Chapter I

INTRODUCTION

Since the captured fisheries as food in the Gulf of Thailand had decreased rapidly for the past few years, the need for cultured fisheries had increased in order to compensate for the loss. The cultivation of many economically important invertebrates such as shrimp, mussel and oyster, is becoming more important and necessary for Thailand. Studies on the biology of these invertebrates had been conducted in relation to its cultivation. While much is known of the biology of these invertebrates, relatively few studies on the biochemical features of these animals had been made. The biochemical composition of an organism is fundamental to an understanding of its physiology. Also biochemical data could be of considerable use in understanding the ecology and overall economy of the animal. The purpose of this study is to present preliminary report of an investigation into the seasonal variations in the composition of the flesh of the green mussel, Mytilus viridis L. An attempt was made to compare the biochemical composition in the field specimens and in those kept in the laboratory for a short period. Attention was focussed on the seasonal changes in some biochemical composition in the mussel in relation to its growth, reproduction, nutrition and some

environmental factors. These data may be fitted as a background for further investigation such as physiological changes in populations kept in the laboratory,physiological changes due to environmental stresses or pollutions, and changes caused by parasitic organisms in the mussel. These further investigations could be useful in cultivating mussels.

Proteins, carbohydrates and lipids (fats) are usually found as the greatest mass of materials which form the tissues and organs, exclusive of skeleton or shells. Thus in this study, emphasis on these three major groups of organic compounds were given. The entire animal was ground up or homogenized and analysed for various substances. This was reported as "total tissue". For a study of the relation of the biochemistry of an animal to its nutritional or reproductive states, analysis of the entire body is less informative than an analysis of each part or body components such as gonad. The body of a mollusc, therefore,was divided by dissecting into the mantle part and the non-mantle part for biochemical analysis. The variations in biochemical composition in many marine invertebrates follow distinct patterns related to the season of the year and the gonad development and spawning. This experiment was designed to determine the biochemical composition (total solid, protein, lipid and carbohydrate content) in mantle part, non-mantle

and total part at monthly intervals for one year, from October 1974 to September 1975. The seasonal change in biochemical composition in various parts of the mussel was discussed. The percentage was given both as wet weight level and dry weight level since these two levels were interconvertible if the total solid or the water content was known.

In bivalves it is sometimes very difficult to separate the viscera from the gonad. In order to analyse the biochemical composition in relation to its reproductive state, the mollusc body was divided into mantle part as "germinal tissue" and non-mantle part as "somatic tissues". The reproductive state in field specimens was studied at monthly interval. The stomach contents of the mussel was also determined at monthly interval to find out whether it was related to the biochemical composition.

It is apparent that most often the specimens chosen for biochemical analyses are large enough to provide material for a whole series of determinations. When specimens are to be kept in aquaria, investigator often chose medium or small mussels because medium or small ones survive better than large ones. Few data exist on the effect of stage in development on the biochemical composition of marine invertebrates except in the early embryonic stages. It would be very interesting to know to what extent the proportion of

various components in the tissues changes with growth. At the beginning of the experiment, the specimens chosen for analysing weresmall (1.0-1.5 cm in length)and newly settled. By the end of the experiment, the specimens were matured (7.0-8.0 cm in length). Some were already spawned. The changes related to growth could also be detected other than the seasonal changes.

Objectives and Scopes of Thesis

1. To determine the seasonal changes in some biochemical composition in the mantle part, the non-mantle part and the total part of the mussel Mytilus viridis collected from the mussel bed at Cholburi Bay, Cholburi Province. Some biochemical composition determined were as followed

1.1 Total solid

1.2 Protein content

1.3 Carbohydrate content

1.4 Lipid content

in relation to growth and some environmental factors.

2. To make a comparison between the biochemical composition of the mussels kept in the laboratory and of those in field specimens of the same size.

3. To follow the reproductive state in field specimens at monthly intervals for one year in relation to its biochemical composition.

4. To determine the intensity of feeding and the types and abundance of food eaten by field specimens at monthly intervals for one year in relation to its biochemical composition.

Literature Survey

The literature on the biology and cultivation of mussels belonging to the genus Mytilus Linnaeus is prolific, but nearly all confined to temperate water species particularly Mytilus edulis Linnaeus. Field (1922)gave excellent descriptions of the anatomy, physiology and embryology as well as the bionomics of Mytilus edulis

Emphasis on the reproduction of the mussel Mytilus spp., Johnstone (1899) made an investigation of the reproductive organs of the common mussel (Mytilus edulis) in relation to the period during which spawning took place on the bed along the Lancashire sea coast, Great Britain, and to the histological changes accompanying the ripening and extrusion of the reproductive products. Battle (1933) studied the rhythmic sexual maturity and spawning of certain bivalve molluscs : Mytilus edulis, Mya arenaria Macoma baltica L. and Yoldia sapotilla (Gould) at St. Andrews, New Brunswick. The gonads of Mytilus edulis and and Macoma baltica littoral forms were throughout the new moon spring tides filled with mature germ cells which were spawned at the time of the subsequent first-quarter neaps,

full-moon springs and third-quarter neap tides. The initiation of spawning may be due to the increase in temperature. Chipperfield (1953) examined regular samples of Mytilus edulis from a number of localities on the British coasts during 1946-1947 for gonad condition and spawning. He described the criteria employed in distinguishing the stages of gonad development. The development of the gonads was referred to in five stages as followed

Stage 0 Neuter or resting spent stage. No follicles in mantle tissue.

Stage I Onset of gametogenesis and appearance of follicles in the mantle.

Stage II Follicles well developed and filled with unripe ova and sperm.

Stage III Ova ripe and capable of fertilization, sperm activated by sea water.

Recently spent stage

Most of follicles empty; a few relict ova and sperm present only.

It does not appear to be much literature on the local mussel Mytilus viridis. Amatyakul (1957) gave a general account on the biology of the mussel Mytilus viridis in the local waters of Thailand. These mussels were usually found from the intertidal level to the depth of 10 metres. They were found attached together on rocks or hard substratums by

the byssus. They were filter-feeders, [illegible] feeding on planktonic organisms. Spawning period began from the end of September to November. Larvae were found most abundant during October to December. In February and March, Larvae were less abundant. The gonad of mussels in the mature condition occupied a great part of the mantle lobes and indeed branched throughout the entire body. The mantle was very thick, of deep orange to reddish colour in the female and white to creamy colour in the male. Brohmanonda (1960) studied some biological aspects and evaluation of the sea mussel production in the waters off the coast of Cholburi Province. The result showed that the spawning season of the sea mussel reached its peak in October and November. Tan (1973) studied various aspects of the biology of the green mussel Mytilus viridis in relation to its cultivation in Singapore and Jahore waters. This study included some aspects of the physical, chemical and biological characteristics of the environment, the distribution of the mussel in local waters, its spawning and development, seasonal stomach contents, growth under experimental and natural conditions, some population studies and spat settlement. Neamsurp (1975) studied the influence of environmental factors on the growth of sea mussel (Mytilus viridis Linn.) both under the laboratory and field condition.

Biochemical studies of marine invertebrates such as: echinoderms, molluscs and crustaceans usually involved the

seasonal changes in biochemical composition in relation to the reproductive cycle. Some studies were made on change caused by physiological stresses. One of the few studies of seasonal changes in all biochemical fractions in marine crustaceans was that of Barnes, Barnes and Finlayson(1963) for the two adult barnacles, Balanus balanoides and. B. balanus. They have followed the seasonal changes in the body weight and composition of these two common boreo-arctic species of cirripedes. Raymont, Austin and Linford (1966) determined the biochemical composition (protein, lipid and carbohydrate content) of the mysid, Neomysis integer at monthly intervals for one year. In echinoderms, Giese(1966) has provided much data on a variety of biochemical constituents of a few echinoderms selected as examples of the five classes. For each echinoderm its content of water, protein, non-protein nitrogen, lipid, carbohydrate and in a few cases nucleic acid was given. Giese (1967) also discussed several methods for the study of the biochemical constituents of marine invertebrates. He attempted to indicate the advantages and disadvantages of some procedures for biochemical analysis mostly confined to gross classes of compounds such as proteins, non-protein nitrogen, lipids and carbohydrates. He also discussed several ways of preparing tissues of marine invertebrates. Some studies have been made of the biochemical composition of the mollusc. Giese (1969) summerized some of the data on biochemical composition of

certain molluscs classes. Comparisons between classes were also discussed. The earlier work of Daniel (1920) was a preliminary investigation of the seasonal variations in the composition of the flesh of the common mussel (Mytilus edulis) from samples obtained on the mussel-beds of Morecambe, Lancashire. Jones (1926) had determined the nutritional value of oysters and other sea food such as shrimp and clam. He concentrated on the vitamin and protein content. Galtsoff (1964) summarized the chemical composition in oyster meat. According to Galtsoff the variations in chemical composition of oysters followed distinct patterns related to the environment and season of the year. These changes were also associated with the gonad development and spawning. He also discussed the variation in glycogen content which was the reserved material in the oyster. The glycogen content in oyster was stored primarily in the connective tissue of the mantle and labial palps. During the rapid proliferation of sex cells the reserve supply was used, and by the end of the reproductive cycle the amount of glycogen was at a minimum and the mantle was reduced from a thick heavy layer to a thin transparent membrane. Soon after spawning, the oysters began to form and store glycogen. Ansell, Loosmoore and Lander (1964) made several studies on the hard-shell clam, Venus mercenaria in British waters. The annual cycle in condition (% wet flesh, % dry flesh) and associated changes in biochemical analysis showed no seasonal change in

the composition of any individual organ. The percentage of carbohydrate content of the gonad was approximately double that of other organs, and changes in the relative proportions of gonad and body resulted in changes in the carbohydrate to protein ratio of the whole animal. Quayle (1969) discussed the nutritive value and chemical composition in the Pacific Oysters. Ansell (1972) had studied seasonal changes in biochemical composition in bivalve Donax vittatus (da Costa) from Millport.

An annual cycle of dry flesh weight, biochemical composition and caloric value on sublittoral Mytilus edulis from natural mussel beds of the Conwy Estuary, North Wales during 1971-1974 had been studied by Dare & Edwards (1975). They found that individual dry flesh weight was highest in summer and autumn, when protein and carbohydrate were maximal, and decreased through winter to a post spawning minimum in spring. Seasonal changes in flesh weight and composition resulted from the storage and utilization of food reserves in relation to the complex interactions of food availability and temperature with growth and reproductive processes. Some preliminary experiments designed to follow certain physiological parameters in populations of the common mussel, Mytilus edulis kept for long periods in the laboratory were done by Bayne & Thompson (1970). Nutritive and temperature stresses in experiments caused a decline in general body condition and dry weight of Mytilus edulis. Carbohydrate and protein

were lost from the body; these losses were greater from the germinal (mantle) than from the somatic (non-mantle) tissues. There was a more rapid loss of carbohydrate than of protein. Similarly Holland & Spencer (1973) had determined biochemical changes in fed and starved oysters, Ostrea edulis L. during larval development, metamorphosis and early spat growth. Gabbott & Bayne (1973) made four experiments in which mussels were subjected to low food levels (mean 5.3 mg dry weight of cells per animal per day) and constant temperature (15°C) for prolonged periods, in order to examine the biochemical effects of temperature and nutritive stress on Mytilus edulis. The changes in protein, lipid and carbohydrate content of the non-mantle and mantle tissues were shown. They found that the seasonal changes in glycogen and protein content of Mytilus edulis were clearly related to the annual reproductive cycle. Reserves were built up during the summer and utilized during the autumn and winter as the gonad develops in the mantle.

Literatures on biochemical composition of marine invertebrates in Thailand mostly deal with the nutritive value. Pochanasomboon (1965) determined protein in different kinds of edible molluscs quantitatively. Her finding were as followed: 11.4925 % in blood clam (Arca granosa Linn.) and 7.3381 % in mussel (Mytilus viridis Linn.). Similarly protein in different kinds of edible molluscs was also determined by Leeranuphan & Saichure (1967) as followed:

blood clam 10.15 %, mussel 11.53 % and clam 8.59 % . Tamiyavanish (1970) in his master thesis reported the protein percentage in blood clam (Arca granosa Linn.) to be 11.14 % and 10.65 % in mussel (Mytilus smaragdinus). In shrimp, Penaeus merguiensis, Rattakul (1970) found the protein percentage in edible parts to be 18.86 % and 18.20 % of the whole. Saowapruk (1970) determined the protein percentage in Penaeus merguiensis when fed with three types of food, namely mussel, blood clam and ray comparing to those reared in shrimp pond. The results obtained were as followed. shrimp fed with ray 21.5120 %, shrimp fed with mussel 20.4324 %, shrimp fed with clam 20.3192 %, starved shrimp 14.9982 % and shrimp collected from the shrimp pond 19.4207 %

Definition of Terms

Biochemical composition

Mantle tissue

Mantle tissue is the general surface covering of the body and is formed of two lobes, one covers the right half and the other the left half of the body. It is dissected out separately for biochemical analysis. The gonad of Mytilus viridis in the mature condition ususlly occupies the mantle lobes and indeed, branches throughout the entire body. Thus the mantle tissue is used as the representative of the germinal tissue.

Non-mantle tissue

Non-mantle tissue is the whole body of the mussel exclusive of shell, mantle and byssus. This is to be determined as the somatic tissue.

Total tissue

Total tissue is a mash of the entire soft body, mantle included.

Dry weight level

Dry weight level is the biochemical data given in percentage of dry weight. This is determined from the total solid percentage of each tissue.

Wet weight level

Wet weight level is the biochemical data given in percentage of wet weight. In this study, the biochemical data were determined on homogenized tissue from pooled individuals.

Total solid

Total solid is the dry flesh weight of the mussel when the water content is removed by drying in the oven at 100°C. Total solid is given in percentage.

Water content

Water content is given in percentage. This value is interconvertible with the total solid percentage.

Proteins

Proteins are defined as total nitrogen in tissue determined by modified Macrokjedahl method. The value for Kjeldahl nitrogen was multiplied by 6.25 to give protein.

Lipids

Lipids are defined as crude fat, substances extracted by petroleum ether. After extraction, lipid are determined gravimetrically.

Carbohydrates

Carbohydrates are determined as glycogen plus "glucose" equivalent, extracted with hot 5% trichloro acetic acid and then estimated by a colour reaction with sulphuric acid.

Reproductive state

Gonad condition index

Gonad condition index shows the stages of gonad development. The stages of gonad development were assessed from the colour and thickness of the mantle and the development of egg and sperm ascertained from the microscopic examination of smears. The mantle tissue was squashed on a slide and graded into different stages. The numbers of animals examined for gonad development that fall into each stage are multiplied by the number of the stage and the sum divided by the number of animals in the sample.

Nutritional state

Nutritional state studied as the degree of fullness in the mussel's stomach and its stomach content.

Stomach content

Stomach content is the living and non-living materials found in the stomach of the mussel.

Degree of fullness

Degree of fullness is graded into different classes as followed,empty, little, moderate and full.