh	Larak Island	Qeshm Island
E. intistinalis	Spring	5	3	8
	Summer	155	17	63
	Autumn	24	8	13
	Winter	0	0	0
U. lactuca	Spring	0	80	0
	Summer	0	0	0
	Autumn	0	0	0
	Winter	0	37	0
G. corticata	Spring	25	38	75
	Summer	7	0	38
	Autumn	0	0	0
	Winter	13	7	26
H. valentiea	Spring	37	90	86
	Summer	0	0	0
	Autumn	5	36	47
	Winter	12	45	55
C. sinuosa	Spring	48	146	130
	Summer	0	0	0
	Autumn	0	0	0
	Winter	6	23	45
S. illicifolium	Spring	863	415	70
	Summer	259	118	28
	Autumn	78	40	5
	Winter	226	103	15

[1]Data extracted from Rohani *et al.* (2004).

Several studies on the biochemical components of seaweeds such as proteins, carbohydrates and lipids (McDermid and Stuercke, 2003; Zubia *et al.*, 2003; Ortiz *et al.*, 2006; Santoso *et al.*, 2006; Dawczynski *et al.*, 2007; Marsham *et al.*, 2007; Chakraborty and Santra, 2008; Matanjun *et al.*, 2009) and minerals content (Rao *et al*., 2007; Sivakumar, 2009) have been carried out in the world. The proximate composition, vitamin C, α-tocopherol, dietary fibers, minerals, fatty acid and amino acid profiles of three tropical edible seaweeds, *Eucheuma cottonii* (Rhodophyta), *Caulerpa lentillifera* (Chlorophyta) and *Sargassum polycystum* (Phaeophyta) were studied in coastal areas of North Borneo, Malaysia (Matanjun *et al*., 2009).

According to their results, seaweeds, especially the brown seaweeds, are naturally rich in vitamin C and α-tocopherol, and presence of essential fatty acids and amino acids in seaweeds allows future development in the search for new sources of specific polyunsaturated fatty acids (PUFA) for nutrition and medicinal use.

In another investigation, Polat and Ozogol (2008) investigated the proximate biochemical composition and the fatty acids of brown and red seaweeds in the northeastern Mediterranean Sea near Turkey. According to their results, seaweed species in Turkey have potential applications for food and the nutraceutical industry. At the same time, Durmaz *et al.* (2008) investigated fatty acids composition and α-tocopherol of *Cystoseira* spp., *Ulva* spp. and *Zostera* spp. in the Sinop Bay (Turkey) of the Black Sea. The result of their study has demonstrated that seaweeds in Turkey could be used as ingredients in functional foods for human consumption.

Ortiz *et al.* (2006) studied the biochemical composition of two edible green seaweeds, *Ulva lactuca* and *Durvillaea antarctica,* in the coastal area of Northern Chile. They reported that the seaweeds *U. lactuca* and *D. antarctica* were high in ash but low in total lipid content, appreciable in protein and dietary fiber content, and relatively high in contents of essential amino acids, PUFA, and tocopherol, and can be a healthy food for human and animal nutrition.

Burtin (2003) demonstrated that, in addition to technological properties of seaweeds, they exhibited original and interesting nutritional properties and therefore can be regarded as an upcoming source of health benefit molecules for the food processing and nutraceutical industry. Dawczynski *et al.* (2007) quantified the

content of amino acid, fatty acid, protein, lipid, and total fiber for 34 edible seaweed varieties (*Laminaria* sp., *Undaria pinnatifida*, *Hizikia fusiforme* and *Porphyra* sp.), and made similar conclusion.

Shanmugam and Palpandi (2008) determined the biochemical composition and fatty acids profile of seaweeds from the Southeast Coast of India. Their investigation verified the presence of several health–promoting and valuable nutrients, such as high content of protein and low content lipid as well as essential amino acids and fatty acids in green seaweed *Ulva reticulata*. At the same time, Chakraborty and Santra (2008) determined the biochemical composition of eight abundant seaweeds in Sunderban, India. They illustrated that seaweeds can be used in food as a source of basic materials in the preparation of nutrient supplement products and in fine chemical synthesis. Later, Manivannan *et al*. (2008a and 2009) mentioned that the protein, lipid and carbohydrate content of seaweeds in India is optimum and can provide potentially good nutritional content as food ingredients.

In order to explore the use of seaweeds as fertilizers, Zubia *et al*. (2003) determined the chemical composition of attached and drift specimens of two brown seaweeds (*Sargassum mangarevense* and *Turbinaria ornata)* from Tahiti, French Polynesia. They suggested that, due to the cost of harvest and the presence of higher amounts of trace metals in drift seaweeds, they could be useable for low-value products such as agricultural products.

Seasonal variation of proximate biochemical composition for 30 common species of seaweeds from Darwin Harbor in Australia was determined by Renaud

and Luong-Van (2006) during summer and winter. The study showed that there was a wide diversity of species in summer and winter seasons, and with the exception of carbohydrate that showed the highest percentages in winter, the highest percentages of protein, lipid and ash were found in summer. They mentioned that although the most nutritionally rich species were found in members of the red seaweeds, in terms of carbohydrate, protein and calculated energy content, it is important that the nutritional contents were not based on chemical analysis only and biological analysis using animal feeding trials would be required to establish the actual and viable nutritional content of seaweeds.

In addition to proximate composition, fatty acids and amino acids, seaweeds are rich in minerals. Manivannan *et al*. (2008b) and Devi *et al*. (2009) determined the mineral composition of some seaweed from the Gulf of Mannar, Southeast Coast of India. Based on the results obtained from their studies, element composition varied with genus and species content. They mentioned that, although the element composition analysis showed that seaweeds were potentially good sources of minerals, more studies are necessary to evaluate the nutritional content of seaweeds as food ingredients.

Santoso *et al.* (2006) studied mineral contents of Indonesian seaweeds and their solubility after cooking. From the results, they concluded that Indonesian seaweeds were high in macro-mineral (Na, K, Ca and Mg) but low in trace-mineral (Cu, Zn and Fe) contents and the solubility of Ca and Mg are significantly increased by boiling, particularly in acid solutions (0.5% acetic acid).

Liberal da Silva and Barbosa (2009) studied the use of seaweeds as a source of nutrient in animal food for the white shrimp *Litopenaeus vannamei* in Brazil. The results demonstrated that two red seaweeds *Hypnea cervicornis* and *Cryptonemia crenulata* were feasible for use in the feeding of *L. vannamei* with positive effect on growth rates, and that there was an increase in feed conversion rate when the dietary contents of seaweed are increased. At the same field, Valente *et al.* (2006) studied the use of two red seaweeds *Gracilaria bursa-pastoris* and *G. cornea* and one green seaweed *Ulva rigida* as food ingredients in European sea bass (*Dicentrarchus labrax*) juveniles, and concluded that these seaweeds can be used as alternative ingredients in European sea bass juveniles diets, without any adverse effects on growth rate and feed utilization efficiency at proportional contents of up to 10% for *G. bursa-pastoris* and *U. rigida* and up to 5% for *G. cornea*.

Mao *et al.* (2009) in North China conducted a study on the potential uses of seaweeds in removal of nutrients from bivalve farming. They concluded that the red seaweed *G. lemaneiformis* has high nutrient mitigation efficiency and absorption capacity in integrating with bivalve *Chlamys farreri*, and could be an effective and environmentally friendly method to reduce nutrient loading by the bivalve farming.

2.2. Microalgae

2.2.1. Microalgae usage

2.2.1(a). Nutritional content of microalgae

The nutritional quality of any microalga species for a particular organism depends on the microalga's cell size, digestibility, production of toxic compounds, and biochemical composition (Brown *et al.*, 1997). Although there are noticeable differences in the compositions of the different microalgae species, protein is always the main organic component, followed usually by lipid and then by carbohydrate. Moreover, microalgae grown to late-exponential growth phase typically contain 30 to 40% protein, 10 to 20% lipid and 5 to 15% carbohydrate (Brown *et al.,* 1997).

Most microalgae species have moderate to high percentages of eicosapentaenoic acid (EPA; 7 to 34 %) present in the diatom species (*Chaetoceros calcitrans*, *C. gracilis*, *Skeletonema costatum*, *Thalasiosira pseudonana*) and the prymnesiophyte *Pavlova lutheri*. The prymnesiophytes (*Pavlova* sp. and *Isochrysis* sp.) and cryptomonads are relatively rich in docosahexaenoic acid (DHA; 0.2-11%). Eustigmatophytes (*Nannochloropsis* sp.) and diatoms have the highest percentages of arachidonic acid (AA; 0 to 4%). Chlorophytes (*Dunaliella* sp. and *Chlorella* sp.) are deficient in both polyunsaturated acids (PUFA), although some species have a little amount of EPA (up to 3.2%). Because of this PUFA deficiency, chlorophytes generally have low nutritional content and are not suitable as a single species diet (Brown *et al*., 1997).

2.2.1(b). Use of microalgae in aquaculture

Table 2.3 shows a list of 8 major classes and 24 genera of cultured algae currently used to feed different groups of commercially important aquatic organisms.

Table 2.3. Major classes and genera of micro-algae cultured in aquaculture (modified from De Pauw and Persoone, 1988).

Class	Genus	Examples of application
Bacillariophyceae	*Skeletonema*	PL, BL, BP
	Thalassiosira	PL, BL, BP
	Phaeodactylum	PL, BL, BP, ML, BS
	Chaetoceros	PL, BL, BP, BS
	Cylindrotheca	PL
	Bellerochea	BP
	Actinocyclus	BP
	Nitzchia	BS
	Cyclotella	BS
Haptophyceae	*Isochrysis*	PL, BL, BP, ML, BS
	Pseudoisochrysis	BL, BP, ML
	Dicrateria	BP
Chrysophyceae	*Monochrysis (Pavlova)*	BL, BP, BS, MR
Prasinophyceae	*Tetraselmis (Platymonas)*	PL, BL, BP, AL, BS, MR
	Pyramimonas	BL, BP
	Micromonas	BP
Cryptophyceae	*Chroomonas*	BP
	Cryptomonas	BP
	Rhodomonas	BL, BP
Xanthophyceae	*Olisthodiscus*	BP
Chlorophyceae	*Carteria*	BP
	Chlamydomonas	BL, BP, FZ, MR, BS BP
	Dunaliella	BP, BS, MR
Cyanophyceae	*Spirulina*	PL, BP, BS, MR

AL, abalone larvae;
BL, bivalve mollusc larvae;
BP, bivalve mollusc postlarvae;
BS, brine shrimp *(Artemia)*;
FZ, freshwater zooplankton
ML, freshwater prawn larvae;
MR, marine rotifers *(Brachionus)*;
PL, penaeid shrimp larvae;

Over 15,000 bioactive compounds originating from microalgae have been chemically determined and most of them are special products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols (Yasumoto and Satake, 1998).

The production of microalgae under artificial conditions is itself a form of aquaculture. In mariculture industries, supplies of food with a high nutritional quality for all growth stages of bivalves, and for the larval stages of crustaceans and fish are directly related to live food sources like microalgae, and therefore, microalgae preparation is one of the crucial activities in hatcheries and consequently supports the growth of demand animals (Cho *et al.*, 1999). Similarly, microalgae are used to produce high quantities of zooplankton (rotifers, copepods, and brine shrimp) which serve in turn as food for larval and early-juvenile stages of crustaceans and fish. All algal species are not equally successful in supporting the growth and survival of a particular filter-feeding animal. Furthermore, the nutritional property of microalgae offered to feed aquatic animals is crucial, as well as is their rapid growth rate and size appropriateness for ingestion (Brown *et al.*, 1997). In addition, it is well known that the productivity of a hatchery is strongly related to the quantity and quality of a suitable food source.

2.2.1(c). Use of microalgae as therapeutic supplements in health management

Microalgae, in addition to seaweeds, show great potential for benefitting human health. For example, the bioactive components such as pigments, vitamins, phytochemicals and trace elements extracted from microalgae and higher plants can help boost the human body's antioxidant defenses (Kelly, 1998).