AN ABSTRACT OF THE THESIS OF

DAVID WINTER ELVIN (Name) for the DOCTOR OF PHILOSOPHY (Degree)

in OCEANOGRAPHY (Major Department) presented on May 29, 1974 (Date)

Title: OOGENESIS IN MYTILUS CALIFORNIANUS

Abstract approved: Redacted for privacy

J. J. Gonor

A two-year field study was made of a population of the California Sea Mussel, Mytilus californianus, located on a protected tidal bench at Yaquina Head on the Central Oregon Coast. This animal was chosen because it is attached to the rocks, filters particulate matter from the water, produces large amounts of eggs, and is a prominent member of the intertidal community.

An equation was developed on both an empirical and a theoretical basis for determining mussel tissue temperature from continuously recorded physical data in order to determine the annual temperature trends and the daily rate of heating. Heating during exposure is an important contribution to mussel temperature during the spring and possibly during the fall.

Particulate organic material 100 to 200 μm in diameter was measured throughout the year and was found to range from 1 to 3 mg dry weight per liter of sea water. During the spring, increases in

particulate oxidizable material are associated with diatom blooms. The rest of the year, detritus associated with the mixing action of waves contributes a significant portion to the suspended particulates.

A gonad index was developed which separated the germinal tissue from the storage tissue in the gonad. The denominator of this index equation was the weight of the somatic tissue rather than the total weight of the animal. The largest amount of reproductive tissue observed was 800 mg per gram dry weight of somatic tissue, but the population generally maintained a germinal gonad index of about 60% of its potential maximum. The storage stage was found to be greatly reduced in this population unlike that found in M. edulis.

The seasonal reproductive progress was studied by quantitatively following three categories of oocyte stages. The number of oogonial clusters was used as an index of mitotic activity. Few clusters of oogonia appeared when numerous mature oocytes were present. The number of previtellogenic oocytes was taken as an index of early meiotic activity. Previtellogenic processes were initiated during the spring when the tissue temperature was rising. Mature oocyte numbers increased as vitellogenesis occurred, and the rate of increase of mature oocytes was best correlated with particulate organic concentration in the water. Partial spawning and resorption of lysed eggs were the predominant fate of mature eggs.

The dynamic aspect of oogenesis was followed using a flow model for the categories of occyte stages. From this model rates of change of each cell type were estimated. Only 40% of the total oocytes produced throughout the year was lost by spawning.

Gonadal tissue was chemically divided into biochemical fractions to determine the chemical characteristics of each reproductive stage and the rates of accumulation of chemical components between bi-monthly samples. The rates of accumulation of protein and lipid increased from 1.7 to 3.6 mg per day per gram animal and 0.6 to 1.8 mg per day per gram animal during previtellogenesis and vitellogenesis respectively. RNA increased at the rate of 0.6 mg per day per gram animal, while the amount of glycogen decreased.

Isochrysis galba, a flagellate, labeled with carbon-14 was fed to mussels under different exposure and thermal regimes to determine the rate of incorporation and the percentage of food allocated to the gonad. For mature gonads undergoing partial spawning, about 10% of the food was found in the gonadal tissue within 3 days. The translocation and synthetic pathways associated with protein and RNA appear to be the most active. The amount of protein per egg determined directly or indirectly was found to decrease as the reproductive year progressed.

A hypothetical scheme for the environmental and physiological control of oogenesis in M. californianus is presented and evaluated in

terms of the data. As a result of the observed timing of events and the variation in the population, elucidation of the relationship between reproductive phenomena and the environment by conventional techniques would require analysis of 50 to 70 specimens every 2 weeks for a period of at least 2 years.

Oogenesis in Mytilus californianus

by

David Winter Elvin

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed May 1974

Commencement June 1975

APPROVED:

Redacted for privacy

Assistant Professor of Oceanography
in charge of major

Redacted for privacy

Dean of School of Oceanography

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 29, 1974

Typed by Mary Jo Stratton for David Winter Elvin

ACKNOWLEDGEMENTS

I wish to express my appreciation to my major professor, Dr. Jefferson J. Gonor, for his interest and many stimulating discussions concerning the thesis, for his encouragement during the difficult portions of the study, and for critically reviewing the manuscript.

Dr. Donald S. Reed read the manuscript and offered many constructive suggestions. Drs. W. D. Loomis and Lawrence W. Small offered helpful suggestions as to methods and procedures during the critical beginnings of the study.

Finally, I would like to acknowledge the unfailing support and encouragement of my wife, Patricia, who gave valuable criticism of the text, prepared some of the histological samples, and typed the rough draft.

This research received support in part from the National Oceanic and Atmospheric Administration (maintained by the U. S. Department of Commerce), Sea Grant Office.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

OOGENESIS IN MYTILUS CALIFORNIANUS

I. INTRODUCTION

The production of eggs by an organism is termed oogenesis and is one aspect of the reproductive process. In many marine invertebrates fertilization takes place in the water following spawning of the eggs and sperm. The release of viable eggs in quantities large enough to sustain a population must occur during seasons when larval survival will be high. Both the timing of cytological events with respect to the environment, and the rate at which oogenesis occurs affect the amount of eggs produced. Establishment of methods which quantify the reproductive state of intertidal organisms is necessary before studies of the mechanisms of oogenesis can be performed.

The California sea mussel, Mytilus californianus Conrad, was chosen to establish a procedure for detecting changes in reproductive state. It is a prominent member of the intertidal community in Oregon and can potentially release a large amount of eggs into the water during spawning. Adults are available throughout the year in quantities necessary for combined histological and biochemical analysis without depleting the population. Feeding can be easily quantified since Mytilus is a filter feeder, and the physical environment can be estimated at all times since the animal is attached to the rocks.

These characteristics of the species simplify the requirements for cattying out the study.

The molluscan genus Mytilus (Pelecypoda: Mytilidae) encompasses a group of species forming abundant populations on coasts throughout most of the world (Soot-Ryen, 1955). The M. edulis species complex has a circumpolar distribution in boreal and temperate waters. M. californianus (Conrad), M. perna (Linne), and M. galloprovincialis (Lamarck) are locally restricted species. The distribution of M. californianus extends from the Aleutian Islands, Alaska to Socorro Island, Baja California (Rankin, 1918). Coe and Fox (1942) state it is found only on open coasts exposed to surf. Although a bed of M. californianus has been reported at depths of 50 fathoms (Berry, 1954), it is predominantly an intertidal species forming a conspicuous upper zone from +1 to +3 feet above mean low low water (MLLW). Comparable reproductive studies have been carried out on Adula, Modiolus, Septifer, and Branchidontes within the family Mytilidae.

The anatomy of the reproductive system of M. californianus is similar to that of M. edulis as described by Field (1924) and White (1937). The sexes are separate, and there is no apparent external sexual dimorphism. The main genital canals open into the mantle cavity on the ventral wall at a genital papilla located anterior to the posterior adductor muscle. They are sealed by lips of tissue and

plugged by granules. Although most of the gonadal tissue is in the mantle lobes, the five principal gonoducts or canals also ramify into the mesosoma, the outer surfaces of the digestive gland, and the floor of the pericardium. One side of the canal is composed of germinal epithelium and the other side of ciliated epithelium. Gonadal tubules or follicles branch from these canals. Tranter (1958) considers the follicular wall in the Australian pearl oyster, Pinctada albina, to be modified connective tissue and hypothesizes that young germ cells arise from germinal primordia which are restricted to genital pores. Growth of the follicles is a method of spreading the germ cells into the mass of connective tissue. In Mytilids, genital development begins dorsally and later extends ventrally into the mantle lobes (Wilson and Hodgkin, 1967).

The other major component of the mantle is the vesicular connective tissue bathed by the haemolymph of the blood sinuses. In M. edulis and M. galloprovincialis three types of cells have been reported in the connective tissue. Amoebocytes similar to the granular leucocytes and hyaline lymphocytes described by Takatsuki (1934) are seen in the lacunae of the blood sinuses (Lubet, 1959). Adipo-granular cells which accumulate lipid and glycogen and the vesicular Langers or Leydig cells which accumulate only glycogen are both present. In M. perna there are only vesicular cells which simultaneously contain reducing sugars, glycogen, neutral lipids, and phospholipids (Lunetta,

1969). Lunetta suggests the origin of the storage connective tissue in M. perna is the blood leucocytes since he has observed a multiplication of them in the mantle.

The cytological development of M. edulis oocytes has been well documented by Lubet (1959). Oogenesis begins when the stem cells of the follicles undergo several mitotic divisions and produce groups of oogonia. These cells are distinguished from somatic cells by a large nucleus and little cytoplasm. Mitosis ceases and meiosis begins. The earliest oocytes (oocyte 1) demonstrate the meiotic leptotene or clumped chromosomal arrangement. Next the chromosomes become very distinct (oocyte 2), but there is still no nucleolus. Previtellogenic oocytes (oocyte 3) exhibit some cytoplasmic development, contain a nucleolus, and are connected to the follicle wall by a wide base. This growth is accompanied by swelling of the nucleolus and a dispersion of the chromatin into a fine reticulum. As the cell increases in size the cytoplasm is enriched with RNA, mitochondria, and Golgi bodies. There is frequently a division of the nucleolus into two nucleoli of 4 to 6 μm in diameter (Lubet, 1959). Nucleolar satellites rich in RNA may be expelled into the perinuclear cytoplasm (Lubet, 1959; Lunetta, 1969). Previtellogenic growth is concluded at this point.

Vitellogenic growth involves considerable increase in cytoplasm particularly in the accumulation of proteinaceous vitellin platelets

(yolk), droplets of chromolipids, and mucopolysaccharide granules. The lipid droplets begin to appear in the region of the cytoplasm between the nucleus and the follicular wall. There is no evidence of glycogen, although Lubet (1959) does show histochemical evidence of granular polysaccharides in very mature oocytes. The vitellogenic oocyte (oocyte 4) becomes elliptical and projects into the lumen of the follicle. The cytoplasm becomes more eosinophilic as yolk accumulates and the nucleus less basophilic as the chromatin disperses. Reverberi (1967) reports numerous multilamellar concentric formations in the cytoplasm of M. edulis occytes which are crowded with ribosomes. He suggests these structures are involved in yolk synthesis. A glycoprotein or mucopolysaccharide coating surrounds the oocyte in M. perna (Lunetta, 1969).

Finally the oocyte becomes free in the lumen to be moved towards the genital canals by the ciliated epithelium. Meiosis, however, is not completed until the egg has been spawned and fertilized. At spawning the germinal vesicle breaks down but the polar bodies are not released until after fertilization. Frequently, a number of large oocytes are not released at spawning and remain along with other debris in the ovary. They undergo lysis and resorption (Lunetta, 1969). The first indication of this phenomena is vacuolation in the cytoplasm followed by breakdown of the cell membrane and the release of the cellular contents (Lunetta, 1969). The residual

oocytes and debris are phagocytised by granular leucocytes from the interfollicular spaces.

The yearly reproductive sequences in the Mytilidae and their correlation with environmental changes have been studied in many ways. Reproduction in M. edulis has been followed by observing the appearance of larvae in the water (Thorson, 1946), the time of larval settlement (Savage, 1956), and the histological appearance of the gonadal tissue (Chipperfield, 1953; Seed, 1969a).

The progress of oogenesis can only be followed by employing histological methods. The whole gonad tissue is monitored by classifying the tissue into stages based on the histological appearance of both the germinal and connective tissues. Either the percentage of stages or a numerical index is then followed. These stages range from an initial stage exhibiting no trace of sexuality to a mature stage in which the lumen of the follicles are packed with mature oocytes, and the surrounding connective tissue is minimal. Lubet (1959) further divides the mature stage into substages based on the appearance of renewed gametogenesis and previtellogenic growth. There may follow a spent or spawned stage and perhaps a phase of resorption. Chipperfield (1953) has studied populations of M. edulis in sheltered areas and estuaries of Great Britain and found a 2 to 4 week spawning period during late spring followed by a 3 month resting phase. Partial spawning was rare. The peak percentage of ripe adults reached

60% synchrony at Conway and Brancaster, and 90% synchrony at Plymouth.

Seed (1969a) followed populations from five marine regions ranging from sheltered bay to wave beaten high shores in Great Britain for 4 years. The peak of maturity occurred once a year during February or March. The highest frequency of mature individuals attained in a population was about 50%. Specimens from high shore stations exhibited completely spent gonads during August while lower shore specimens always had developing or residual oocytes present. Low shore populations also indicated a tendency for multiple spawning.

M. edulis and M. galloprovincialis in three locations in France have been examined by Lubet (1959). From September to May the populations were in the mature stage. There were short periods of resting or spent stages during June to July, and development of oocytes occurred between July and August. For the oceanic station the duration of the indifferent or resting stage is reduced compared to the bay stations. The duration of the mature state in M. galloprovincialis is correspondingly increased from 8 months at the bay location to 10 months at the oceanic location and in M. edulis from 5 to 8 months.

The reproduction of M. perna in the Southern Hemisphere was followed by Lunetta (1969). This population inhabits a region of

Brazil characterized by a permanently high temperature without sharp limits between the seasons (26°C in January and 20°C in June). Complete spawning occurs from February to March (fall) and again in July to August (spring) during which time synchrony was 68% and 63% respectively. A small percentage of the population was observed to spawn throughout the year. All of the individuals collected in March demonstrated egg lysis.

Wilson and Hodgkin (1967) studied five Mytilids in Western Australia. They found that the "five species differed in time and duration of spawning, in the frequency and amplitude of synchronized spawning peaks, and in the nature of the histological changes." M. edulis planulatus (Lamarck) is a winter and spring breeder. Gametogenesis begins in the autumn with falling temperatures. There is an extended period when the gonads are ripe. In July and September two synchronous spawnings occur followed by 4 months of asynchronous spawning and gonad regression. They contrast this picture with that of the summer-autumn breeding tropical species, Branchidontes variabilis and Septifer bilocularis. These animals spawn briefly in April coincident with decreasing temperatures and gametogenic activity begins in October with rising temperatures.

There is considerably less literature concerning the seasonal reproductive progress of M. californianus. The reproductive cycle was first described for a population at Mussel Rock, San Mateo,

California by Stohler (1930) who observed the "fullness or emptyness" of the mantle. Two spawning periods occurred from November 18 to January 14 and June 25 to August 1. Whedon (1936) investigated seasonal spawning in the San Francisco Bay region by observing whether or not gametes flowed when the mantle was punctured. He found that spawning occurred at all times of the year, but was attained by 85% of the individuals during October to February with a slight increase in June to 45% of the population. The rest of the year only 20% to 30% of the individuals were ripe. Coe and Fox (1942) state "spawning may take place at any season of the year on the coast of Southern California but occurs most commonly in spring and autumn and least frequently at mid-winter and mid-summer." Young (1942) followed spawning in the field by observing the macroscopic appearance of the mantle for "fatness" and for "the presence or absence of embryos in the gills of incubating specimens." He tested groups of mussels for their spawning response to the mechanical shock of pulling the byssal threads. The period of maximum spawning lasted from November until April with 36% to 56% of the individuals responding. Barlett (1972) was the first to follow the reproductive sequences by histological procedures. He found 56% and 67% of the population from Dillon Beach, California to be ripe during June and January respectively. There was little variation between spawning of populations located at different heights on the shore.

In summary, Mytilids may or may not have a period of resorption. Population synchrony may be as low as 50% to 70% during peaks of maturity. There are usually spawning periods in the spring, but the mature state may last 10 months and partial spawning is known. Figure 1 summarizes the documented observations on M. californianus and demonstrates the variation in the published data.

Environmental temperatures are known to be one of the most important exogenous factors influencing reproduction. Thermal influences on reproduction are reviewed by Giese (1959) and Kinne (1970). Loosanoff and Davis (1950, 1952) carried out one of the first studies directly relating water temperature and reproductive phenomena in lamellibranchs. They were able to condition the clam, Venus mercenaria, and the oyster, Crassostrea virginica, to spawn out of season by maintaining them at low temperatures and then slowly raising the water temperature when they wished spawning to take place. The average time required for the oyster to reach maturity was described by an exponential formula. Price and Maurer (1971) have shown that the effect of temperature on maturation and spawning in the oyster, C. virginica, can be described in terms of degree days. Chipperfield (1953) found a correlation between the rate of increase of sea water temperature and ripening in M. edulis. Wilson and Hodgkin (1967) emphasize species range and found for the winter-spring breeders that gametogenesis coincided with falling air and sea

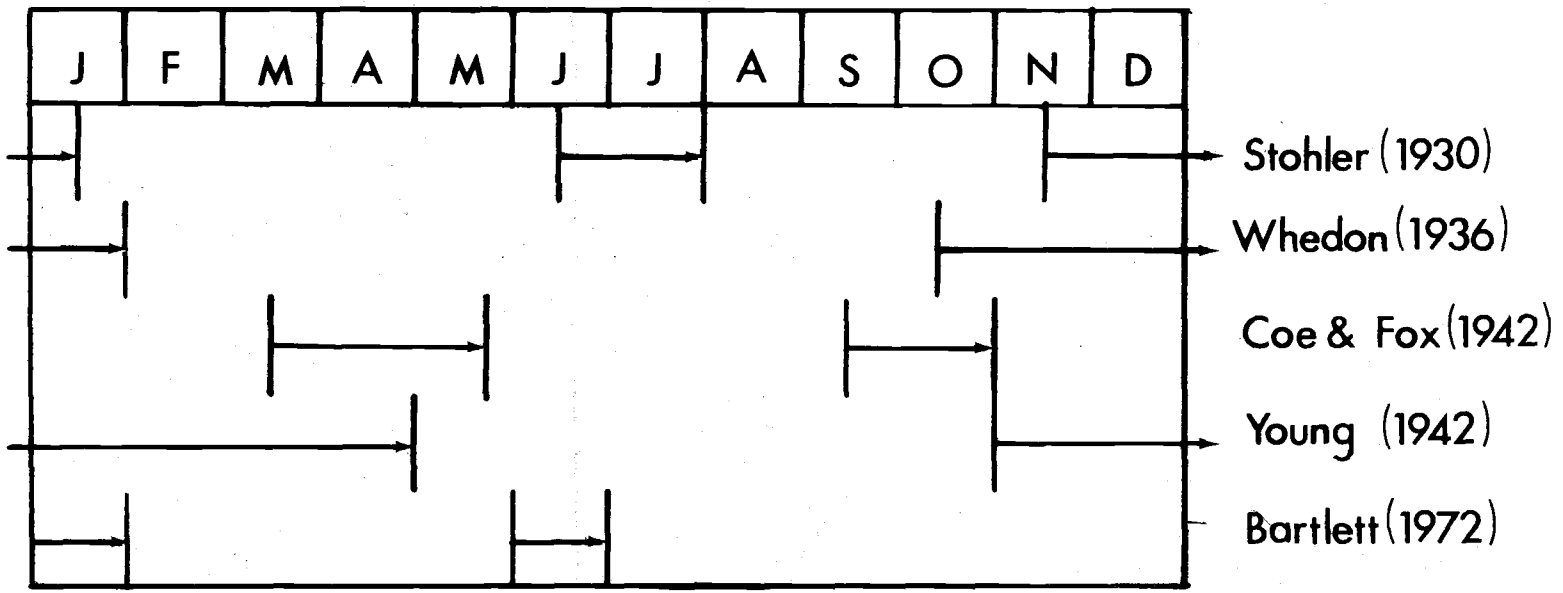


Figure 1. Summation of the literature documenting spawning periods for Mytilus californianus. Arrows indicate the extent of spawning.

water temperatures (25°C to 15°C) while spawning is associated with rising temperatures.

Lubet (1959) has shown the complexity of the effects of temperature. The length of the gametogenic cycle in M. edulis is a function of mean annual water temperature with gametogenesis arrested below 7°C. The duration of the storage or resting stage appeared to be increased by temperatures above 22°C. The beginning of gametogenesis is favored by a lowering of the temperature below 19° to 20°C. Barnes (1963) also found a low temperature condition requirement for the barnacle, Balanus balanoides. Lubet (1959) further found that a slow elevation of temperature enhances the gametogenic process after it has been initiated, whereas a rapid elevation in temperature results in the emission of gametes. Coe and Fox (1942), Young (1942, 1946), and Whedon (1963), however, found no evidence that spawning of M. californianus is induced by temperature or temperature changes.

The influence of other environmental factors on reproduction has been studied to a lesser degree. Young (1942) suggests mechanical shock including wave action is the best spawning stimulus for M. californianus. Campbell (1969) notes that the duration of exposure at low tide is highly correlated with spawning of M. edulis. Only 18% of the specimens which had experienced 1 to 2 hours a day of exposure showed signs of spawning, while 70% of those with a 12-hour exposure period had spawned. Mason (1972) cites some evidence that darkness

or reduced sunlight is beneficial for the growth of M. edulis. Medcof (1965) also notes that light influences growth of oysters and mussels. Neither of these papers, however, mentions the effect of light on reproduction as has been shown for barnacles (Barnes, 1963). Sastry (1968) states that oocyte growth of the scallop, Aequipecten irradians, at Beaufort, North Carolina is limited by temperature (minimum 11.8°C) and food abundance. At 15°C oogonia were produced, but the oocytes did not grow and finally disintegrated. At 20°C oogonial production and growth occurred in the fed specimens, but no development of oogonia took place in the starved specimens.

The relationship between the biochemical composition and the reproductive stages has been investigated in terms of seasonal changes of the constituents, comparison between annual constituent maxima, timing and rates of increase in gonadal and somatic tissues, and total amount of the component in relationship to habitat. Giese (1969) reviews the literature concerning the biochemical composition of molluscs and notes that glycogen is the predominant storage material in lamellibranchs in contrast to lipid in the other classes of mollusca. He further remarks that nutrient storage occurs in all body components rather than in a localized storage depot.

Seasonal changes in the biochemical constitution of M. edulis were first followed on a basis of percent total dry weight of the soft tissues of both sexes by Daniel (1921, 1922, 1923) in England. One

peak of protein was consistently found in the spring. Two glycogen maxima were observed in summer and late fall. Total lipid exhibited a steady rise from June to April.

More recently, Williams (1969) examined the biochemical composition of M. edulis from Great Britain over a 2-year period. His data show small differences between the years in the maximum values. These differences for 1966 and 1967 were: protein (December to June) 70% and 62%, carbohydrate (June to December) 22% and 20%, and lipids (January to March) 12% and 16%.

Gabbot and Bayne (1973) followed the major components in the mantle and non-mantle tissues in both male and female M. edulis. Their data are given as total amount for a standard 5.6 cm long animal. The mantle protein reached 140 mg/animal and the lipid 25 mg/animal. Glycogen increased about 20-fold in both mantle and non-mantle tissues to 150 mg/animal. Their glycogen data are supported by data of Zwann and Zandee (1972) who found glycogen increases in M. edulis from 10 to 140 mg/animal in the mantle and 45 to 210 mg/animal in all the other tissues.

Lubet (1959) followed the components of both sexes on a basis of total wet weight for bay and oceanic populations of M. edulis. Low protein content was associated with the resting stage in summer and increased with maturation. Two glycogen peaks coincided with the increased phytoplankton concentrations during the spring (3% total wet

weight) and fall (7% total wet weight). These glycogen peaks also correspond to the fall storage stage and the spring mature stage. In contrast to the previous works, lipid content was maximum during the summer resting stage with smaller maxima during the spring. Lipid content was greater in M. edulis than in M. galloprovincialis and greater in oceanic populations than in those in brackish waters.

In M. perna, glycogen concentration was also correlated with development of Leydig tissue in the spring (Lunetta, 1969). Lipid concentrations were not seen to increase until after the fall spawning occurred and then were histochemically observed as globules in the connective tissue. Fraga and Lopez-Capont (1958) report upon the biochemical composition of M. edulis at Vigro, although Lubet (1959) suggests that they were really dealing with M. galloprovincialis. Fraga and Lopez-Capont (1958) found both the oligosaccharide (1.1% wet weight) and glycogen maxima (7.5%) to occur together in October.

The only documentation of biochemical levels in M. californianus is given by Giese (1966) who found 50.7% of the dry weight of the ovarian tissue to be protein, 12.9% glycogen, 7.1% lipid, and 3.8% non-protein nitrogen.

There are two studies on the relationship in bivalves between the biochemical state of the mature gonad and the success of the resulting embryos. Collyer (1957) found no correlation between glycogen content and either larval size or yield in Ostrea edulis.

Bayne and Thompson (1970) demonstrated that 85 days of stress conditions to adult M. edulis resulted in abnormal embryos. Stress was associated with a 60% decrease in protein and an 80% decrease in carbohydrate from the mantle tissue. No comparable biochemical studies have been done on M. californianus at this time.

None of the above studies gives a value for maximum production in terms of total eggs produced per year as has been done by Barnes and Barnes (1968) for the intertidal barnacle, Balanus balanoides. They found the largest number of eggs per animal in protected and fully marine areas. Moore et al. (1963) found the total annual spawn of the tropical sea urchin, Tripneustes esculentus, was about five times the maximum amount found in the gonads.

Evidence for neurosecretory control over oogenesis and possible mediation of environmental cues through a neuro-hormonal system comes from correlations of reproductive events with the appearance of neurosecretory granules in ganglia, observations of the effect of cerebral ganglia removal on reproductive processes, and correlations between the appearance of neurosecretion with environmental changes.

Gabe (1955) found neurosecretory cells in the cerebral-pleural and visceral ganglia of pelecypods and followed the cytological formation of neurosecretory granules. Lubet (1959) presents evidence for an active neurosecretory phase in the cerebral ganglia during gametogenesis in M. edulis. A release of neurosecretion occurs at

spawning, and the neurosecretory cells renew their contents during the net oocyte growth and development phase (restoration). After successive spawnings, a reduction of neurosecretion coincides with the appearance of the storage phase. Umiji (1969) also found that the cerebral, visceral, and pedal ganglia in M. perna contain a large amount of neurosecretory cells and that neurosecretory activity of small neurons is related to the mature stages of the reproductive cycle.

When Lubet (1959) performed bilateral excision of the cerebral ganglia during the resting stage, the multiplication of oogonia and the initiation of gametogenesis were retarded for 2 months. If the excision was carried out during the summer, the connective tissue was poorly developed, and glycogen and lipid were not accumulated. Removal of the cerebral ganglia from mature mussels resulted in more rapid spawning then in traumatized controls (Lubet, 1969). Subsequent restoration phases were extended. Adipo-granular cells were rarely seen in the connective tissue and contained less neutral and phospholipids. The presence of the visceral ganglia is necessary for spawning, but it should be emphasized that removal of these latter ganglia results in death within 3 or 4 months. Lubet (1959) did not differentiate between direct effects of the excision on metabolism and the indirect effects on filtration rate. He found specimens without cerebral ganglia are more receptive to external stimuli than either

controls or field samples. Specimens lacking cerebral ganglia and its inhibitory effect spawned in February at 5°C. This temperature is far below the lower limit required for normal spawning. Umiji (1969) found removal of the cerebral ganglia in three species of Mytilus had no effect on follicle development, but byssal gland function was impaired. Ablation of the cerebral ganglia in the oyster, Crassostrea virginica, and the mussel, Branchidontes recurvus, also hastens spawning (Nagabhushanam, 1962, 1963). In M. perna, Umiji (1969) found that the neurons in the ganglia were predominantly empty during summer and winter, whereas a large amount of small neurons was completely packed with neurosecretory material in April (fall) and August (spring). Spawning occurs in fall and spring in this animal.

Lubet (1959) records that a sudden thermal change causes release of the neurosecretory material in the cerebral ganglion of the fresh-water mussel, Lamelliens marginalis. A salinity stress causes immediate release of neurosecretion from the cerebral ganglia in M. galloprovincialis (Lubet, 1959). In summary, the activity of the cerebral ganglia appears to be necessary for the reproductive processes and is correlated with environmental changes in all the Mytilids examined.

The effects of the environment on reproduction cannot be separated from consideration of material and energy budgets. Widdows and Bayne (1971) have established caloric budgets for M.

edulis under three thermal regimes and three food levels. Total weight-specific oxygen consumption was higher at 10°C in November when there is an increase in metabolic tissue than in June at 15°C. M. edulis is not able to maintain an energy balance with temperature increases, and warm acclimation results in stress. Assimilation efficiency was lowest at the high temperature (15°C) and high food concentrations.

Gabbot and Bayne (1973) separated mantle and somatic tissues when they did seasonal studies on temperature and nutritive stress in M. edulis. Some implications can be drawn on the seasonal mobility of reserves. Glycogen was lost from the mantle only during August to September, and no significant decrease occurred in the somatic tissues. Lipids were lost from the mantle from January to June in both tissues. Protein decreased in the mantle only from January to April, but from non-mantle tissue between January to June and October to November. Transfer of assimilated food material to oocytes in a scallop has been studied by Sastry and Blake (1971). The rate of transfer of reserves to gonads appears to be related to the initiation of the growth phase of the oocytes. Based on the results of experiments employing carbon-14, they suggest that amino acid transfer is slow and is regulated by the developing oocytes.

The physical environment experienced by intertidal animals has been extensively discussed. Gislen (1943) summarizes the

physiographical conditions from Ventura, California to Ensenada, Mexico. He records seasonal air and sea water temperatures, sunlight, humidity, winds, currents, and waves, tidal differences, and discusses briefly the habitat of M. californianus. Daily data for sea surface temperatures and salinities, and air temperatures at shore stations along the Oregon coast are available from the reports of Gonor and Thum (1970) and Gonor et al. (1970). A summary of the nearshore oceanography of the Pacific Northwest is presented by Bourke et al. (1971). Newell (1970) reviews the subject of thermal budgets for intertidal organisms. Actual tissue temperatures of M. californianus were recorded by Gonor (1968), who found ranges in the beds up to 7°C and maximum temperatures up to 30°C.

The habitat of M. californianus, the exposed open coast, offers a different environmental regime than the bays in which M. edulis predominates. Furthermore, M. californianus is one of the largest members of the genus and is a dominant member of the open coast rocky intertidal community from Alaska to Baja California (Glynn, 1965; Dayton, 1971). This species offers good experimental material for elucidation of both the mechanisms involved in the reproductive processes of pelecypods and the study of intertidal populations. This study presents a detailed quantitative description of the reproductive events of M. californianus and establishes methods to calculate rates

of chemical accumulation and egg production. The thermal regime of the tissues is documented in order to correlate environmental and reproductive events.

II. MATERIALS AND METHODS

Collection of Physical Data

Data on insolation, sea water and air temperatures, and tidal water heights were collected on an hourly basis from August 1970 to August 1971. Data prior to this period were taken from the work of Gonor (1968) and Bourke et al. (1971). In the period following August 1971, weekly sea water and air temperatures were taken at Yaquina Head on the Central Oregon Coast. Air temperature data obtained by the U.S. Weather Bureau, Newport, Oregon were also used to estimate temperatures.

Total short wave solar insolation up to 4 μm was continuously recorded with an unshielded horizontal surface Eppley pyroheliometer situated on the Marine Science Center dock at Yaquina Bay. Air and sea water temperatures were taken from a continuously recording protected thermistor chain located at Whale Cove, 10 miles north of Yaquina Head (Gonor et al., 1970). Sea water temperature was taken from a -2 foot probe at the time of high tide and air temperature from a +10.6 foot probe during the two low tide periods.

Direct measurements of mussel tissue temperature were made in the field using a thermoprobe (Yellow Spring Instrument Co.). Substrate temperatures were taken 15 cm into the mussel bed. The long wave back radiation scatter from mussels warms the air to 8 cm and

thus affects the temperature gradient (Gonor, 1968). Therefore, shaded air temperatures were taken at 30 cm above the bed. Internal tissue temperatures were obtained by inserting the hypodermic thermoprobe into the visceral mass of mussels perpendicularly packed into beds. Relative humidity was measured using a wet-dry bulb sling psychrometer.

Tidal exposure data for the +3 foot level above mean low low water (MLLW) were taken directly from the charts kept by the U. S. Weather Bureau located at Yaquina Bay. Storm swell during December at Yaquina Head can raise the water level by 1 m relative to May, and therefore decreases the exposure period relative to that recorded in the bay (Panshin, 1967).

A water sample was collected weekly from channels of moving water at Yaquina Head in polyethylene containers to determine particulate oxidizable carbon. The sample was filtered through a 200 μm mesh screen to eliminate zooplankton, wood chips, and large pieces of debris. The water was then poured into 1 liter cylinders and allowed to settle at 4°C. Sets of cylinders containing 5 ml of Lugols Iodine solution were used for weight measurements and diatom counts with a Sedwick-Rafter cell. The sample for gravimetric determination was decanted, concentrated by centrifugation with a clinical centrifuge, washed quickly with distilled water once to reduce salt, and placed in a pre-weighed aluminum pan. This sample was then dried at 68°C for

a day, ashed for 1 hour at 580°C (Paine, 1964), and weighed to give the weight of inorganic particulates per liter of sea water. Another set of cylinders was allowed to stand and was used for dichromate-organic analysis (Strickland and Parsons, 1968). The supernatant water was filtered through a cleaned Mitex LC 10 μm teflon filter, and the precipitate in the cylinders collected by centrifugation. The filter and precipitate was washed twice with a few milliliters of sodium sulfate (45 g/liter) to remove chloride. The samples were heated in a boiling water bath with 1 ml of reagent phosphoric acid to volatilize any remaining chloride. Three ml of dichromate solution were added (7.5 g potassium dichromate to 500 ml concentrated sulfuric acid), the tubes were heated again for 30 to 60 minutes, cooled, and 3 ml of water added and mixed. The centrifuged solutions were then read at 600 mμ on a Beckman Spectronic 20 colorimeter. Blanks and glucose standards were run with each analysis, and the amount of oxidizable material was given in glucose units.

Observations on Field Specimens

Three types of observations were carried out on the same individual so that correlations between the different reproductive indices could be made. All studies were done on female specimens. Up to 25 specimens of 70 to 90 mm in length (0.9 to 1.3 g dry flesh weight) were taken at random from the mussel bed located at +3.5 feet on the

intertidal bench at Yaquina Head, and the mussels kept in the laboratory water system or stored out of water in a 9°C cold room for no more than 2 days. For sampling, they were drained, the flesh was quickly removed and placed on a damp paper towel, and the wet tissues were separated into somatic tissue and gonadal tissue. Gonadal tissue included the inner lobes of the mantle (exclusive of the pallial muscle) and the gonad proper plus as much of the other diffuse gonadal tissue as possible. Nearly all of the gonadal tissue can be obtained in the following manner. The mantle lobes are easily cut out. Then the connective tissue overlying the retractor muscles is cut with a sharp scalpel, and the muscles are pulled free by the foot and byssus. One cut then separates most of the gonadal material from the digestive gland. Some leakage of eggs occurs from very ripe gonads, and the haemocoelic fluid is certainly lost. The tissue specimens were placed on pre-weighed aluminum pans, and a known portion of the gonad was preserved in Bouin's fixative. A second portion was placed in acetone to be used for biochemical analysis. The remaining portion was weighed and allowed to dry in an oven at 68°C for 1 week.

Histological Indices

Care was taken to always select a central portion of the mantle for histological purposes. One cm pieces of the mantle were placed in Bouin's fixative, embedded in paraffin, cut into sections 7 μm thick,

and stained with Mayer's haematoxylin and eosin using standard techniques. Sections from the center of each specimen were examined.

The percentage area of the section composed of Leydig tissue and of germinal tissue was estimated, and the presence of amoebocytic granular cells was also noted. The percentage of the follicles with oogonial clusters (defined as groups of more than four oogonia) was determined from 50 to 100 follicle cross sections. Cross sections of follicles in the center of the histological sections were chosen and their diameters measured. The diameters of all the oocytes whose nuclei were observed in that follicle were measured. Each individual histological sample was subjected to a minimum of at least five to eight follicle counts. The oocyte data were divided into four groups: 1) small oocytes still associated with nests and having a diameter up to 10.5 μm; 2) previtelligenic oocytes with 12 to 29 μm diameters which have a basophilic cytoplasm, enlarged nuclei, but little cytoplasm; 3) intermediate oocytes 30 to 38 μm in diameter; and 4) mature vitellogenic occytes larger than 39 μm and containing pink staining, yolk-filled cytoplasm.

Correction must be made for the effect of the different size classes of oocytes on the true number of cells. Since all the cells counted are larger than the 7 μm section thickness, their numbers and diameter measurements are in error according to their size. A large oocyte is observed in more sections than a small cell. This

problem becomes more important when different size oocytes are used together for estimating the growth rate from one size to another. The nucleoli which measure 7 to 7.5 μm in diameter are closer to a point and would be a better determining feature for counting than the nucleus, but there would not be enough cell counts for statistical analysis. Corrections can be made by comparing nucleolar counts with the counts used. The oocyte counts were found to be overestimated by the amounts in Table 1.

Table 1. Correction factors for oocyte counts in 7 μm sections.

Oocyte size (μm)	Counting error (% observed/true)	Correction factor
12-29	120	0.83
30-38	170	0.59
39-	200	0.50

For the purposes of grouping the samples into reproductive stages for biochemical analysis, a gonad was designated as in the storage stage if more than 70% was Leydig tissue, as a developing gonad if more than 60% of the eggs were previtellogenic oocytes, as mature if more than 60% of the eggs were mature, and in the resorption stage if more than 50% of the follicles had cells undergoing lysis.

Biochemical Composition

A maximum of nine specimens of gonadal tissue were placed immediately into acetone to dehydrate the tissue, stop enzymatic degradation, and begin the lipid extraction (Loomis, 1972). The separation scheme is given in Figure 2. Since the amount of tissue in an individual is limited and at least three types of analyses on the same sample were required, a decision was made as to the allocation of the sample sizes. The best use of the tissue could be made by taking 500 mg pieces of wet gonadal tissue for biochemical analysis.

The separation of lipids from the tissue was based on the procedure developed for brain lipids (Folch et al., 1951, 1957). The acetone extractions were followed by two 10 ml extractions of 2:1 chloroform-methanol mixture. This method removes more polar lipids than the ether alcoholic extractions (Giese, 1967). The combined acetone and chloroform methanol extracts were washed by layering the top with at least 40 ml of distilled water or 0.9% NaCl and allowing the tubes to set until the layers have cleared. The aqueous soluble components go into the water and the lipids into the chloroform layer. A white layer of proteolipids and possibly some phospholipids forms between the two layers (Folch et al., 1957). After the aqueous layer is removed, the white layer is made miscible with the chloroform layer by adding a few milliliters of methanol.

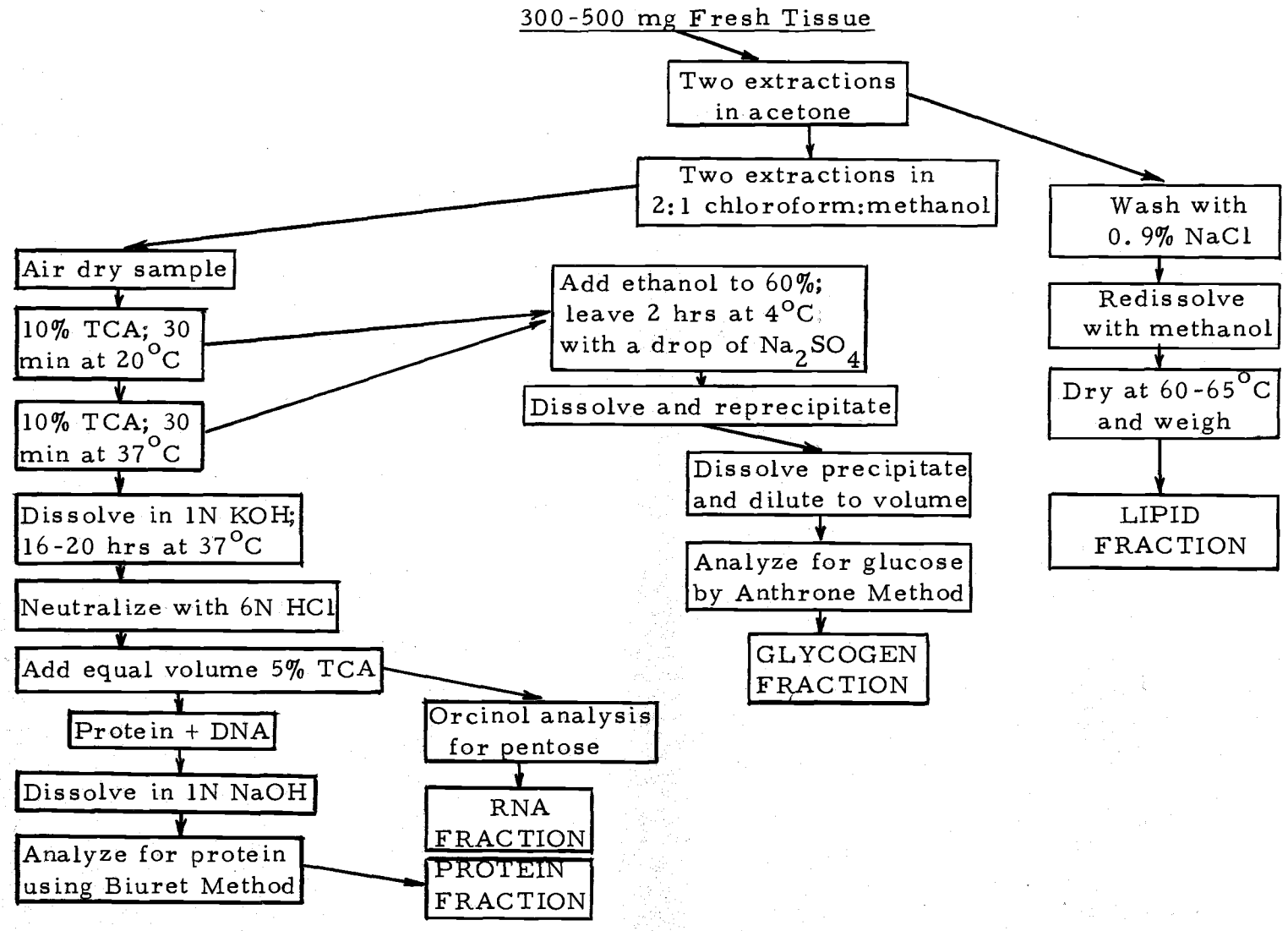


Figure 2. Scheme of separation for biochemical components in gonadal tissue of Mytilus californianus.

This remaining solution is placed in a pre-weighed aluminum pan, dried at 68°C, and quickly weighed. The weight of the remaining solids is called "total lipid." This fraction includes some protein associated with the proteolipids and some loss of lipids volatile at 68°C.

The tissue remaining from the lipid extraction was allowed to air dry and was extracted with 10% trichloroacetic acid (TCA) at room temperature for 30 minutes and again with TCA at 37°C for 30 minutes. The combined extracts contained polysaccharides, sugars, amino acids, organic acids, nucleotides, and coenzymes (Roberts et al., 1957). The glycogen polysaccharides were separated from this extract by precipitation in 60% ethanol (Good et al., 1933). An added drop of saturated sodium sulfate increases the recovery by coprecipitation (Van Handle, 1965). The precipitation is carried out in the cold for several hours, redissolved in water, and precipitated again in 90% ethanol.

Aliquots of each sample were assayed in triplicate for glucose using the anthrone procedure (Sattler and Zerban, 1950). Two and one-half ml of the sample was added to 5 ml of anthrone reagent (0.2 mg anthrone/100 ml concentrated sulfuric acid) in a large test tube. The tubes were mixed and immediately placed into a boiling water bath for exactly 3 minutes. They were then plunged into cold water and the optical density read on a spectrophotometer at 620 mμ.

The factor 0.9 was used to convert units of glucose to equivalent glycogen units.

The remaining TCA precipitate contains protein and nucleic acids. This precipitate was dissolved in 1 N NaOH, diluted with water, and subjected to Biuret analysis. One ml of sample was added to 4 ml of Biuret reagent, mixed, and allowed to stand for 20 minutes. Absorbance was read at 560 mμ (Layne, 1955).

Analysis for ribonucleic acids (RNA) was initiated by dissolving the TCA precipitate in 1 N KOH for 16 to 20 hours at 37°C, neutralizing with 6 N HCl, and adding an equal volume of 5% TCA. The protein and DNA form a precipitate, and the RNA remains in solution. Quantitative analysis was carried out using the orcinol method for pentoses (Schneider, 1955).

The procedures were calibrated with standards of bovine serum albumin, palmitic acid, glycogen, and ribonucleic acid (Sigma Chemical Co., St. Louis, Mo.).

Incorporation of Carbon-14

In order to obtain some idea of the rate and the amount of incorporated food that finally reaches the gonad and to establish what points along the physiological pathway from food to egg might be limiting, carbon-14 labeled flagellates were employed as a food. The requirements for the food to be used were that it be particulate,

uniform in both chemical composition and distribution of the label throughout the components, readily available, and an acceptable food to M. californianus. These characteristics were met with Isochrysis galba, a flagellate which was cultured in the following way. Nutrients and trace metals were added to 500 ml of ultraviolet irradiated, filtered sea water of 32 $^{o}/_{oo}$ salinity (Appendix I). One hundred ml of algal stock culture (20 to 50 x 10^4 cells/ml) was obtained from the Oregon State University Pilot Oyster Hatchery and added to the beaker. Air was continuously bubbled into the culture and fluorescent lamps were placed 6 inches to one side. The temperature was maintained at 23 ^{o}C. This culture technique resulted in the composition listed in Appendix II. The progress of the culture was followed by direct count with a haemocytometer. After culturing for a day to assure that the cells were dividing, 10 ml of $Na_2C^{14}O_3$ (2.5 μCi/ml, 67 μg Na_2CO_3/ml) was added to give a final culture activity of 42 μCi/liter. The culture was allowed to grow for 2 to 3 days before use in order to permit the label to become evenly distributed among the components. About 36% of the label was assimilated by this time.

After collection, M. californianus specimens were cleaned of encrusting barnacles and held in the laboratory for 2 to 4 days. Thus they were considered to be field acclimated. A 1 cm window was cut into the shell valve with a hacksaw without injury to the mantle so that the sex and reproductive state of the individual could be ascertained.

Those animals which were exposed had the hole resealed with glass coverslips and vaseline for about a week until a conchyolin layer had grown over the hole. This operation did not appear to harm the mussels.

For an incorporation study, a specimen was placed into a constantly aerated and lighted plastic tank containing 15 liters of sea water with a concentration of labeled flagellates on the order of 10 million cells per liter. This is approximately the maximum phytoplankton concentration found in the field. Concentrations greater than this amount result in the production of pseudofeces.

The animals were left in labeled flagellate cells for about 3 hours at which time they had removed an amount of cells containing at least 100,000 cpm. Incorporation was initiated at times coinciding with local high tides since it was found that filtration rates were usually higher at this time. The mussels were then kept in tanks with unabeled food concentrations of 1 to 5 x 10^4 cells per ml for up to 23 days. Based on field data, the mussels were subjected to four experimental conditions. Group I was continuously submerged at 9°C and Group II continuously submerged at 15°C. Group III was submerged for 6 hours at 9°C alternating with 6-hour periods of air exposure at 9°C, and Group IV was submerged at 9°C alternating with 6-hour periods of air exposure at 20°C giving the same total heat as the 15°C continuously submerged animals. The internal temperature

of the mussels during exposure was monitored by a thermoprobe in an exposed animal of similar size.

The amount of labeled flagellate cleared from the medium was determined by filtering a large sample (500 to 1000 ml) through a 0.8 μm millipore filter, rinsing with Na_2SO_4 (45 g/liter) to remove soluble activity, air drying, and counting with a Gieger-Muller tube. The resulting activity was compared to a 100 ml sample of the control medium. Values for replicate samples were within 1.4% or less of each other. A calibration curve of $BaC^{14}O_3$ precipitate was extrapolated to zero thickness giving a correction factor for both non-point source and counting efficiency (Wang and Willis, 1965). The counting efficiency was determined to be 21.2%. The total amount of activity (in dpm, disintegrations per minute) invested was calculated as:

$$\text{Total dpm} = (\text{Sample volume})\left(\frac{\text{Change in concentration in cpm}}{}\right)\left(\frac{100}{\text{Counting efficiency}}\right).$$

This figure was converted to total cells ingested by:

$$\text{Total Cells} = \text{Total dpm} \times \text{cells/dpm},$$

where the value for cells/dpm was obtained from the control. The rate of filtration was calculated using Jorgensen's formula (Jorgensen, 1966).

$$\text{Rate} = \frac{(\text{Volume})\,(\ln C_o - \ln C_t)}{\text{Elapsed time}}.$$

Fecal ribbons are fairly resistant to disintegration and were collected periodically. Any remaining heavy particulate matter was collected on a glass filter (Gelman, Type A, 0.3 μm). Both samples were air dried and counted to 10,000 counts with a Gieger-Muller tube. One set of fecal ribbons and a sample of labeled flagellates were analyzed for carbon and nitrogen using an F and M Model 185 Carbon-Hydrogen-Nitrogen Analyzer.

The individuals were sacrificed at periods up to 23 days from the time of label incorporation. The digestive gland and stomach, gonadal tissue, and remaining muscle and somatic tissue were separated and weighed. The entire somatic and digestive tissues were placed into equal volumes of 30% KOH. A histological sample of gonad was taken, and the remainder of the gonadal tissue was then subjected to separation into protein, glycogen, lipid, RNA, and residual fractions (Figure 2). Each biochemical fraction was further divided into an aliquot for counting and an aliquot for biochemical analysis. The final preparations for scintillation counting of these fractions are given in Table 2. Counting efficiency for each type of sample was determined by adding a small volume of known activity and observing the recorded activity. The resulting counting efficiencies were about 81% for the lipid fraction, 61% for the protein fraction, 73% for both the glycogen and RNA fractions, and 79% for the residual supernatant fraction. Since the amounts of chemical and water quenching

Table 2. Preparation of scintillation samples.

Fraction	Origin[1]	Preparation
Lipid	Chloroform:methanol	Dilute to 10 ml with methanol 1 ml aliquot into vial Air dry 10 ml Aquasol[2] added or 10 ml Fluor mixture[3] added
Glycogen	60% Ethanol precipitate from TCA soluble fraction	Dilute to 4 ml with water 1 ml aliquot Add 10 ml Aquasol
RNA	Supernatant of 5% TCA fraction following KOH incubation	Dilute to 15 ml with water 1 ml aliquot 10 ml Aquasol
Supernatant	Combinations of a. lipid wash water b. 60% ethanol soluble fraction in 10% TCA supernatant c. ethanol/ether wash of 10% TCA precipitate if carried out	Dilute to 15 ml with water 1 ml aliquot 10 ml Aquasol
Protein	TCA precipitate	Dissolve in 1 N NaOH Dilute to 10 ml 1 ml aliquot Add 2.5 ml water 11.5 ml Aquasol Shake to form gel[4]

[1] See Figure 1 for separation scheme.

[2] New England Nuclear, "Aquasol, Universal LSC Cocktail."

[3] Radin, N. S. 1972.

[4] New England Nuclear, "Aquasol, Universal LSC Cocktail."

agents in the extract remained proportional, the channel ratio method of efficiency estimation could be employed (Bush, 1964). Plots of efficiency against the ratio of counts from the low energy:high energy discrimination windows were constructed. The dpm of the whole tissue and biochemical fractions were estimated by the formula

$$\frac{\text{cpm - background}}{\left(\begin{matrix}\text{Daily machine}\\ \text{effic.}\end{matrix}\right)\left(\begin{matrix}\text{Mixture}\\ \text{effic.}\end{matrix}\right)\left(\begin{matrix}\text{Quench}\\ \text{effic.}\end{matrix}\right)} \left(\begin{matrix}\text{Aliquot}\\ \text{factor}\end{matrix}\right)\left(\begin{matrix}\text{Sample}\\ \text{factor}\end{matrix}\right) = \text{Total count}$$

All calculations of carbon-14 retention were based on net food retention (ingested minus fecal). This method then allowed the activity of the various fractions to vary independently of each other, and normalized the values for slightly different amounts of food assimilated. The amount of biochemical component accumulated was estimated by dividing the dpm of the component from the gonadal tissue by the specific activity of the component in the food. It was realized that this method does not account for metabolic interconversions. The specific activity was determined by dividing the observed dpm of the component by the total weight of that fraction.

III. RESULTS

Environmental Characteristics

The beds of M. californianus at Yaquina Head are found at about the level of +3 feet above MLLW. In the semi-protected backshore where the study was carried out there is an average of 1, 120 mussels measuring about 70 mm in length per square meter. The zone containing the mussels averages 8 m in width.

The maximum daily air temperatures recorded at Whale Cove range from 0° to 21°C and are highest during August (Figure 3). Surface sea water temperatures at Whale Cove generally remain between 8° and 12°C throughout the year (Figure 4). Summer values are quite variable due to periods of upwelling of cold water. The highest temperatures occur during August or September. The linear correlation between 16 pairs of data for Yaquina Head and Whale Cove taken throughout the year is given by the equation

$$T_{yh} = 0.99T_{wc} + 0.03$$

where T_{yh} and T_{wc} are the temperatures at Yaquina Head and Whale Cove respectively, and the correlation coefficient for the 16 pairs was 0.83. Thus the temperature data from Whale Cove could be used for Yaquina Head observations.

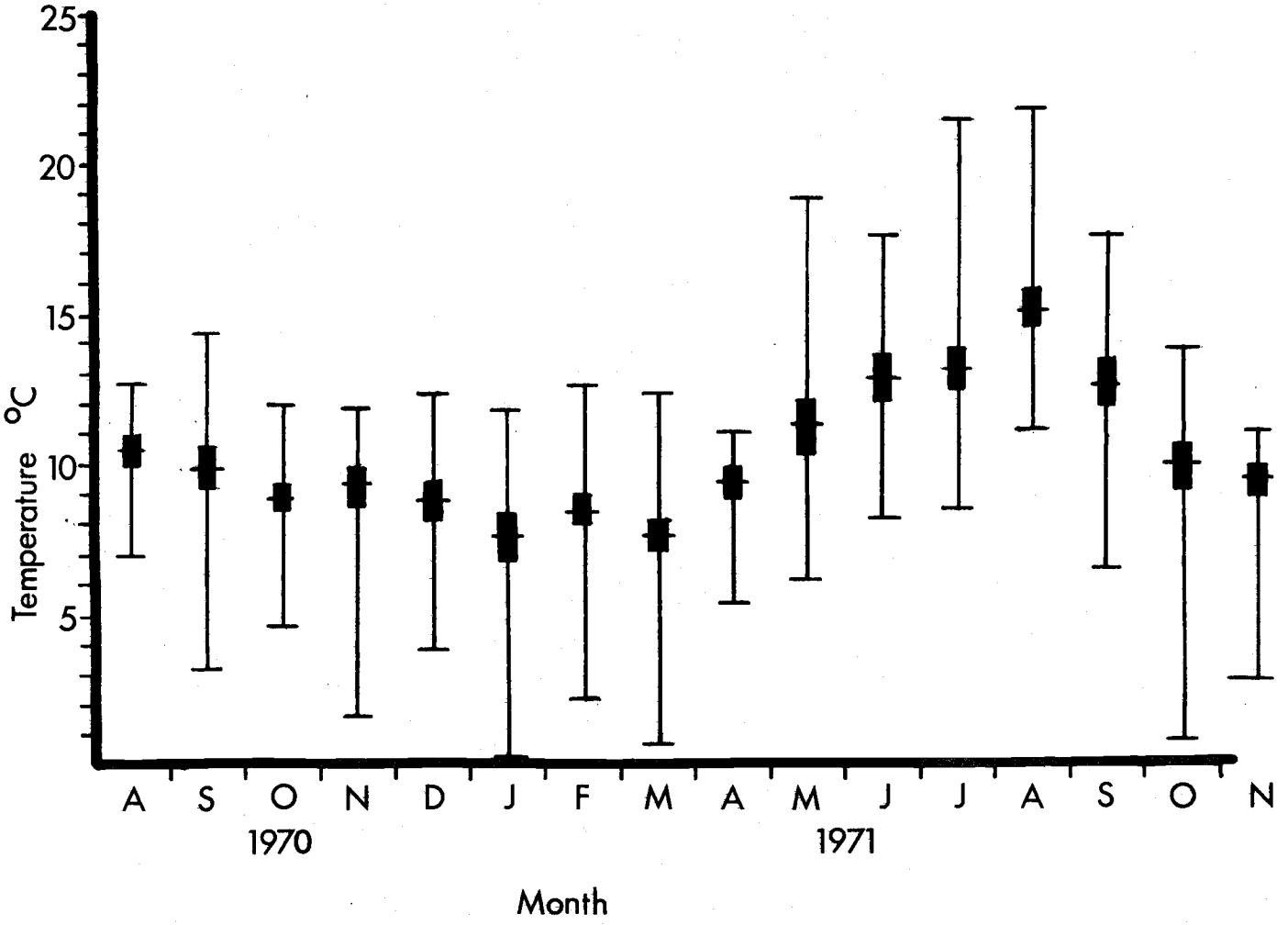


Figure 3. Maximum daily air temperatures observed during low tides at Whale Cove, Oregon. Monthly means, 90% confidence interval and ranges are shown.

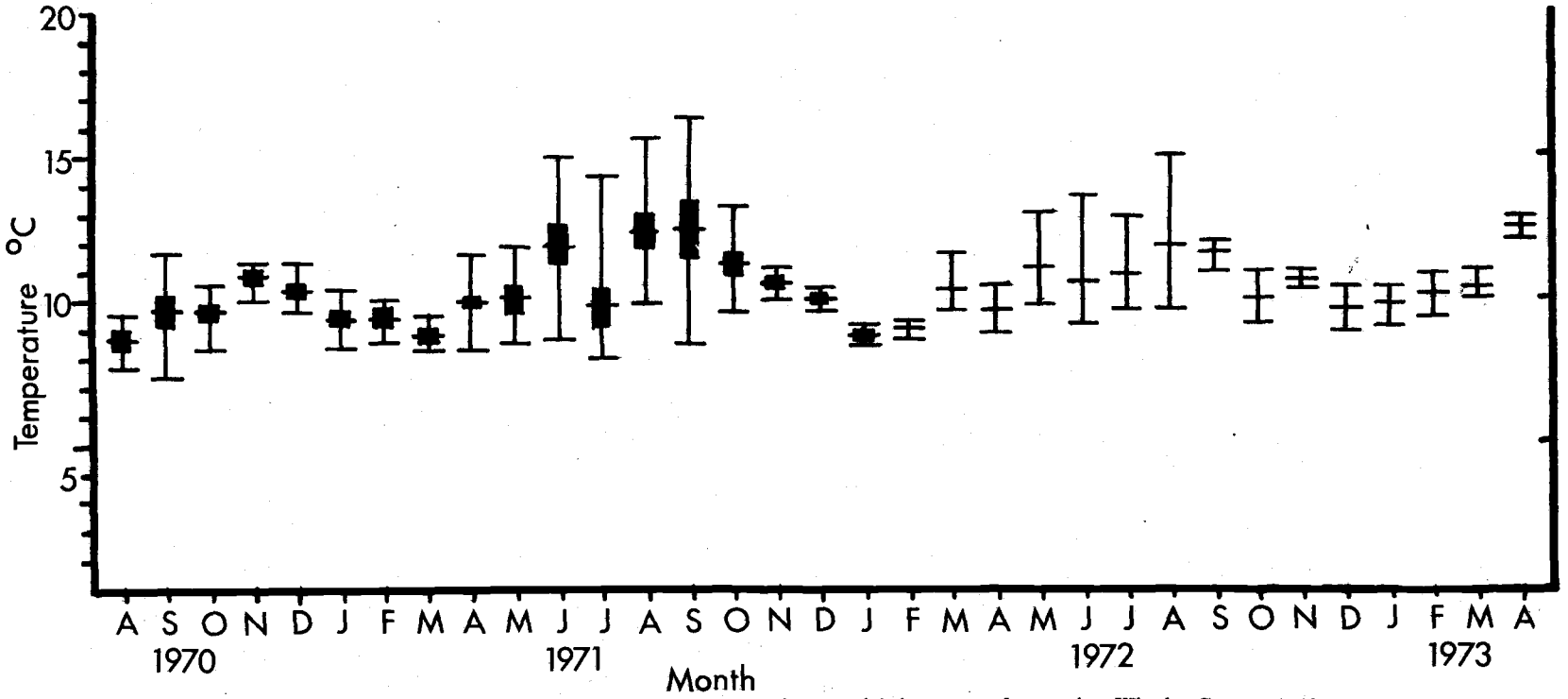


Figure 4. Surface sea water temperatures taken at high water from the Whale Cove station. Monthly means, 90% confidence intervals and ranges are presented.

The average daily insolation (langleys per day) measured at Yaquina Bay is presented in Figure 5 and was found to be quite constant from year to year. The sunlight throughout the day (langleys per minute) is given in Figure 6. Occasional morning fogs at Yaquina Bay result in a drop in incident radiation that is not seen at Yaquina Head. The time and duration of exposure of the +3 foot level was plotted in Figure 7. The time of exposure passes through the middle of the day beginning in March and ending early in June and through the middle of the night from August to February. The average exposure period is 6 hours. When the maximum langleys per minute (corrected for cloud cover) falling on the +3 foot level were integrated over the time of exposure for every day of the year, the total amount of insolation falling on this level was obtained. The results of this calculation are presented in Figure 8. The greatest number of langleys per month occurred during late May to June.

Estimation of Mussel Tissue Temperature

Empirically it was found that mussel tissue temperature was adequately explained by

$$T_{mussel} = T_{air} + 5.07 \text{ (langleys/min)}$$

in which T_{mussel} and T_{air} are the mussel tissue temperature and the shade air temperature respectively. The data on air temperature,

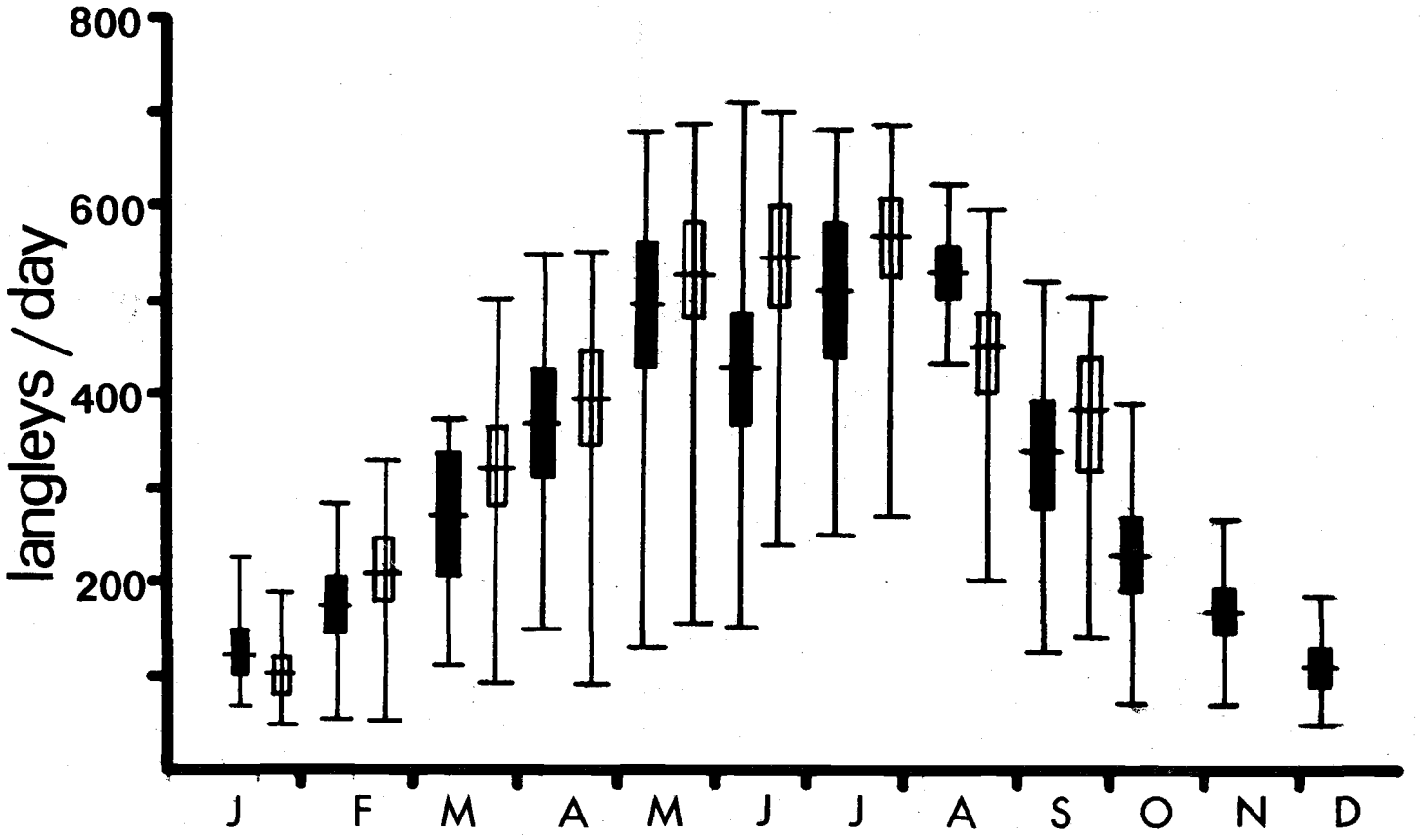


Figure 5. Average daily insolation for one year. Filled bars: 1969. Open bars: 1970. Monthly means, 95% confidence intervals, and ranges are shown.

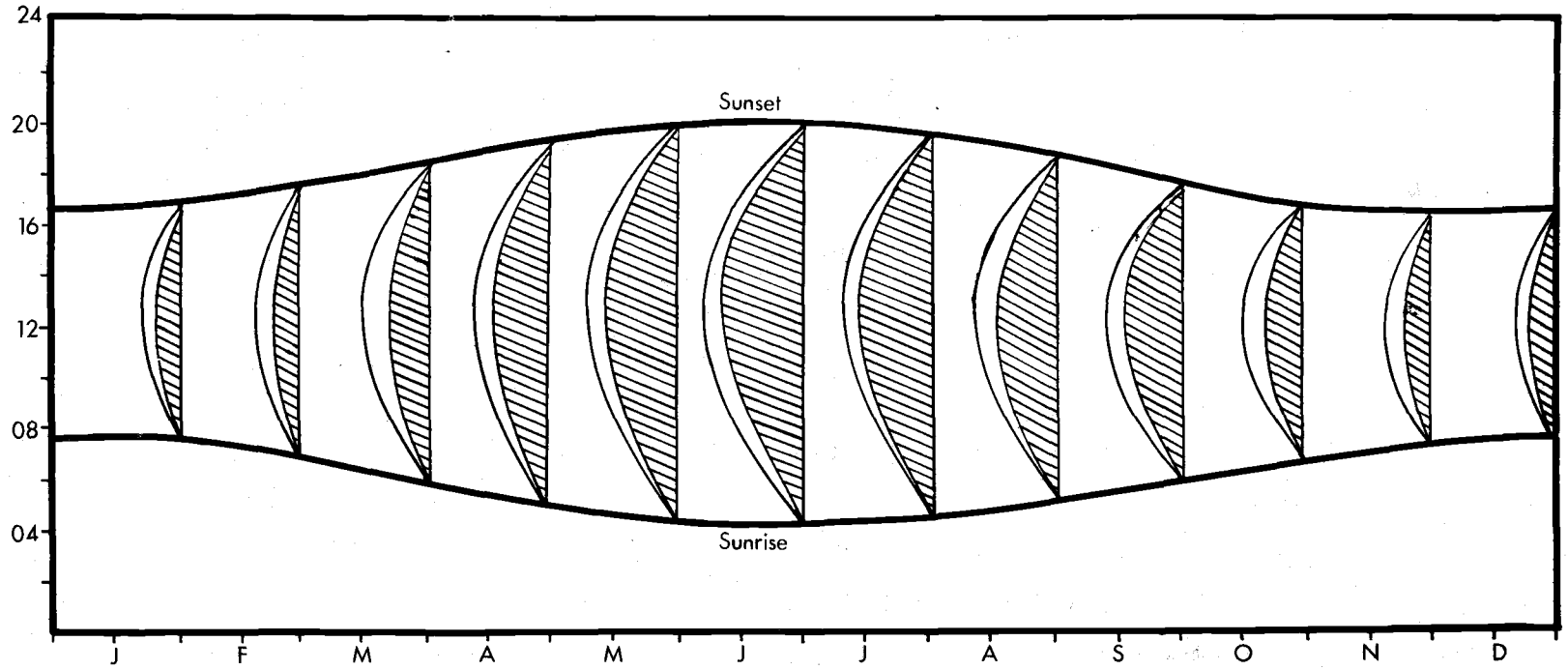


Figure 6. Daily insolation throughout the year. Height of the parabola indicates the langleys per minute. Lower parabola is the value corrected for cloud cover. Scale: 1 cm height = 0.5 langleys per minute.

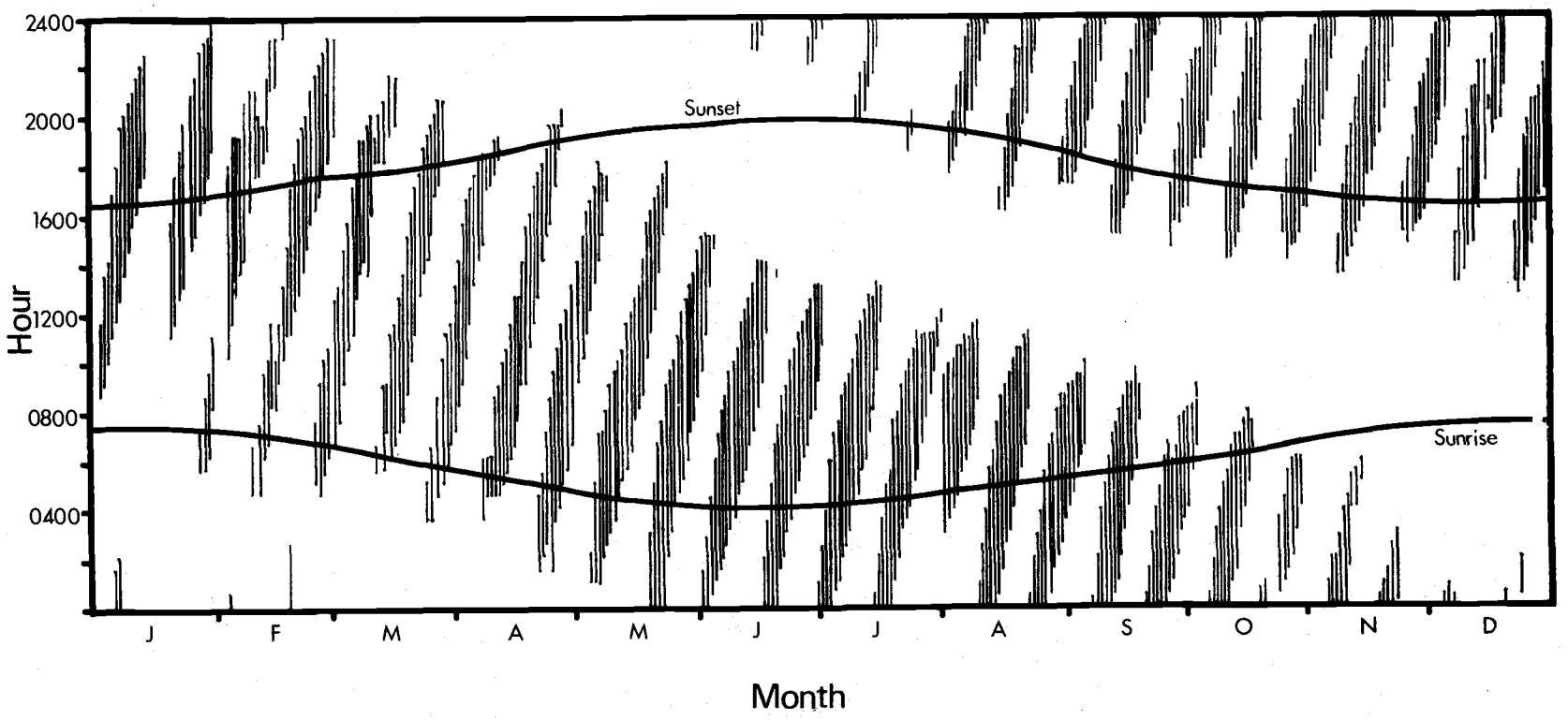


Figure 7. The time and duration of air exposure for the level of +3 feet above mean low low water at the Marine Science Center Tide Gauge, Yaquina Bay, Oregon.

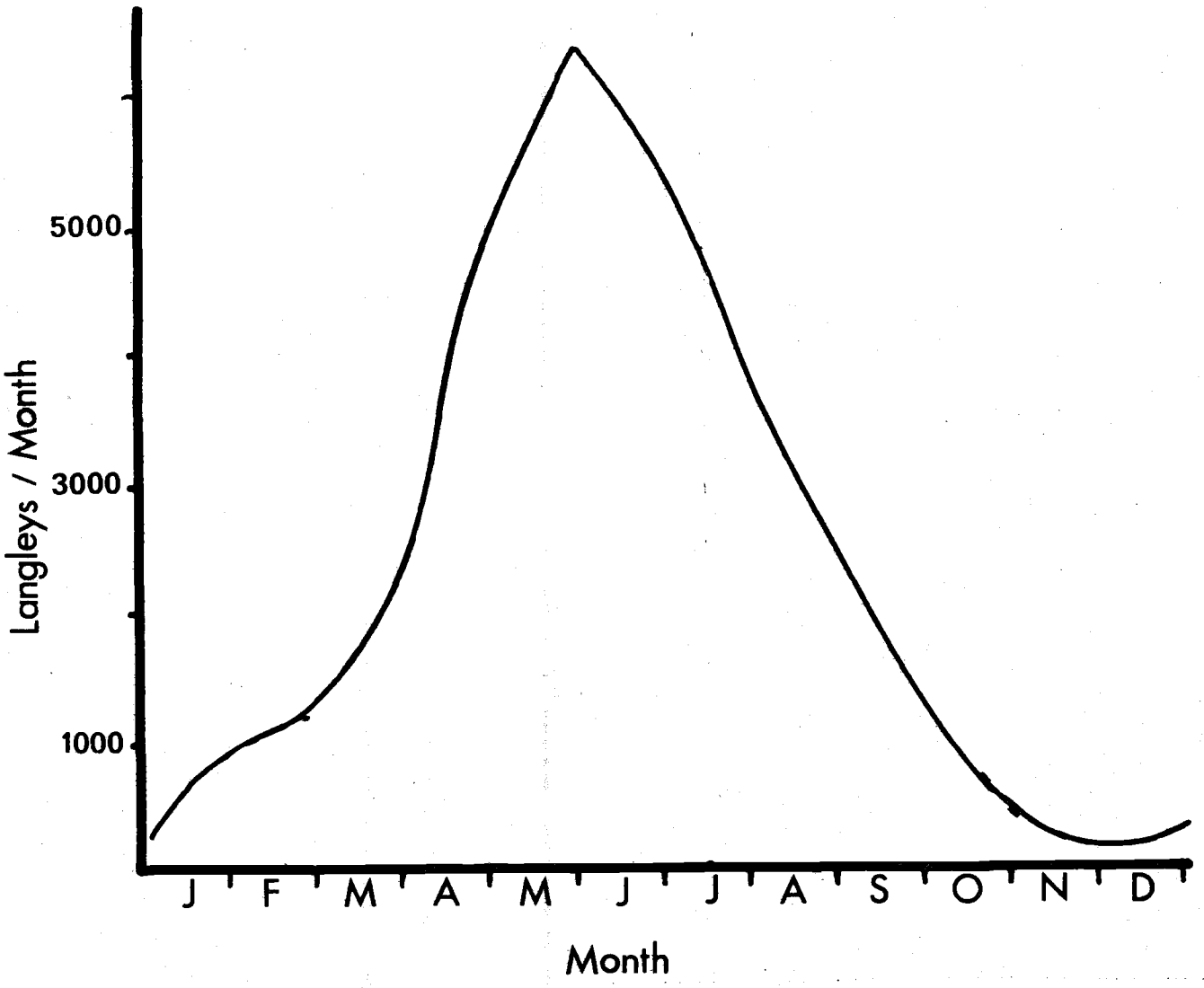


Figure 8. The total monthly insolation falling on the shore level of +3 feet above mean low water during exposure.

sea water temperature, and insolation permit continuous calculation of the mussel tissue temperature using this equation. This formula was theoretically justified by evaluating a heat budget equation for the periods of exposure (Appendix VII). A regression between observed and calculated values gave the 90% confidence limits as $\pm$ 3.1°C and a correlation coefficient of 0.97. The confidence interval reflects the variation actually found in the mussel bed (Appendix III). There was no correlation between deviations of the calculated temperatures from the observed and the differences in vapor pressures. Therefore, it was felt that further refinement of the equation, in particular with respect to evaporation, would not reduce the variation.

Continuous half-hourly estimates of mussel tissue temperature during exposure were determined from the physical data for 1 year in the following way. While the mussel was submerged, its temperature was made equal to that of the water. The mussel temperature equation was used when the animal was exposed during the day. The range of the averages of the calculated tissue temperatures is presented in Figure 9 along with the sea water temperature for comparison. It is seen that sea water temperatures are not adequate to describe the thermal regime of the mussel bed.

The rates of heating or cooling at the beginning of exposure were also calculated and are presented in Figure 10. From this chart it is evident that the heating rates during exposure to sunlight

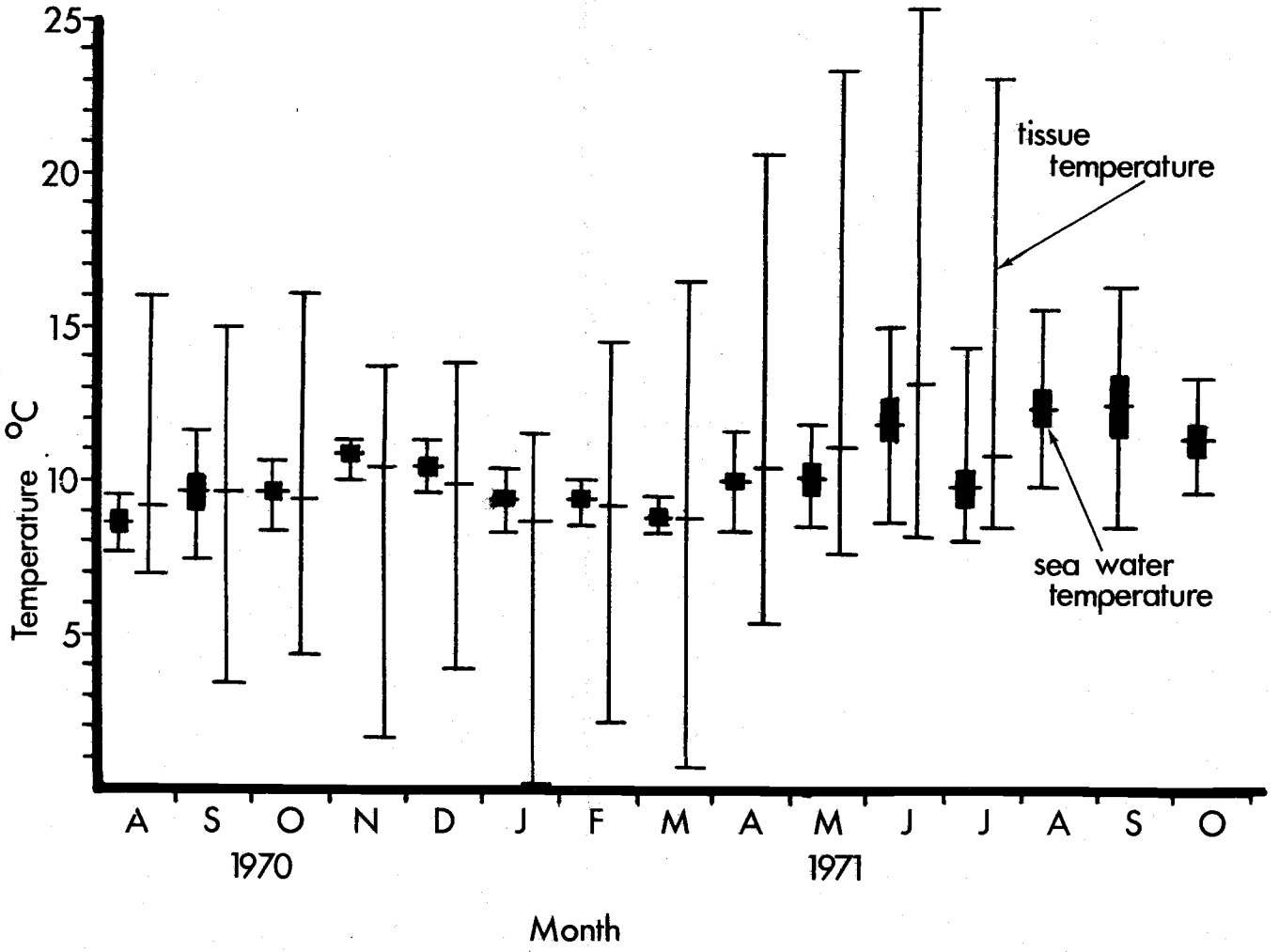


Figure 9. Average daily calculated tissue temperatures for mussels at Yaquina Head at the level of +3 feet above mean low water. Means and ranges are presented. Means, 90% confidence intervals and ranges of sea water temperatures are given for comparison.

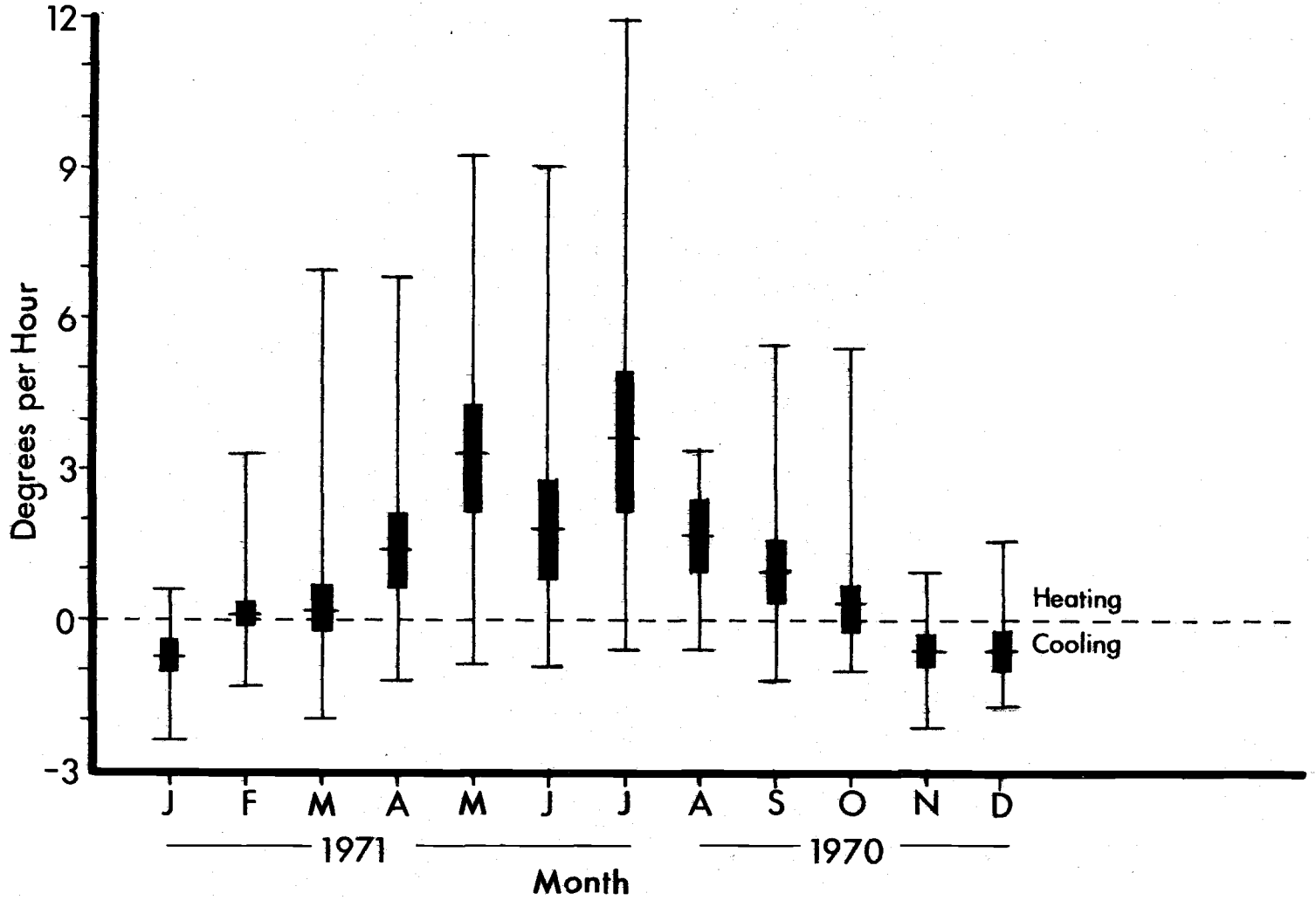


Figure 10. Average calculated heating and cooling rates of mussels at the +3 foot level throughout the year.

can be as much as 8° to 12°C per hour. Cooling rates observed during the winter and fall, when the mussel is exposed to night air colder than the sea water, are slower and can be estimated by

$$\text{Cooling Rate} = 0.25 \left(\frac{T_{\text{mussel}} - T_{\text{air}}}{\text{Time}} \right).$$

During re-immersion the change of temperature from air to sea water is nearly instantaneous.

Finally, since it is the time-temperature aspect that is often important rather than temperature itself, the daily degree hours defined as

$$\text{degree hours} = \text{time } (T - 5^{\circ}\text{C})$$

were calculated for exposure and submergence. Since no fecal ribbons were produced at 5°C, it was selected as the temperature of zero physiological activity. The tissue temperature of the mussel was calculated every half-hour for 1 year. An estimate of the contribution of exposure or submergence to the temperature regime of the animal was made. The heat units or degree hours per day throughout the year are shown in Figure 11b. The increased heating due to exposure during March to May increases the total heat the mussel experiences over that which would be obtained from sea water alone. This increased heat is mainly due to insolation rather than air temperature as can be seen by comparing the theoretically derived plot (Figure 8) with the observed data (Figure 11b).

Figure 11a. Estimated differences between temperatures during exposure and submergence.

Figure 11b. Calculated heat units (degree-hours) experienced each day by mussels at the level of +3 above mean low water.

-------- average contribution from time submerged in water

———— average contribution due to exposure

●———● combined degree-hours (calculated)

o------o maximum calculated degree-hours

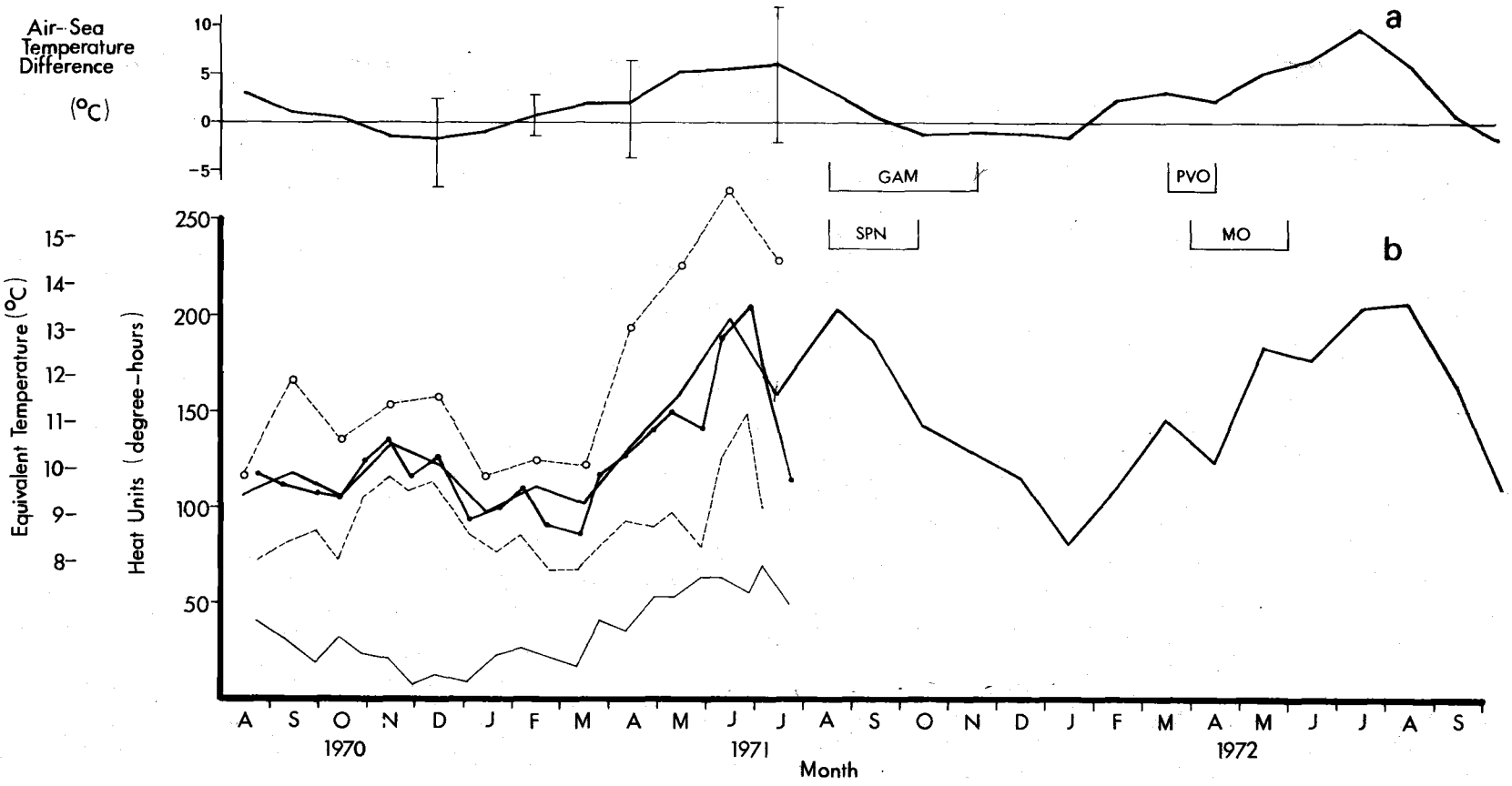
———— estimated degree-hours based on monthly sampling

GAM - observed period of oogonia production

M - observed period of intense mature oocyte production

PVO - observed period of intense previtellogenic oocyte increases

SPN - observed period of fairly complete spawning


Air-Sea Temperature Difference (°C)
10
5
0
-5
a
GAM
PVO
SPN
MO
b
Equivalent Temperature (°C)
15
14
13
12
11
10
9
8
Heat Units (degree-hours)
250
200
150
100
50
A S O N D J F M A M J J A S O N D J F M A M J J A S
1970
1971
1972
Month

Available food supply is the other major environmental feature. The monthly average of chemically oxidizable particulate carbon per liter of sea water is given in Figure 12a. It can be seen that the values are quite variable, but spring and winter values are the highest. The values remain between 1 and 3 mg of glucose equivalents per liter (1 mg glucose = 0.673 calories) but can change within a 3-day period. Some inferences can be made about the quality of the food by studying the quantity of diatoms, the species present, and the amount of non-oxidizable inorganic material present. A diatom peak of about 10^5 cells per liter is seen in the spring compared to summer and winter values of 10^3 cells per liter. Furthermore, the spring diatoms were all large species belonging to the genera Chaetocerous, Biddulphia, and Rhizosolenia, while the summer and winter groups are mainly composed of small pennate species. Table 3 shows a better correlation between the oxidizable particulate carbon and the weight of inorganic material (Figure 12b) than between oxidizable particulate carbon and phytoplankton numbers.

The concentration of inorganic particulates is a function of the degree of stirring of the bottom sediments. The implication is that oxidizable particulate carbon is mostly composed of detritus due to bottom stirring by storm waves.

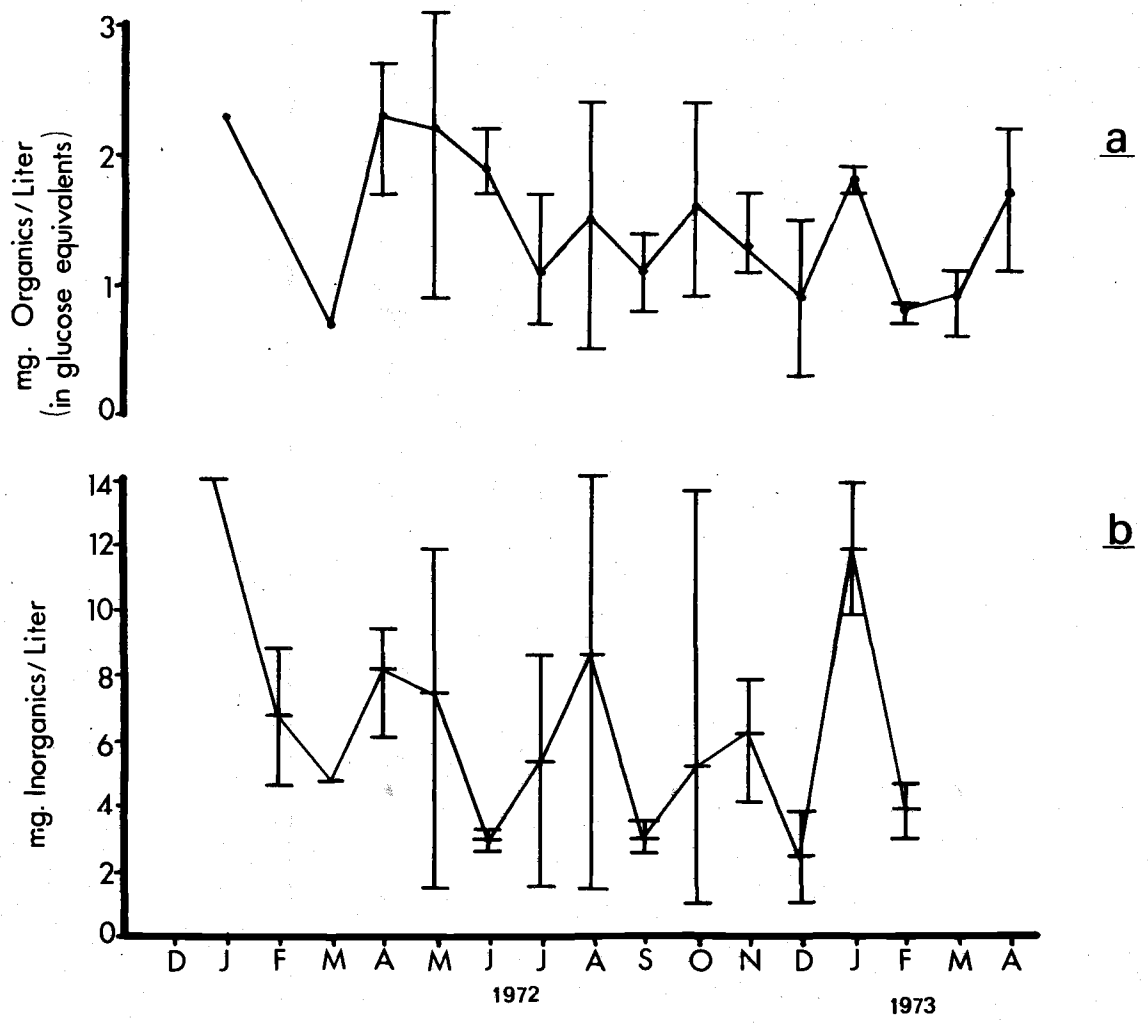


Figure 12a. Milligrams of oxidizable particulated carbon per liter as determined by the dichromate oxidation method. Mean and ranges given.

Figure 12b. Milligrams of inorganic particulates per liter of sea water.

Table 3. Results of correlation analysis of sea water characteristics at Yaquina Head, Oregon.

Correlation	df	r	Significance level (r different from 0)
Chemically oxidizable carbon vs log of phytoplantkon number	14	0.41	less than 90%
Chemically oxidizable carbon vs mg of inorganic particulates	28	0.75	greater than 99%
Chemically oxidizable carbon vs material oxidized by ashing	28	0.62	greater than 90%

Anatomical Observations

The gross anatomy of the reproductive and nervous systems is shown in Figures 13 and 14, and is essentially similar to that described by White (1937) for M. edulis. The muscular genital canals are found at the inner base of the gills and open into the mantle cavity at a genital papilla near the mesosoma. A small nerve connects the visceral ganglia and the genital canal. At the base of the foot the main genital canal turns into the mantle and immediately branches. The first follicles are attached to the canal at the third branching. They average 250 μm diameter by 750 μm in length. Generally the longer axis is perpendicular to the surface of the mantle. It is clearly seen that the canals and follicles are bathed by a fluid. Areas in which neither follicles nor connective tissue are present appear as channels throughout the mantle. Upon dissection of the gonadal regions, one may observe clumps of bright red-orange material. These clumps are islands of connective or storage tissue, and the color is a result of small, 9 μm diameter cells.

Gonad Index

Changes in the amount of gonad must be normalized to account for different sized animals. The gonad index used is defined as the dry weight of the gonadal tissue divided by the dry weight of the

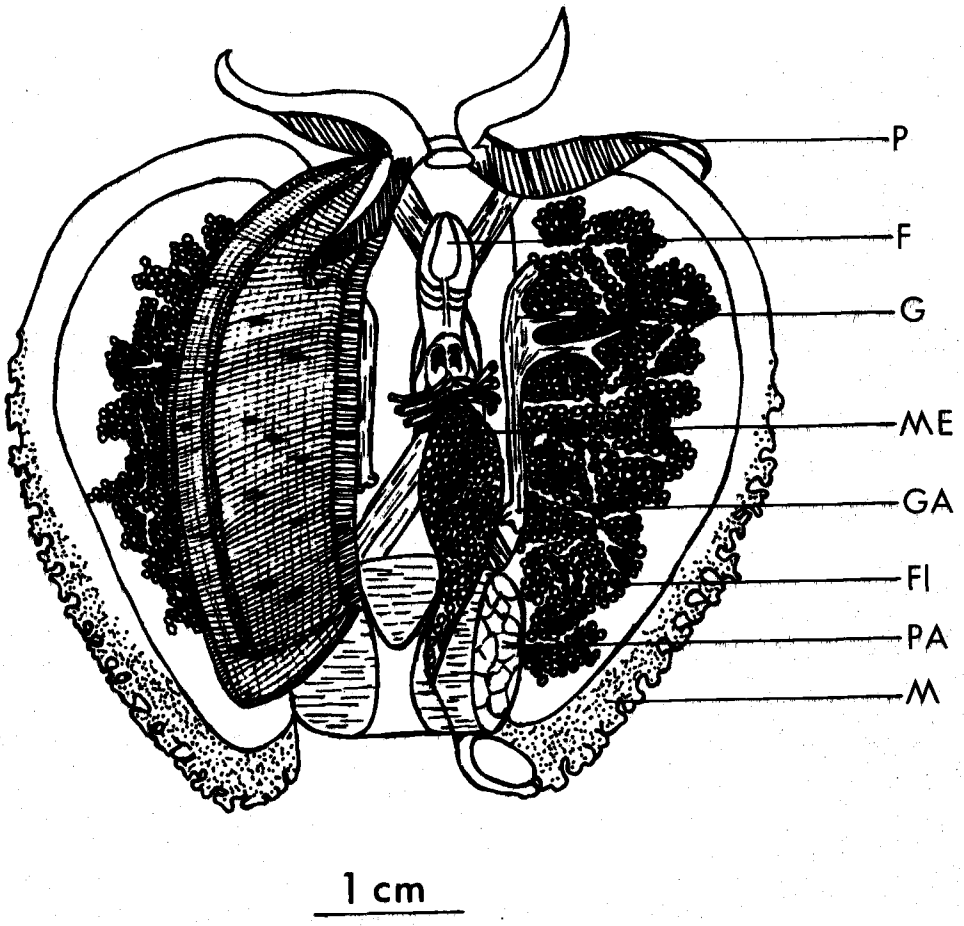


Figure 13. Ventral view of the major organs of Mytilus californianus.

G - gonoduct
GA - genital aperture
F - foot
Fl - follicles of gonad
M - mantle
ME - mesosoma containing gonad
P - palp
PA - posterior adductor muscle

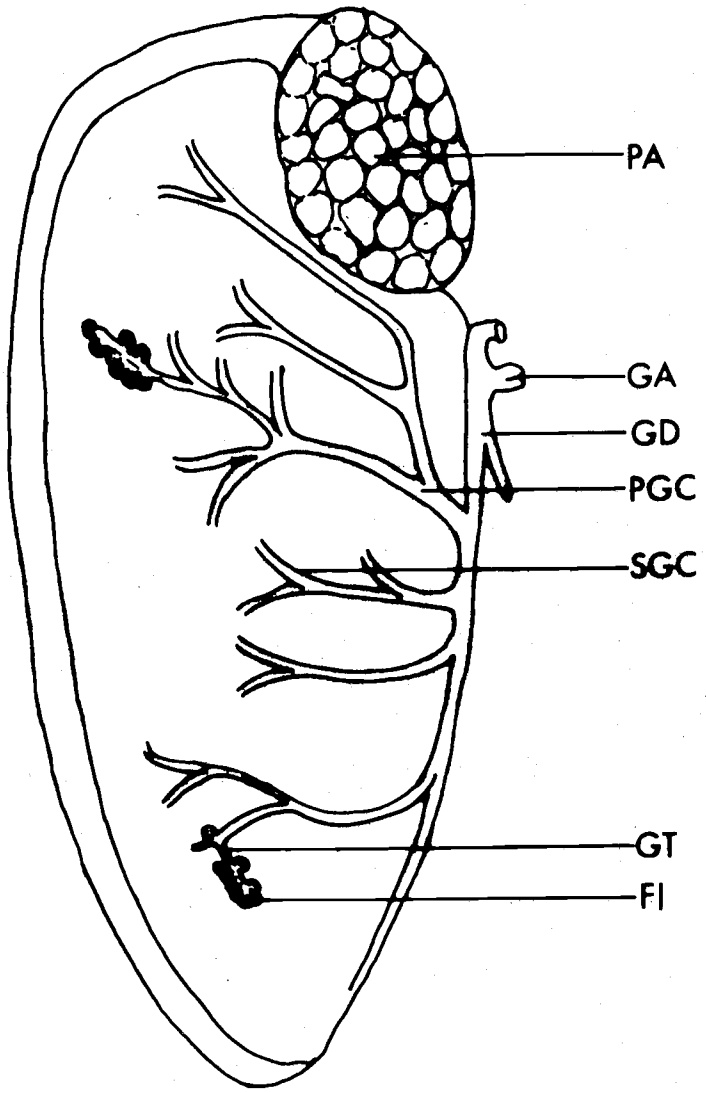


Figure 14. Lateral view of the reproductive system in the mantle of Mytilus californianus.

Fl - follicles containing oocytes
GA - genital aperture
GD - gonoduct
GT - gonadal tubule
PA - posterior adductor muscle
PGC - primary gonadal canal
SGC - secondary gonadal canal

somatic tissues. Such an index assumes that somatic tissue is constant. To check this assumption the average dry weight of the tissue in a 70 mm mussel was calculated for each sample. The formula, Log (weight) = K Log (length) + C, presented by Fox and Coe (1943) was used. There were 20% differences throughout the year but unfortunately the variation was too great to show significant differences. The differences were not large enough to change any of the results using the gonad index (Table 4).

The characteristics of monthly and bimonthly gonad indices are shown in Figure 15. No seasonal trend was repeated during the 18 months this index was followed. For a mussel having 1 g of somatic tissue, the average value of the index was between 200 and 400 mg during 1972 and between 400 and 500 mg in early 1973. The maximum amounts of gonad produced were between 700 and 800 mg. After 75 days of starvation, the gonad index only decreased from 350 to 278 mg/g. In fact if the decrease in somatic tissue is considered, the values are nearly the same.

Since the "gonad" is really a compound tissue, the weight was further allocated according to the percentage of germinal and Leydig tissue seen in the histological sections. The germinal gonad index (derived in Appendix IV) was obtained for each specimen by using the formula

$$\text{G. G. I.} = \left[\frac{0.24\ (\%\ \text{germinal tissue})}{0.13 + 0.11\ (\%\ \text{germinal tissue})} \right] [\text{gonad index}]$$

Table 4. Average somatic weight of 70 mm specimens of Mytilus californianus.

Date	N	Weight	$\pm t_{90}$ (S. E.)
1972			
2 Jan.	6	850	116
4 Feb.	5	870	246
8 Apr.	5	820	231
16 Apr.	8	930	120
2 May	4	815	150
27 May	6	955	146
28 June	7	1,120	155
9 Aug.	10	910	161
23 Aug.	4	690	251
8 Oct.	5	1,000	161
3 Nov.	6	775	153
1973			
6 Feb.	5	690	327
17 Mar.	5	890	228
30 Mar.	6	775	113
11 Apr.	5	885	128
Starved 71 days (Sept. -Dec.)	4	690	137

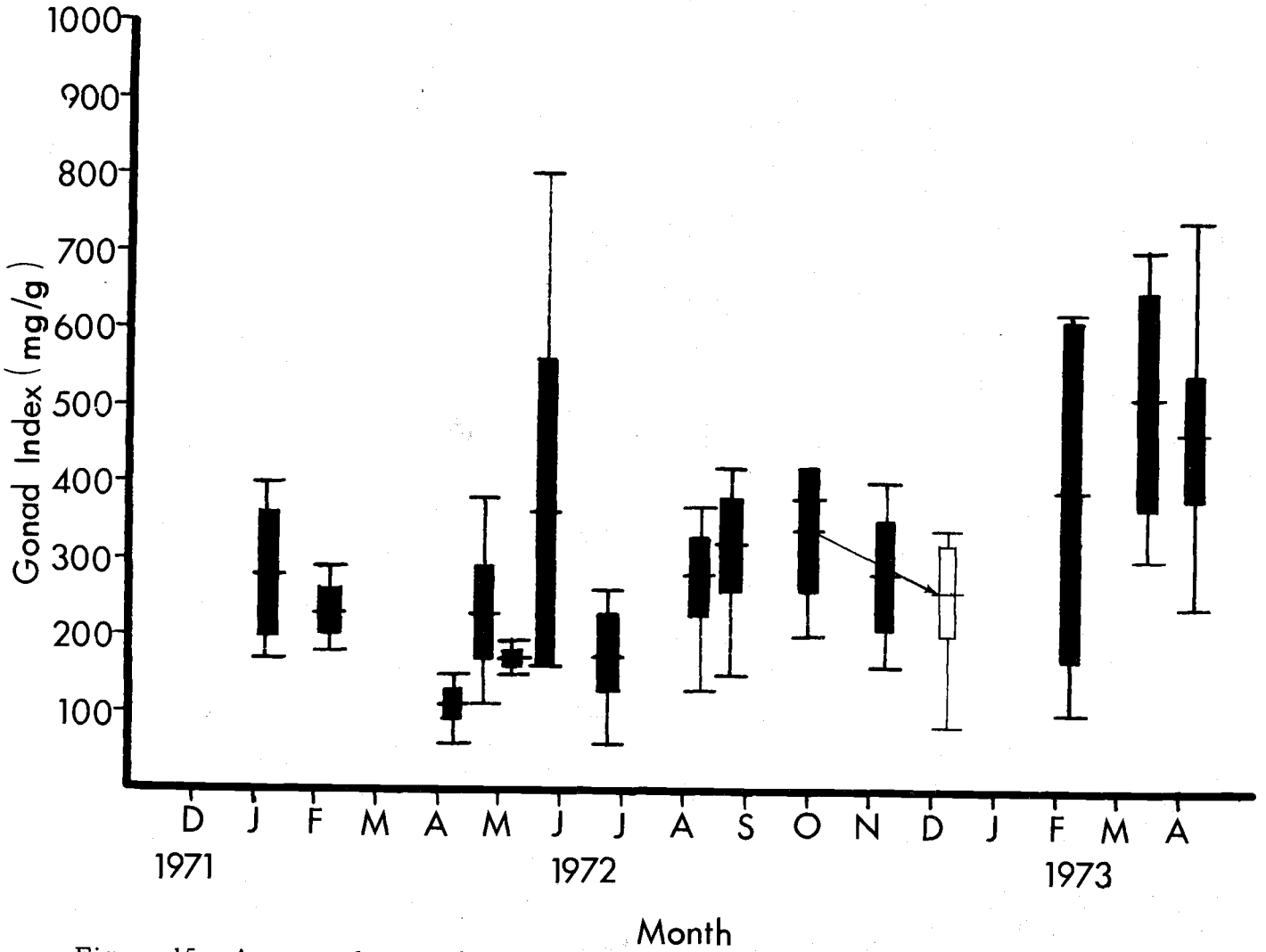


Figure 15. Average dry weight gonad index for bimonthly samples. Empty box indicates the results of a 75 day starvation experiment. Means, 90% confidence intervals, and ranges shown.

Over the 2-year period, Leydig tissue (Figure 16a) was found in small amounts, whereas germinal tissue (Figure 16b) tended to follow the total gonad index.

During the entire collecting period, 2 out of 440 females (0.5%) sampled were hermaphrodites.

Histological Appearance

The histological progress of oogenesis in M. californianus closely resembles that of the other Mytilids. The sequence of reproductive stages and their duration are quite different, however. The mesosoma and inner lobes of the mantle are composed of storage tissue, muscle, and gonadal tubules. The tissues are surrounded by a low columnar epithelium on the outer surfaces of the mantle and a ciliated epithelium on the inner surfaces. The haemocoelic fluid of the open circulatory system bathes the entire system.

The storage stage of the reproductive sequence is shown in Figure 17. The sexes cannot be distinguished at this stage. The mantle is composed of Leydig cells 45 to 50 μm in diameter and eosinophilic granular cells 6 to 12 μm scattered throughout the tissue. Haemocoelic spaces are occasionally seen. With standard paraffin embedding techniques, the Leydig cells appear to contain strands of material. The periodic acid-Schiff test for glycogen was positive for these cells, and the staining for lipid with Sudan black was negative.

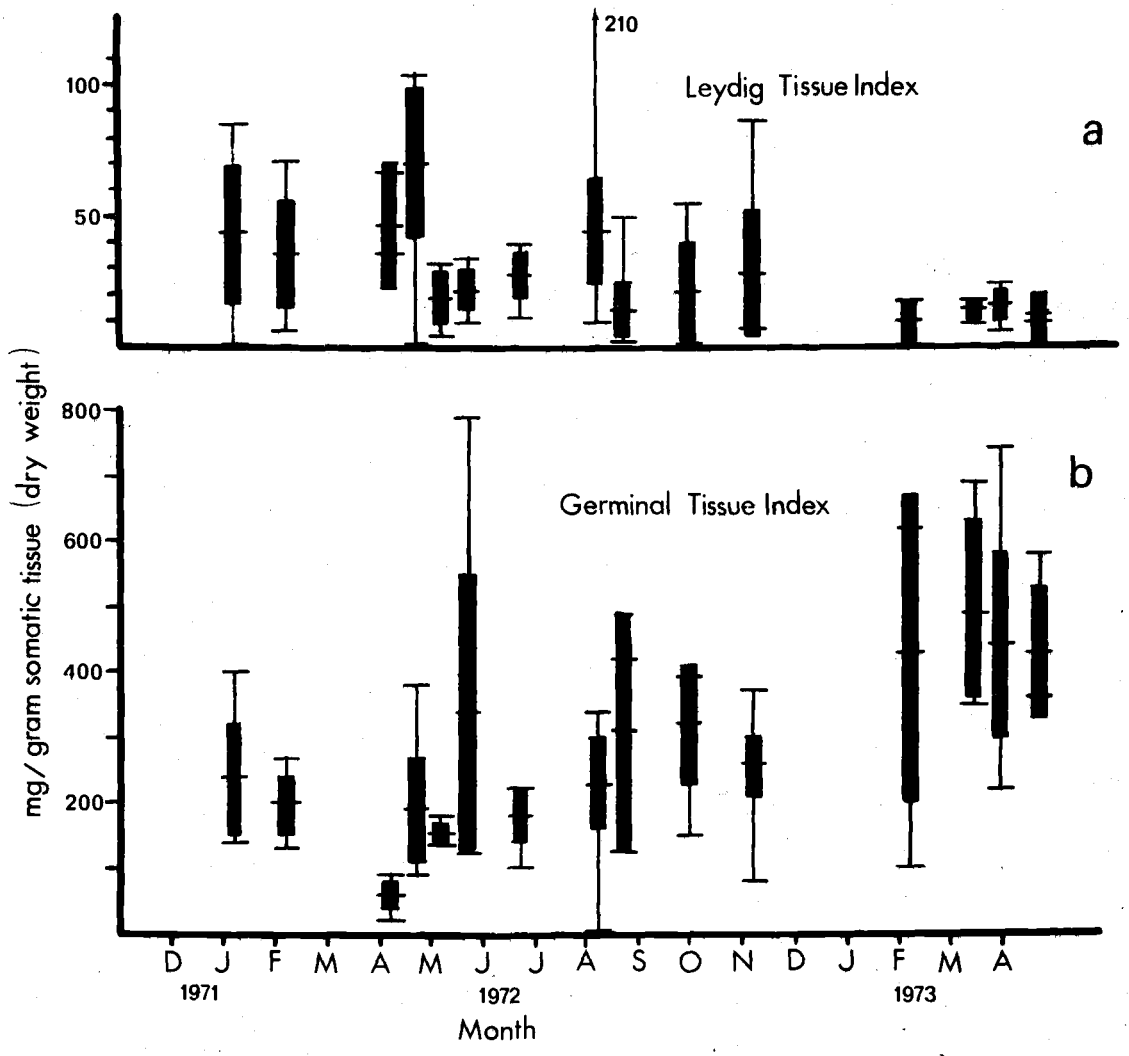


Figure 16a. Average dry weight Leydig tissue index for bimonthly samples. Means, 90% confidence intervals, and ranges shown.

Figure 16b. Average germinal gonad index for bimonthly samples. Means, 90% confidence intervals, and ranges presented.

Figure 17. Leydig tissue in the mantle during the storage stage. Granular cells can be seen throughout the tissue.

Figure 18. An early stage of the growth of a gonadal tubule into the Leydig tissue of the mantle. The arrow indicates an area of possible mitotic activity.

c - cilia in tubule wall

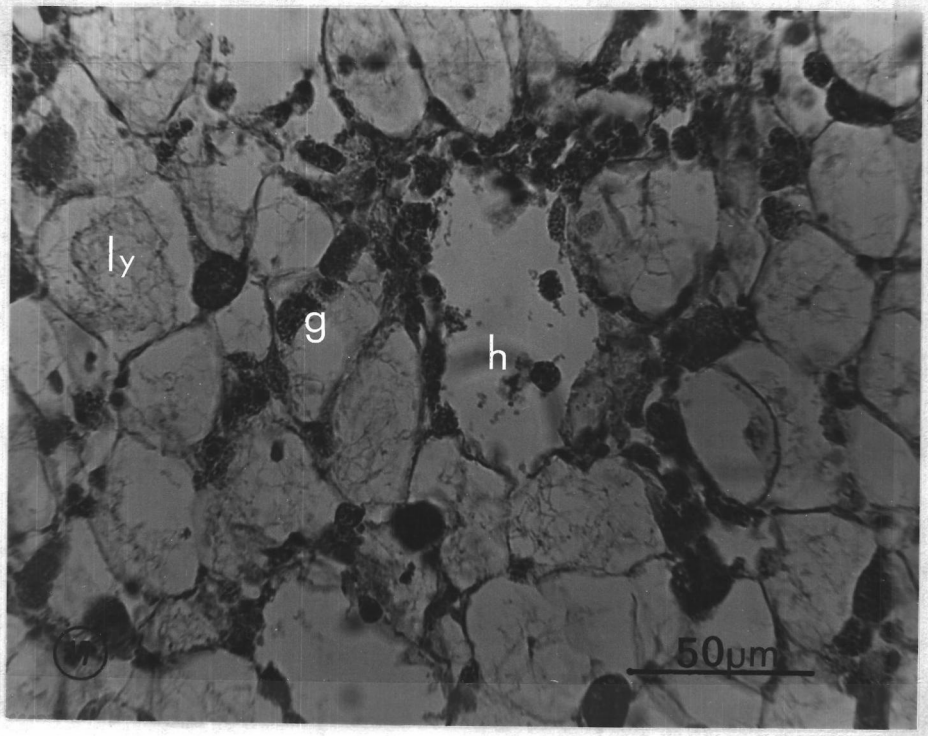
g - granular cell

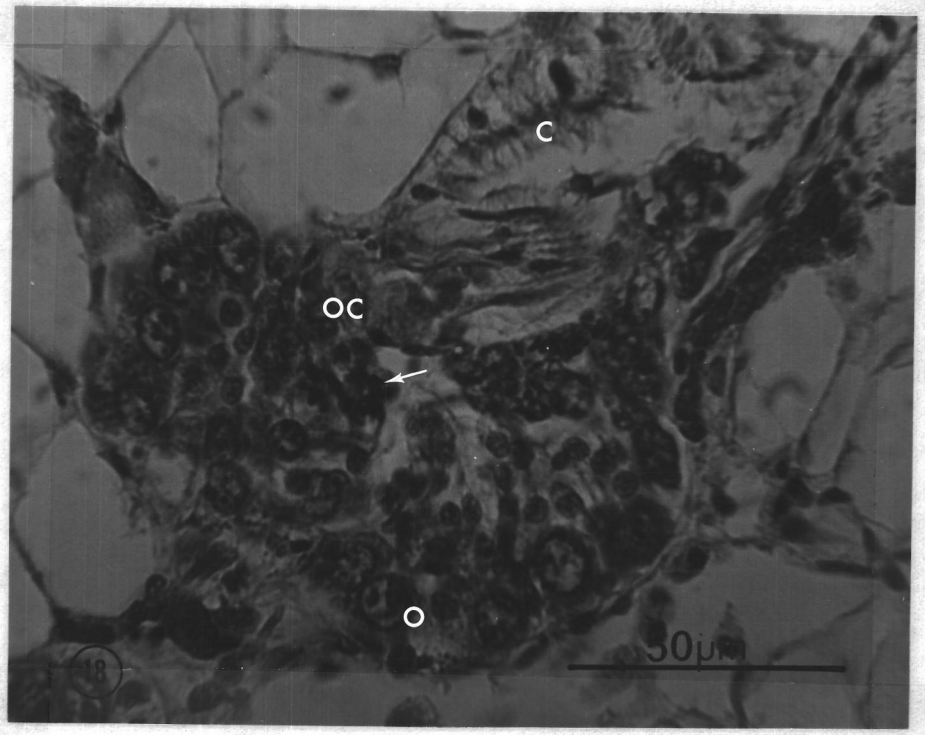
h - haemocoelic space

ly - Leydig cell

o - oocyte

oc - oogonial cluster


ly
g
h
50µm


c
oc
o
30µm

The granular cells at this stage are few and scattered. They are packed with eosinophilic granules and in fresh material have a bright orange color presumably due to carotenoids. No trace of the undeveloped gonadal tubules can be found during the storage stage. The gonadal canals are a permanent feature and can best be seen at this time.

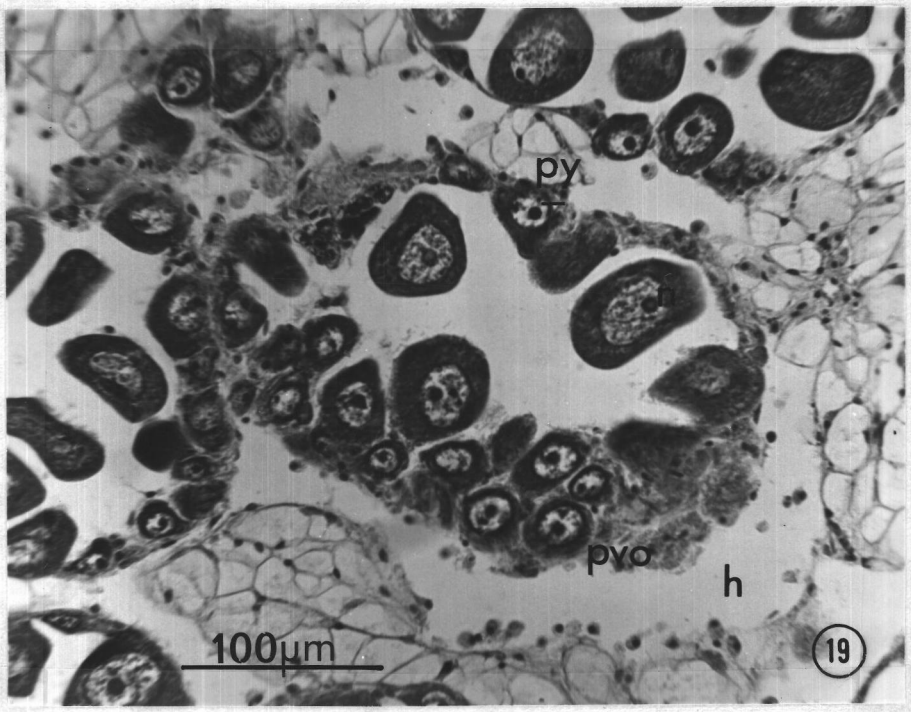
During the first reproductive stage young tubules proliferate into the storage tissue (Figure 18). The germinal tissue appears continuous in the tubules at this stage except for the ciliated epithelium on one side. The amount of cilia decreases as one moves away from the apex of the mantle. Tubules differ from follicles by the presence of cilia and muscle tissue. Follicles begin as 30 to 45 μm diameter outgrowths of oogonia. The oogonia can be distinguished from somatic cells by their clear, round nuclei, 4 μm in diameter, which may show mitotic figures or clumped chromatin, have very little cytoplasm, and occur in groups. The nuclei of the somatic tissue in the follicle wall are more darkly stained and tend to be flattened.

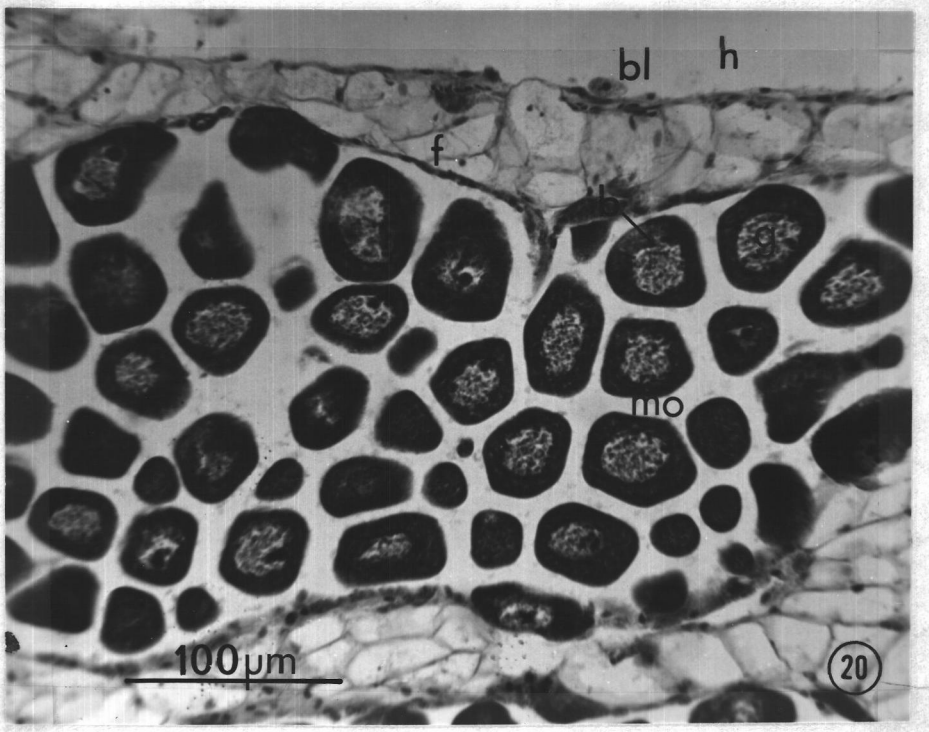
During subsequent development the gonad contains both the Leydig and germinal tissue. Previtellognic oocytes are first seen as 9 μm diameter cells with a 7.5 μm diameter germinal vesicle containing clumped chromatin (Figure 19). A nucleolus is present and often has a clear area in the center. The minimal cytoplasm is very basophilic. These previtellogenic cells are found only attached to the wall

Figure 19. Developing oocytes in a gonadal follicle.

Figure 20. A mature follicle packed with mature oocytes.

b - basophilic bodies in the cytoplasm
bl - blood leucocyte
f - follicle cell
g - germinal vesicle
h - haemocoelic space
mo - mature oocyte
n - nucleolus
pvo - previtellogenic oocyte
py - pyramidal previtellogenic oocyte


py
pvo
h
100µm
19


bl
h
f.
mo
100µm
20

and may take on a pyramidal appearance with the nucleus closest to the follicle wall. The follicles have grown to about 150 to 200 μm in diameter.

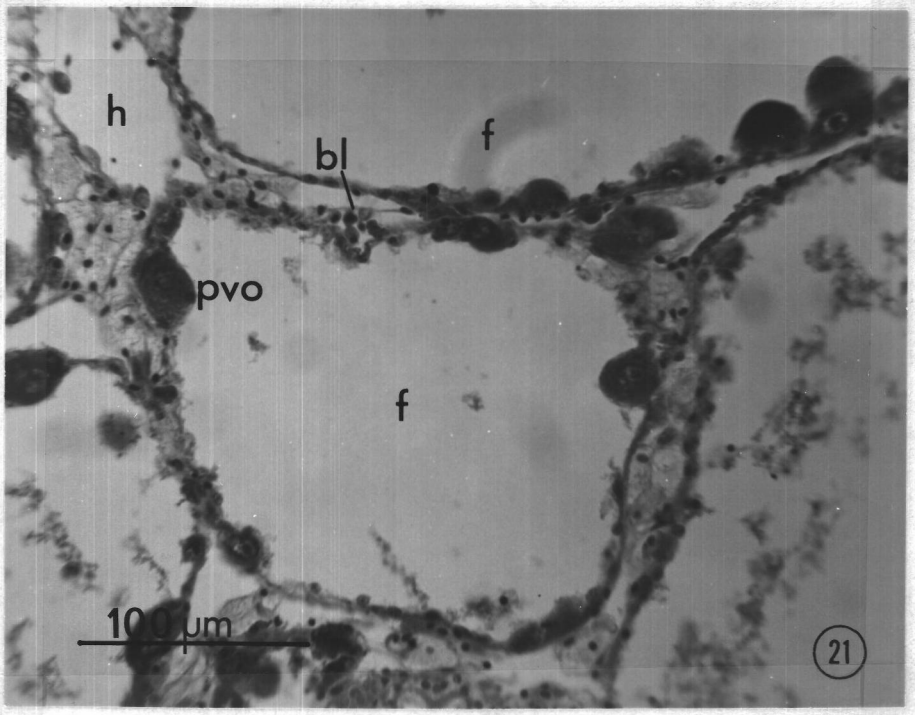
The mature ovary has 200 to 300 μm diameter follicles packed with oval oocytes which may reach 50 x 70 μm in size (Figure 20). The follicles have expanded so that they are separated from each other by only a thin sheet of connective tissue cells. The walls of the follicles at this point may break, essentially leaving the eggs on stalks of connective tissue. The mature oocytes within the follicles have a clear germinal vesicle with diffuse strands of chromatin and a nucleolus. They have undergone yolk formation or vitellogenesis, and the cytoplasm has become acidophilic. Stalked occytes are common, but some of the eggs are now free in the lumen. Considerable shrinkage has occurred due to histological technique as is noted by comparing paraffin embedded samples with fresh tissue.

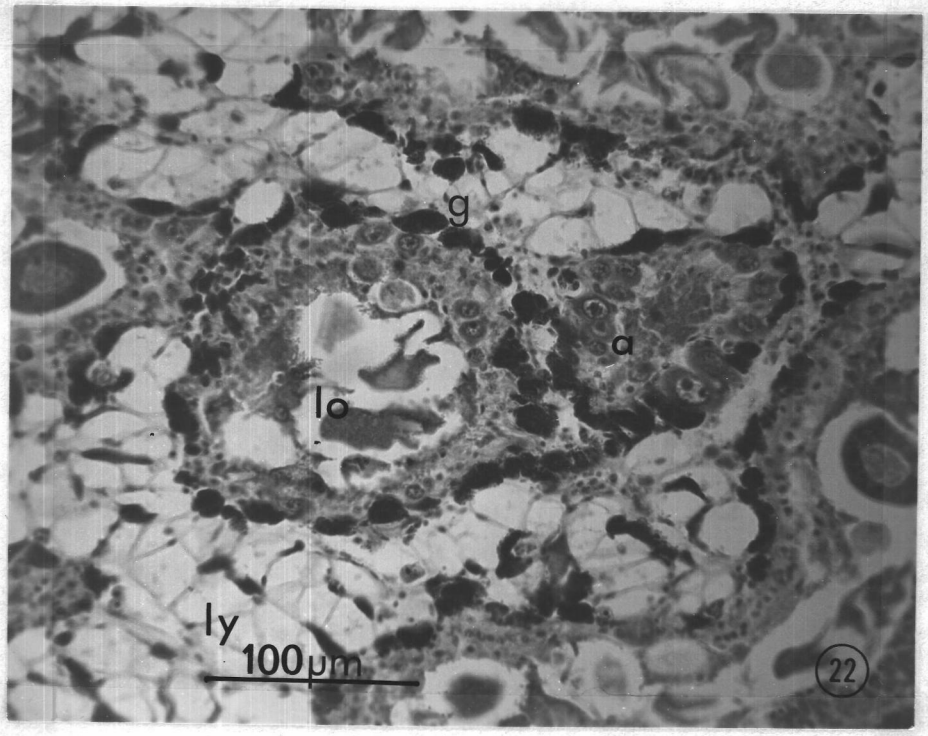
A mature mussel may spawn completely, but this phenomena was observed in only 3 of the 400 specimens examined (Figure 21). If complete spawning has occurred, the follicles collapse and a storage stage begins again. Groups of oogonia can be seen in partially spawned and completely spawned animals before the collapse occurs. During the years 1971 to 1973, only partial spawning was noted. Mussels observed to spawn in the laboratory released about four million eggs, but their histological samples gave the appearance of a

Figure 21. Empty follicles following total spawning.

Figure 22. Mature oocytes undergoing resorption. Note the granular cells surrounding the follicles.

a - amorphous material
b - basophilic bodies in cytoplasm
bl - blood leucocytes
f - follicle lumen
g - granular cell
h - haemocoelic space
lo - oocytes undergoing lysis
ly - Leydig cell
pvo - previtellogenic oocyte


h
bl
f
pvo
f
100 µm
21


g
a
lo
ly
100 µm
22

partial spawn. In such cases previtellogenic oocytes still remain on the walls in reduced numbers. Such oocytes may later develop, although there is little storage tissue left. The more general situation is resorption which begins as a degeneration of the unused oocytes which become extremely vacuolar in appearance. The follicle becomes filled with an amorphous material and may become surrounded with the eosinophilic granular cells (Figure 22). The result of this process is intermediate follicles which have an appearance similar to the developing follicles in Figure 19.

Quantitative Histology

In addition to staging the reproductive state of the gonad in the conventional manner, the different oocyte growth stages were dealt with independently of each other. The histological sample was always taken from the center of the mantle. The apex region occasionally has empty follicles, and the distal portion of the mantle may have a high percentage of Leydig tissue.

Figure 23 presents the average per cent of the follicles in a mantle containing at least one cluster of oogonia. There were some oogonia present in all the specimens throughout the year. There was a significant increase in the fall of 1971 which was correlated with heavy spawning at this time. The percentage remained high from October 1971 through May 1972, but was considerably lower from

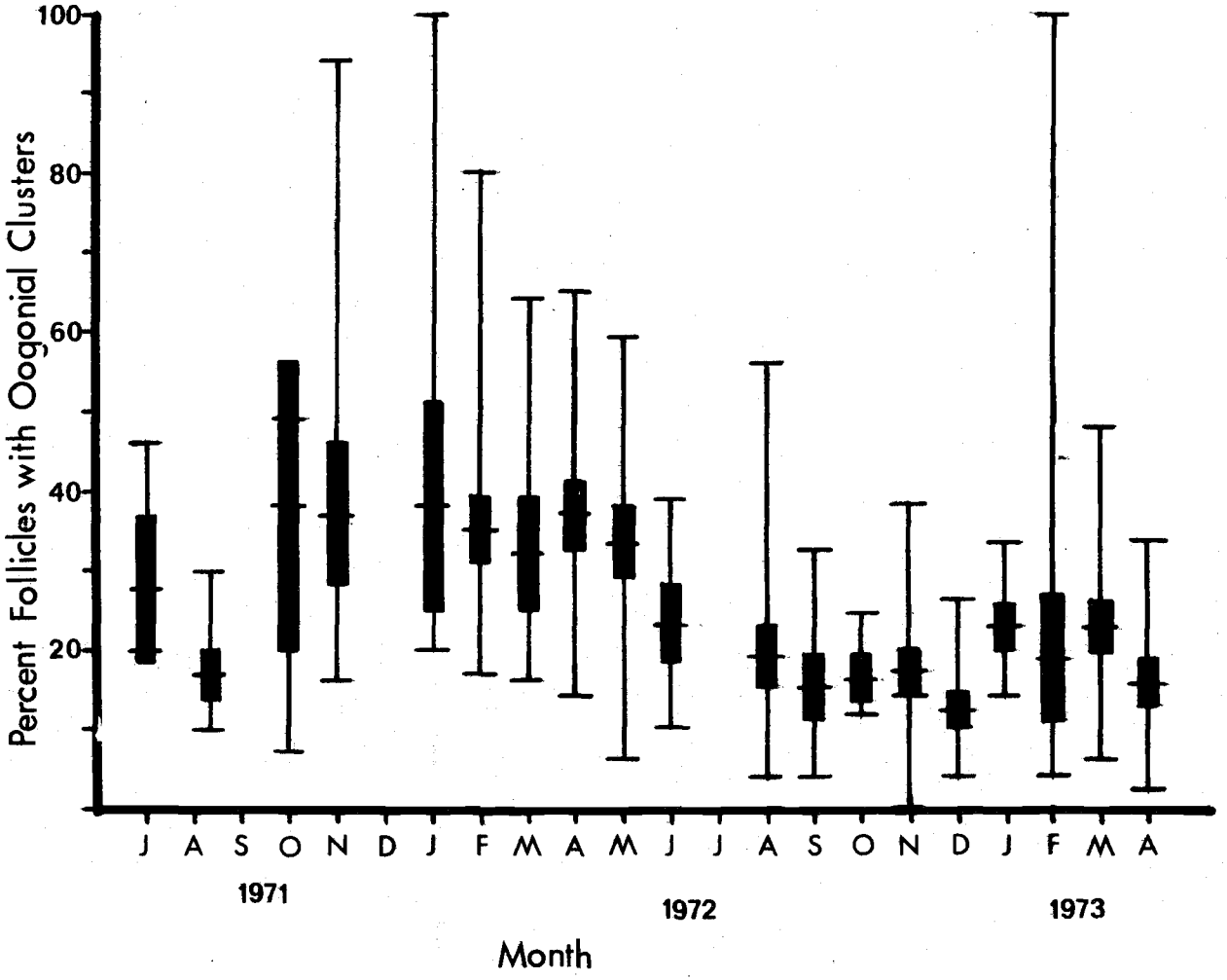


Figure 23. Average percentage of follicles containing at least one cluster of oogonia or young oocytes. Means, 90% confidence intervals and ranges are shown.

June 1972 to April 1973. This decrease corresponded to the increase in mature oocytes. Possibly an asynchronous increase occurred during February, 1973.

The numbers of oocytes in the follicles were counted and their diameters measured. The data were then separated into previtellogenic oocytes with basophilic cytoplasm and diameters of 12 to 29 μm and mature oocytes with eosinophilic cytoplasm and diameters greater than 39 μm. Since previtellogenic oocytes are confined to the walls of the follicle and their numbers might be limited by the available follicle wall area, a correlation was considered between the number of cells and the circumference of the follicle section. No significant correlation was found for follicles ranging from 90 to 400 μm in diameter (N = 35 pairs, r^2 = -0.083). Furthermore, the coefficient of variance was greater for the number of previtellogenic oocytes per unit of circumference than for the number of oocytes per follicle section. The density of previtellogenic oocytes is presented in Figure 24. Over the 2-year period of observations only one significant increase in the number of previtellogenic oocytes per follicle was seen. The entire increase occurred in about 3 weeks, and the estimated sample size required for a 90% confidence interval within 10% of the mean was found to be 73 specimens (Table 7). An asynchronous increase appeared during the same period in 1973.

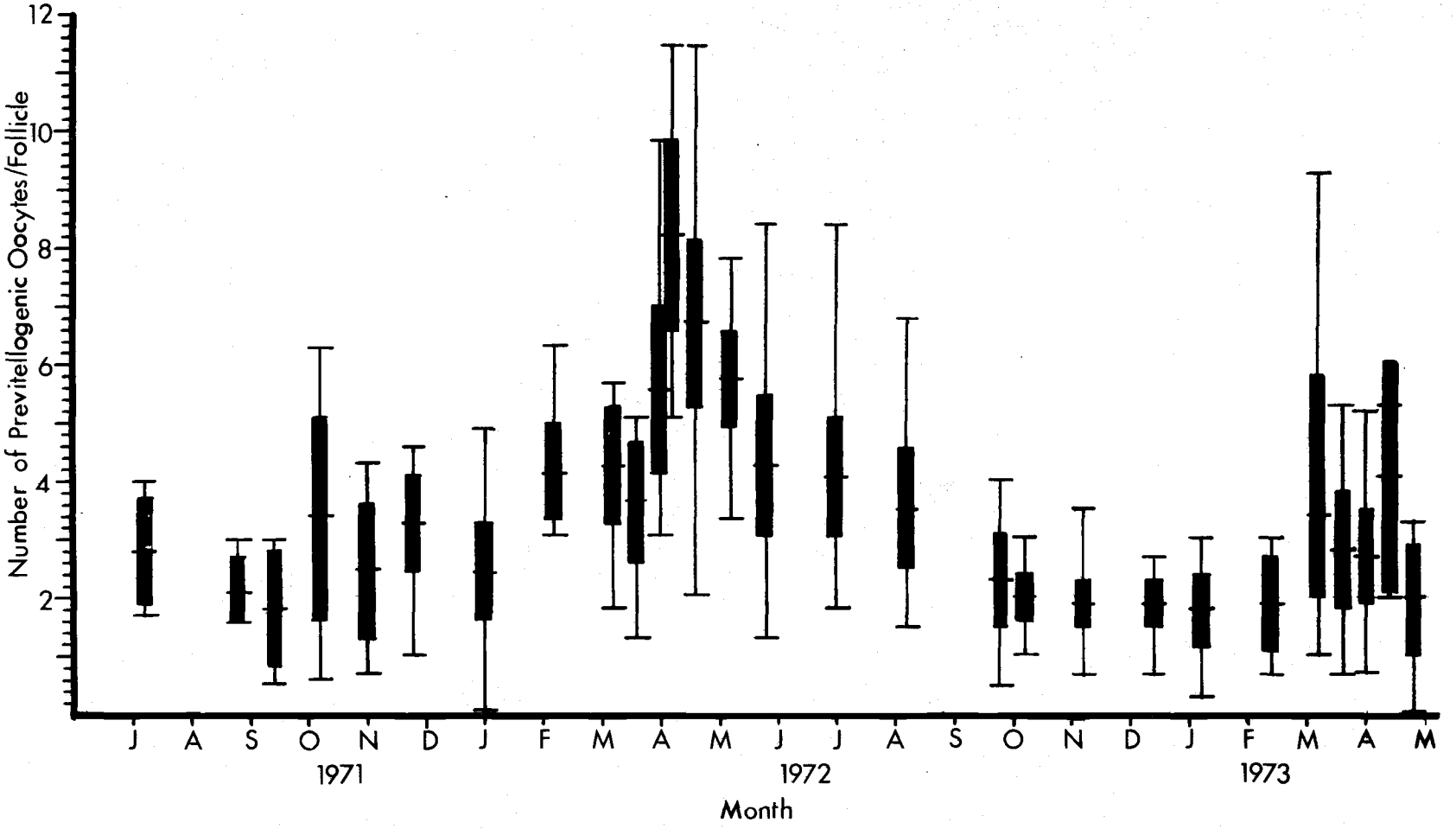


Figure 24. Average number of previtellogenic oocytes per follicle section. Means, 90% confidence intervals, and ranges are presented.

The mature oocytes are not confined to the walls of the follicles and eggs may be free in the lumen. A correlation between the number of mature cells per follicle and cross sectional area of the follicle was found to have a significant positive correlation for follicles ranging from 135 to 360 μm in diameter (N = 11 pairs, $r^2 = 0.87$). The density of mature oocytes is given in Figure 25. A decrease is seen in October, 1971 which is associated with a major spawning. A second decrease in the number of eggs is seen early in the spring of 1972. Examination of the histological sections reveals that the latter decrease is associated with lysis and resorption of eggs rather than spawning.

The major spawning in fall was not repeated in 1972, and the density of mature oocytes remained high throughout the year. The production of mature oocytes was fairly rapid, taking about 8 weeks, both after the spawning in October, 1971 and again after lysis and previtellogenic development in April, 1972. Variation within a sample requires that 51 specimens be collected in order to produce a 90% confidence interval within 10% of the mean (Table 4).

Cell densities are misleading because the total number of oocytes can increase without a change in cell density. The number of previtellogenic oocytes per animal was determined by:

$$N_{pvo} = (D_{pvo})\ (F)\ (GGI)\ (1.06 \times 10^3),$$

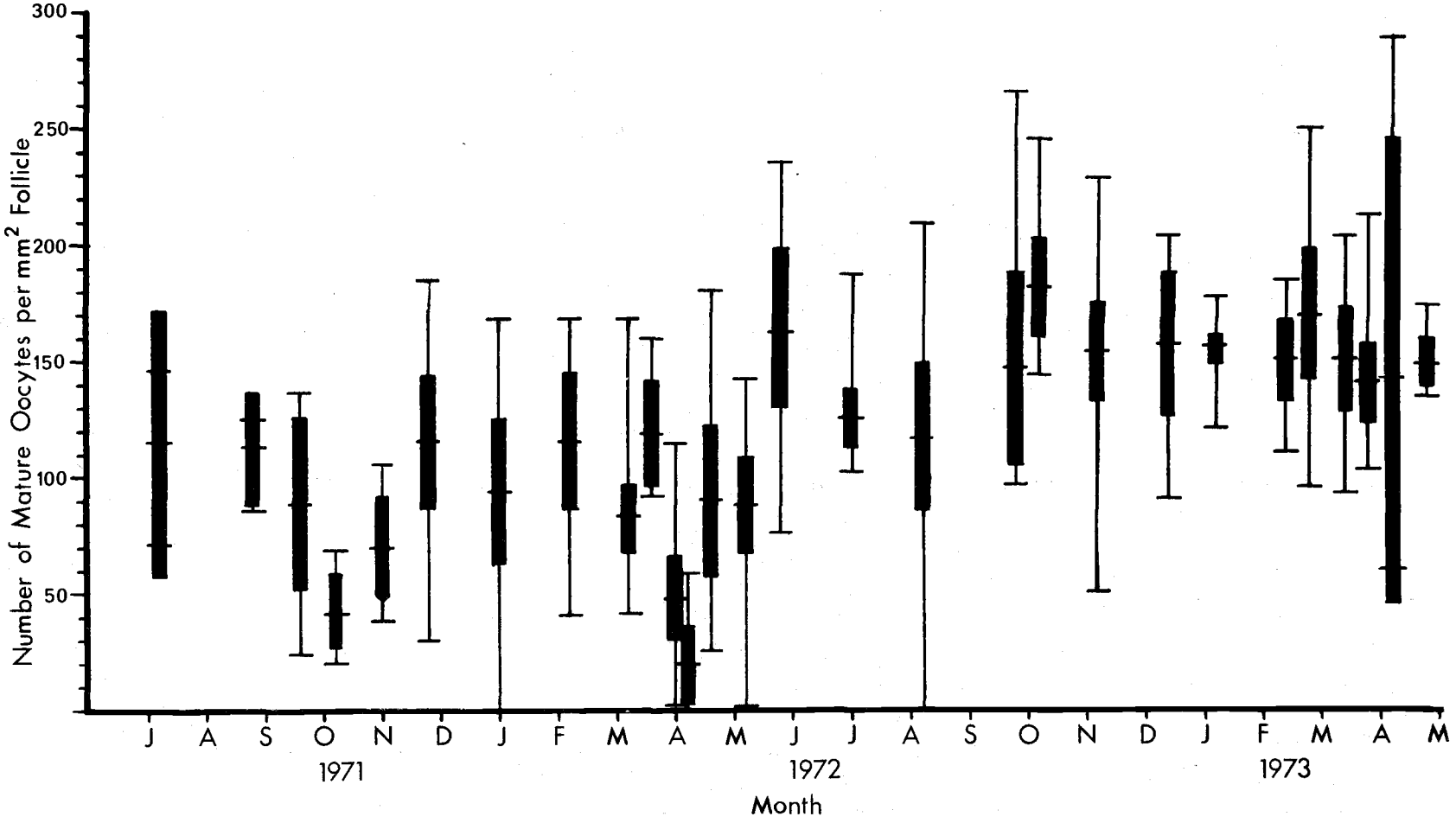


Figure 25. Average number of mature oocytes per square millimeter of follicle cross section. Means, 90% confidence intervals, and ranges are shown.

and the number of mature oocytes per animal by:

$$N_{mo} = (D_{mo}) (GGI (6.36 \times 10^{2}) ,$$

where D_{pvo} and D_{mo} are the cell densities, F the number of follicle sections per square millimeter of germinal tissue, and GGI the germinal gonad index. The formulas are derived in Appendix V. The total numbers of oocytes per animal are presented in Figure 26, and from these data rates of changes in the categories of oocytes can be estimated.

Rates of change in the numbers of mature oocytes were calculated by taking the difference in the total numbers for two successive samples and dividing by the number of days elapsed. Rates of change in the number of previtellogenic oocytes were calculated in the same manner. However, the amount of previtellogenic oocytes converted to the mature oocyte category must also be included. These rates are given in Table 5. The increase in previtellogenic oocytes must also include those lost by becoming mature oocytes. During the spring of 1972, the rate of previtellogenic oocyte production increased to almost 14×10^{4} oocytes per animal per day. Generally, throughout the rest of the year there was a continuous production of about 2×10^{4} previtellogenic oocytes per day. The appearance of negative rates in May is possibly due to the rapid conversion of cells to mature oocytes relative to the rate of conversion of oogonia to previtellogenic oocytes.

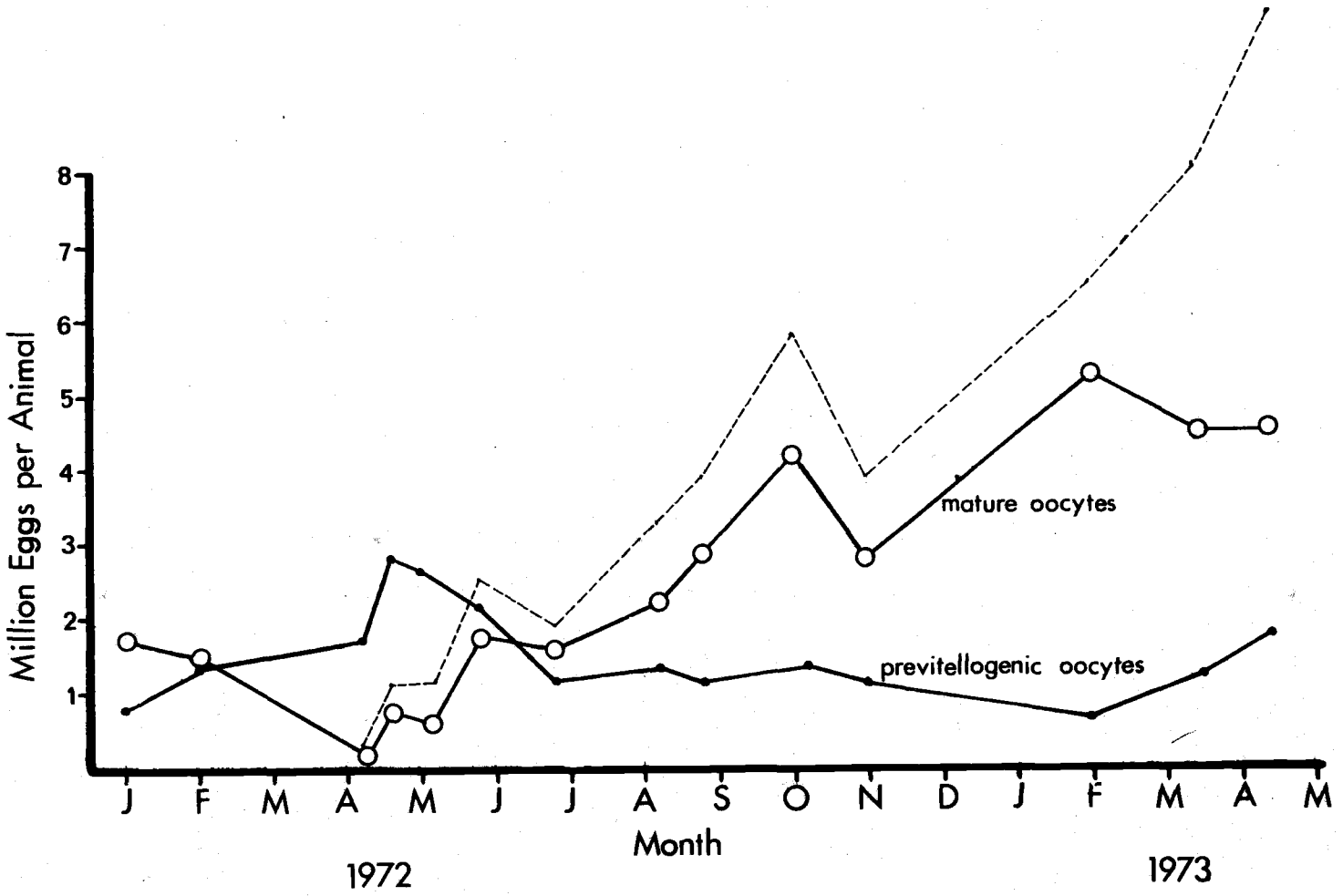


Figure 26. Total numbers of oocytes per standard one gram animal.
●——● average amount of previtellogenic oocytes
o——o average amount of mature oocytes
------ maximum observed amount of mature oocytes

Table 5. Calculated rates of oocyte production (10^4 oocytes per day).

Date	Rate of previtellogenic production	Rate of mature oocyte production	Rate of mature oocyte loss by spawning
1972			
Jan. 2-Feb. 4	1.63	-1.00	
Feb. 4-Apr. 8	1.37	-4.10	
Apr. 8-Apr. 16	13.50	+8.60	
Apr. 16-May 2	-1.20	-1.00	
May 2-May 27	2.16	4.50	2.52
May 27-June 29	-2.81	-0.86	
June 29-Aug. 9	2.09	+1.66	
Aug. 23-Oct. 8	2.65	1.41	0.75
Oct. 8-Nov. 3	-1.08	-1.40	2.48
Nov. 3-Feb. 6	1.98	2.49	
1973			
Feb. 6-Mar. 17	1.70	-1.90	
Mar. 17-Apr. 11	1.96	0.04	

The seasonal trend in the numbers of mature oocytes can also be seen from Figure 26. The maximum amount observed was 10.5 million eggs in April, 1973.

A major spawning would be indicated by a sudden and complete loss of mature oocytes. Occurrence of a partial spawning is implied by a decrease in the average total number of eggs, and can be verified by checking the histological section for large follicles partially filled with mature oocytes. No major spawning was detected during 1972 and early 1973. The sum of the partial spawns is equivalent to 41.5% of the total amount produced. The total number of mature oocytes produced accounts for 84% of the previtellogenic oocytes. This difference results from the elimination of the data on intermediate cells, 28 to 39 μm in diameter, from the mature oocyte category in order to avoid confusing them with previtellogenic oocytes. Table 6 compares the production of mature oocytes to temperature and organic particulates. Higher rates are concurrent with higher food ingestion but not with changes in temperature. The percentage of females spawning in response to 0.2% potassium chloride treatment was found to be 47% in September, 1972 (N = 19) and 0% to 6% from November, 1972 through April, 1973 (N = 20 to 50).

Biochemical Analysis

Seasonal biochemical analysis was performed on the gonad with

Table 6. Rates of mature oocyte production and environmental features.

Date	Average sea water temperature (°C)	Estimated tissue temperature (°C)	Average organic particulates (mg/l)	Food ingestion (mg/day)	Rate of mature oocyte production (10^4 oocytes/day)
1972					
8 Apr. - 16 Apr.	9.6	10.5	2.3	100	8.6
2 May - 27 May	11.0	12.5	2.2	167	4.5
9 Aug. - 8 Oct.	11.7	13.5	1.4	50	1.4
1973					
3 Nov. - 6 Feb.	9.9	9.0	0.95	45	2.5
17 Mar. - 11 Apr.	12.5	-	1.4	-	0.04

particular attention paid to reproductive state. The statistical characteristics of the sample are given in Table 7. The percentage recoveries of protein, lipid, and glycogen standards were 91%, 94%, and 96% respectively, and the coefficients of variation for analysis of the standards were 5%, 4%, and 11% respectively.

The percentage dry weight is related to the amount of water in the tissue. Average values of dry weight ranged from 18.8% to 26.1% of the wet weight. Leydig tissue is about 13% dry weight and gonadal tissue is about 24%. The ash weight averages approximately 6% of the dry weight.

The percentage composition of the reproductive tissue during four stages of oogenesis is given in Table 8. The chemical differences between successive stages reveal the predominant biochemical activities associated with each transition. There is a doubling of protein and RNA, and lipid increases 4.5 times during the processes leading from storage to previtellogenesis. Total carbohydrate, mostly glycogen, is reduced by half. Yolk formation leading to mature oocytes results in a 40% increase in protein, doubling of lipid, 25% increase in RNA, and loss of the remaining carbohydrate. The resorptive phase appears to involve no loss of protein, about 80% loss of lipid, 20% loss of RNA, and a five-fold increase in carbohydrate.

The parameter of percentage composition has limited use. The total amount of the components in a standard animal is a more reliable

Table 7. Observed sample variations and estimated required sample sizes for obtaining mean with 10% confidence intervals.

Character	Units	Greatest observed s.d. individuals	Greatest observed s.d. population	Representative value	Coefficient of variation (%)	Required sample size
Follicle with oogonia	%	-	9	38	24	17
PVO[1] density	#/follicle	7	3	6	50	73
MO[2] density	#/mm^2	75	51	118	42	51
Total PVO	10^6 PVO/animal	-	0.2	3	7	1
Total MO	10^6 MO/animal	-	0.5	5	10	3
Gonad index	mg/g	-	120	400	30	26
Germinal gonad index	mg/g	-	100	350	29	25
% Protein	mg/mg	3.8	-	47.0	8.1	2
% Lipid	mg/mg	2.95	-	14.5	20.3	12
% Glycogen	mg/mg	0.017	-	0.112	15	7
% RNA	mg/mg	1.3	-	5.7	22.8	15
Total protein	mg/g	-	71	140	50.7	75
Total lipid	mg/g	-	31	43.5	71	147
Total glycogen	mg/g	-	8.2	17.2	48	66
Total RNA	mg/g	-	14.7	18.6	79	181

[1]PVO = previtellogenic oocyte
[2]MO = mature oocyte

Table 8. Percentage composition of reproductive tissues (percentage dry weight).

Stage	Protein	Lipid	Carbohydrate	RNA
Storage	22	2	57	2
Developing	42	11	27	4
Mature	59	23	2	5
Resorption	59	15	12	4
	Differences between stages (%)			
Storage-development (previtellogenesis)	+20	+ 9	-30	+2
Development-mature (vitellogenesis)	+17	+12	-25	+1
Mature-resorption	0	-18	+10	-1

measure of the biochemical state. Tables 9-12 present the total amounts of the biochemical constituents of the various reproductive stages. It is clear that glycogen is associated with the Leydig tissue of the storage stage. Lipid and RNA in mature gonads appear extremely variable. The average amount of RNA in mature mussels is found to be about four times that in animals with developing gonads. A comparison of the required sample size in Tables 7 and 12 shows that separation into reproductive stages reduces the variation. Tissue in the resorptive stage retains at least 60% of the protein and can have large amounts of glycogen present. Mature mussels which had been starved for 71 days showed losses in lipid, but about 80% of the protein was retained. An interesting increase in glycogen was found in mussels classified as partially spawned during February 1973. In spite of large amounts of glycogen, the storage stage appears to contain only half the energy of the mature gonad. Starvation results in loss of caloric value from the gonad but not protein.

The seasonal variation in biochemical levels has meaning only when the reproductive stage distribution of the sample is known. Figure 27 shows the monthly changes in protein. The rate of protein accumulation during previtellogenic development was 1.7 mg per day and in late spring during yolk formation was 3.6 mg protein per day per standard 1 g animal. The rest of the year, the mature and partially spawned gonads had between 150 and 200 mg per animal. During this

Table 9. Gonadal protein levels of the reproductive stages (mg/g).

Stage	N	Mean	90% Confidence interval	Range	Coefficient of variation (%)	Required sample size
Storage	2	40	-	35-46	-	-
Developing	23	71	62-81	24-118	33	33
Mature						
1972	13	131	104-157	89-192	36	37
1973	11	194	160-227	110-271	28	23
Resorption	5	109	90-128	74-128	19	11
Starved	4	119	108-130	109-132	-	-

Table 10. Gonadal lipid levels of the reproductive stages (mg/g).

Stage	N	Mean	90% Confidence interval	Range	Coefficient of variation (%)	Required sample size
Storage	2	6.9	-	4.1-9.6	-	-
Developing	19	23.0	18-28	4-44	44	57
Mature						
1972	13	42.5	31-54	17-100	47	64
1973	15	98.3	86-111	66-138	24	17
Resorption	5	44.3	26-62	24-75	45	59
Starved	4	36.7	29-45	33-48	-	-

Table 11. Gonadal glycogen levels of the reproductive stages (mg/g).

Stage	N	Mean	90% Confidence interval	Range	Coefficient of variation (%)	Required sample size
Storage	2	50.0	-	23-77	-	-
Developing	11	19.7	13-27	5-44	58	99
Mature						
1972	11	26.7	19-34	9-53	46	61
1973	13	4.3[1]	3-6	1-7	53	83
Resorption	3	41.0	0-84	6-79	87	224
Starved	4	5.3	2-9	2-9	60	106

[1]Excludes February, 1973. Mean = 133

Table 12. Gonadal ribonucleic acid levels of the reproductive stages (mg/g).

Stage	N	Mean	90% Confidence interval	Range	Coefficient of variation (%)	Required sample size
Storage	1	2.2	-	-	-	-
Developing	2	5.6	-	3.6-7.5	-	-
Mature						
1973	11	18.7	11-26	4.1-38.4	67	132
Resorption	2	14.1	-	11.6-16.6	-	-

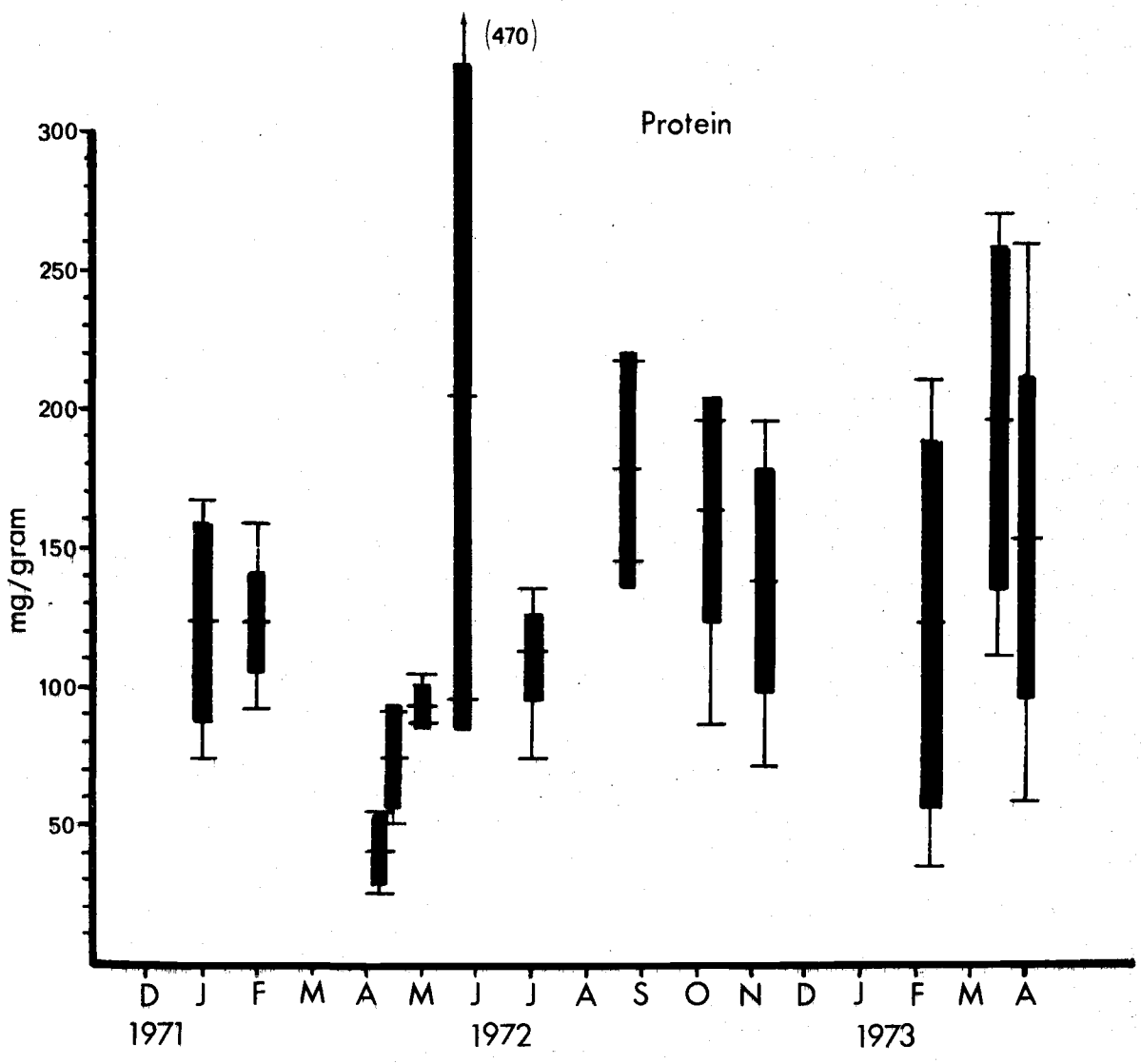


Figure 27. Average amount of protein per standard one gram animal. Means, 90% confidence intervals, and ranges shown.

same period lipid was accumulated at a rate of 0.59 mg per day and 1.8 mg per day respectively and was otherwise found to have a value between 50 and 100 mg per animal (Figure 28). Glycogen was generally less than 50 mg per animal (Figure 29). An interesting significant increase in glycogen was noted in February 1973 associated with the mature gonad. Glycogen does not appear to be closely related to reproductive state.

Finally, the biochemical content of mature eggs was ascertained by direct analysis of spawned eggs and indirect calculation using total oocyte estimates and total biochemical levels. The calculations were made by solving the following equation derived in Appendix VI.

$$\text{Amount} = K_1(N_{pvo}) + K_2(N_{mo}) + \text{residual}.$$

For protein, the equation becomes:

$$\text{Protein}/10^6 \text{ eggs} = \frac{A_{protein} - 19.5(N_{pvo})}{N_{mo}}$$

where $A_{protein}$ is the total amount of protein and N_{pvo} and N_{mo} are the number of previtellogenic oocytes and mature oocytes respectively. The estimate can be applied to mature gonads which have little residual material. The biochemical content of mature eggs is presented in Tables 13 and 14. There are strong implications that the amount of protein per egg decreases after the fall even though the number of eggs is increasing. Eggs spawned in early spring have

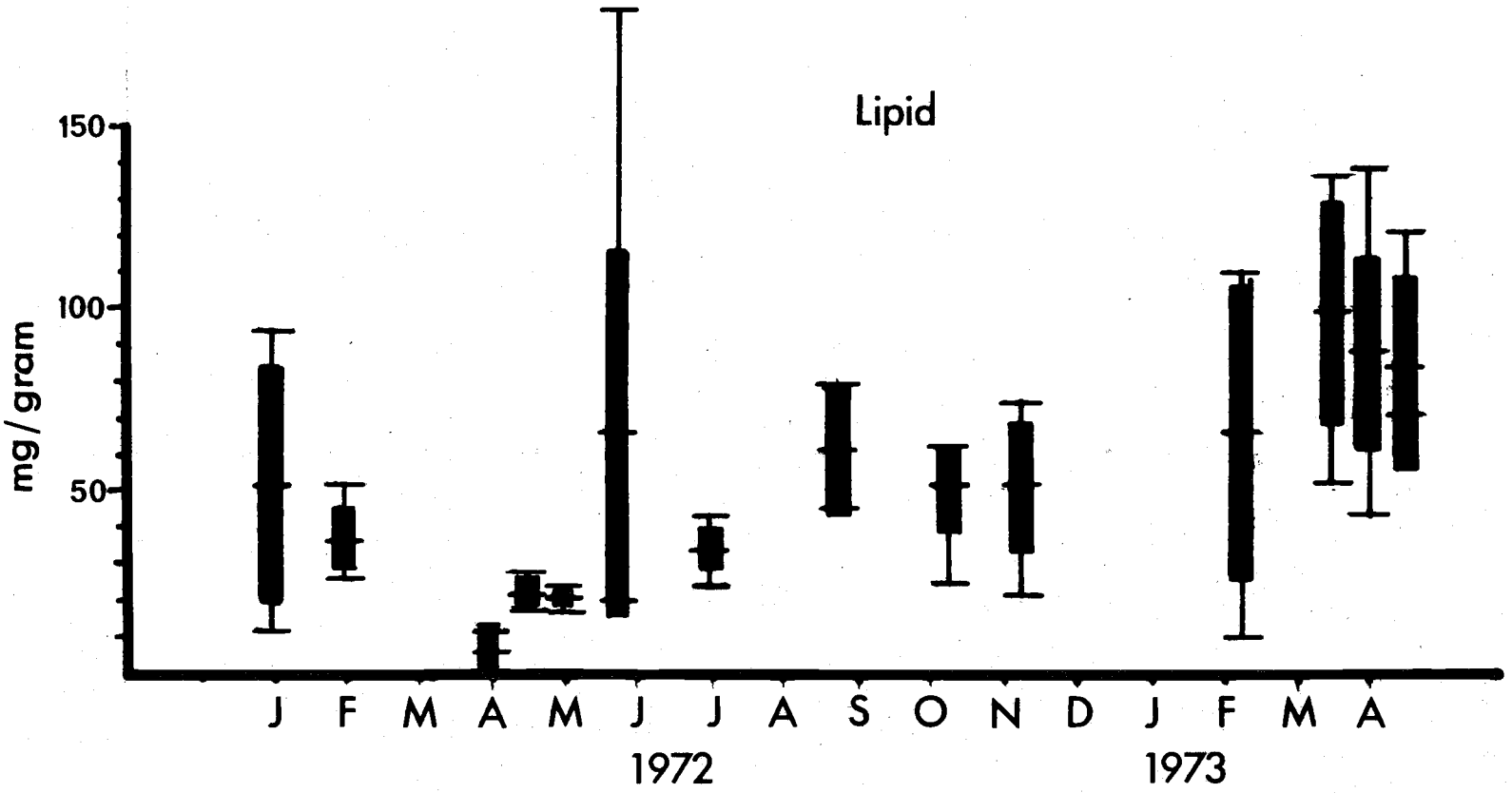


Figure 28. Average amount of lipid per standard one gram animal. Means, 90% confidence intervals, and ranges shown.

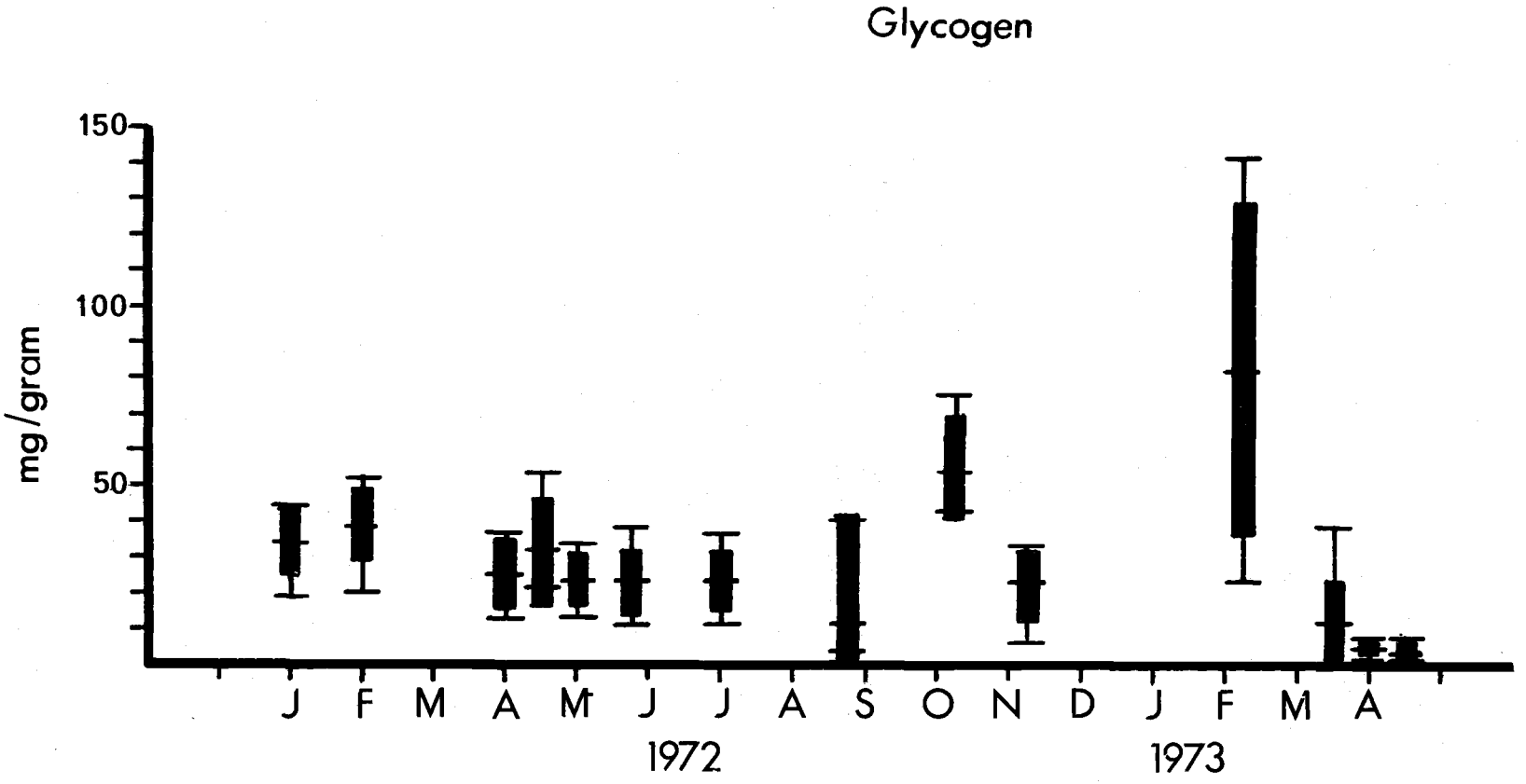


Figure 29. Average amount of glycogen per standard one gram animal. Means, 90% confidence intervals, and ranges shown.

Table 13. Biochemical composition of eggs in mg/million eggs directly calculated from spawned samples.

Date	N	Protein	Lipid	Glycogen
30 Sept. 1972	9	36	13	2.0
19 Nov. 1973	1	28	11	0.1
6 Feb. 1973	2	19	11	0

Table 14. Protein composition of eggs in mg/million eggs indirectly calculated from histological and biochemical samples.

Date	N	Average protein	Range
2 Jan. 1972	5	70	43-111
4 Feb. 1972	6	52	27-80
16 Apr. 1972	4	38	9-65
2 May 1972	4	81	21-208
27 May 1972	6	125	32-430
29 June 1972	6	64	54-78
23 Aug. 1972	3	56	41-75
8 Oct. 1972	5	35	28-44
3 Nov. 1972	5	43	36-78
6 Feb. 1973	3	32	22-47
17 Mar. 1973	5	42	32-69

about half the amount of protein of eggs spawned in early summer to fall.

Incorporation of Carbon-14

The histological and biochemical observations on rates and percentage accumulation were supplemented with information based on incorporation of a carbon-14 labeled flagellate, Isochrysis galba. The radiotracer work provided information on feeding rates, food incorporation, the amount of food allocated to the gonad, and the distribution of the food in the biochemical fractions of the gonad in relation to temperature.

Food incorporation was quantified by measuring the decrease in the number of labeled cells in the water. Feeding rates could be calculated from the same data used for food incorporation studies if the length of feeding time was noted. Figure 30 presents the filtration rates observed during the incorporation study at the times of high tide. The average feeding rates were found to double during the spring, increasing from 2 liters to 4 liters per hour per gram dry weight of animal. When these feeding rates are multiplied by the average number of hours the animals are submerged per day and then by the concentration of particulate organics, a theoretical food intake in the field can be established (Figure 31). The maximum

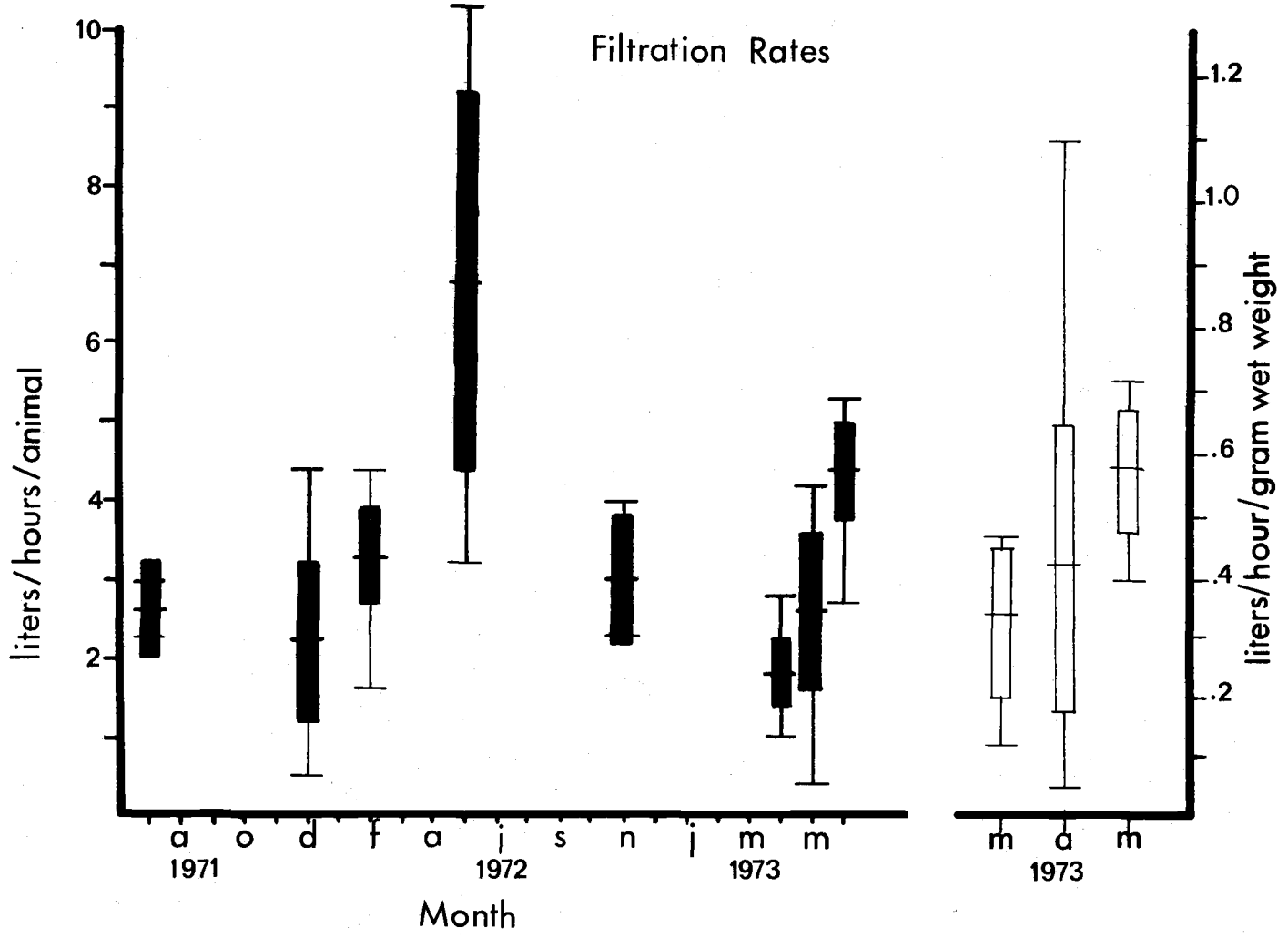


Figure 30. Observed filtration rates at high tide in the laboratory. Means, 90% confidence intervals, and ranges shown.

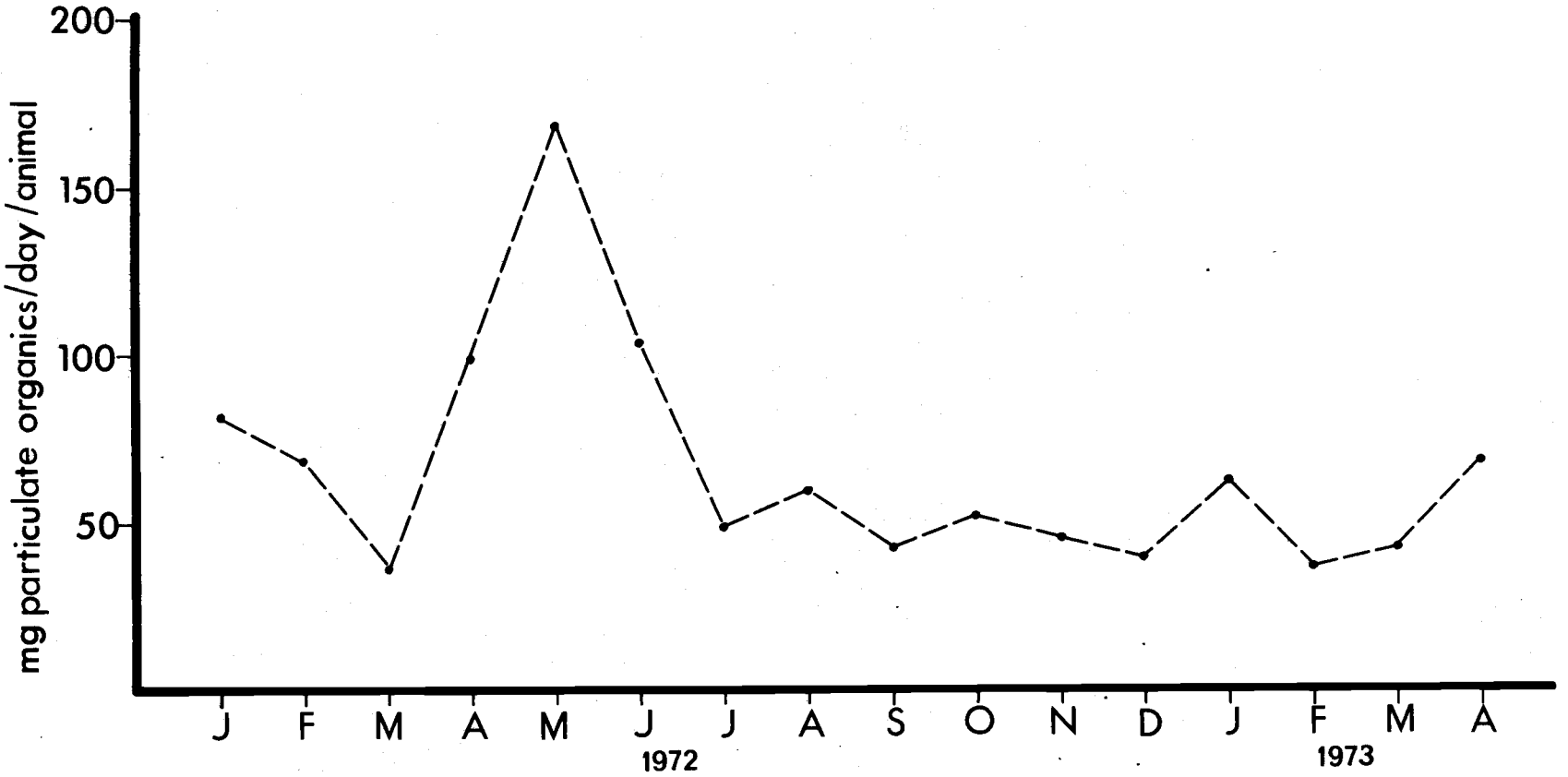


Figure 31. Theoretical amount of particulate food ingested per day by a standard one gram animal.

incorporated food changes from a low in February to March of 36 mg per day to a high value in May of 167 mg per day.

Net food incorporation was determined by subtraction of fecal radioactivity from total radioactivity filtered from the water. By the end of 48 hours, most of the total fecal radioactivity has been released. At the food concentrations used, there was no correlation between rates of feeding and the percentage of the incorporated label in the feces. Tables 15 and 16 present the observations on the initial amounts of radioactivity in the feces for two food levels. The percentage of fecal material contains 10% to 20% of the amount ingested, depending on conditions. The percentage in the feces is influenced by the amount of food ingested. At an intake greater than 2×10^8 cells (2.52 mg dry weight), the group continuously submerged at $9^{\circ}C$ had a lower net assimilation than either of the two exposed groups or the submerged group at $15^{\circ}C$.

The remaining small amounts of radioactivity were released at the rates shown in Table 17. The exposed groups have a significantly lower rate of release than the submerged groups at either temperature at the significance level of 70% and conversely hold material for a longer period.

The carbon-14 in fecal material does not have a direct correspondence with the true amount of incorporated food material. Table 18 gives the carbon and nitrogen characteristics of a fecal sample

Table 15. Percentage of ingested label released in fecal ribbons during the first 48 hours for two food levels at each condition.

Conditions	Total amount of food ingested (10^8 cells)	N	Average (%)	90% Confidence interval	Range
9°C submerged	0-1	11	4.5	2.2	1.3-15.0
	2-5	9	22.2	5.2	10.0-32.5
9/9°C exposed	0-1	6	4.8	1.0	4.1- 7.2
	2-5	5	11.4	1.0	10.5-11.5
9/20°C exposed	0-1	4	4.6	2.0	1.8- 6.6
	2-5	5	11.3	2.0	7.7-13.4
15°C submerged	0-1	3	5.4	0.2	5.3- 5.7
	2-5	4	12.0	1.4	10.6-13.7

Table 16. Results of t test for differences in the data in Table 15.

Groups	df	t	Level of significance	Remarks
All groups at low amount of food	-	-	none	no difference
9°C Submerged vs 15°C	11	2.5	95%	9°C is higher
9°C Submerged vs 9°C exposed	12	2.1	95%	submerged is higher
9/20°C exposed vs 9/9°C exposed	8	0.09	0	no difference
15°C submerged vs 9/20°C exposed	7	0.53	38%	no difference

Table 17. Percentage per day of ingested label in fecal ribbons released after 48 hours.

Condition	N	Average	90% Confidence interval	Range
9°C submerged	12	0.26	0.16	0.1 - 1.03
9/9°C exposed	5	0.08	0.02	0.06 - 0.11
9/20°C exposed	5	0.12	0.04	0.08 - 0.21
15°C submerged	4	0.23	0.04	0.18 - 0.29

Table 18. Results of carbon and nitrogen analysis of cultured Isochrysis galba and fecal ribbons from Mytilus californianus.

Sample	µg C/mg	µg N/mg	C:N	Specific activity dpm/µg C
Isochrysis galba	127	21	6.3	11,600
Fecal ribbons + 5 hours	60	6.6	9.0	740
Fecal ribbons + 10 hours	104	22	4.8	2,520

from a specimen continuously submerged at 15°C. It is clear that fecal material is not composed completely of unused food cells since the carbon:nitrogen ratio and the specific activity change. During the first 5 hours there is a relative decrease in nitrogen. The absolute decrease in both components is due to inorganic debris in the mussel. Furthermore, considerable non-radioactive carbon has been added at this point as is seen from the decreased specific activities. In the next 5 hours both elements have risen to nearly that amount and ratio found in the food. However, some of this material is still non-radioactive as is seen by the still low specific activity. Although a steady state was not observed, the difference between incorporated and fecal activities was considered a good estimate of net assimilation.

The amounts of carbon-14 found in the various tissues in relation to the four experimental conditions were then determined. Figure 32 illustrates the amount of carbon-14 remaining with time in the somatic tissues, digestive gland, and gonad. As expected, the amount of radioactivity decreases with time from incorporation. Therefore, comparison between samples can only be made with specimens with the same time lapse from incorporation. At 9°C about 30% of the carbon-14 is retained in the whole animal and about one-third of this carbon-14 is in the gonad. The rest is lost presumably by respiration and soluble excretion. The loss occurs mostly from somatic and digestive gland tissues rather than gonad. Figure 32 also shows that

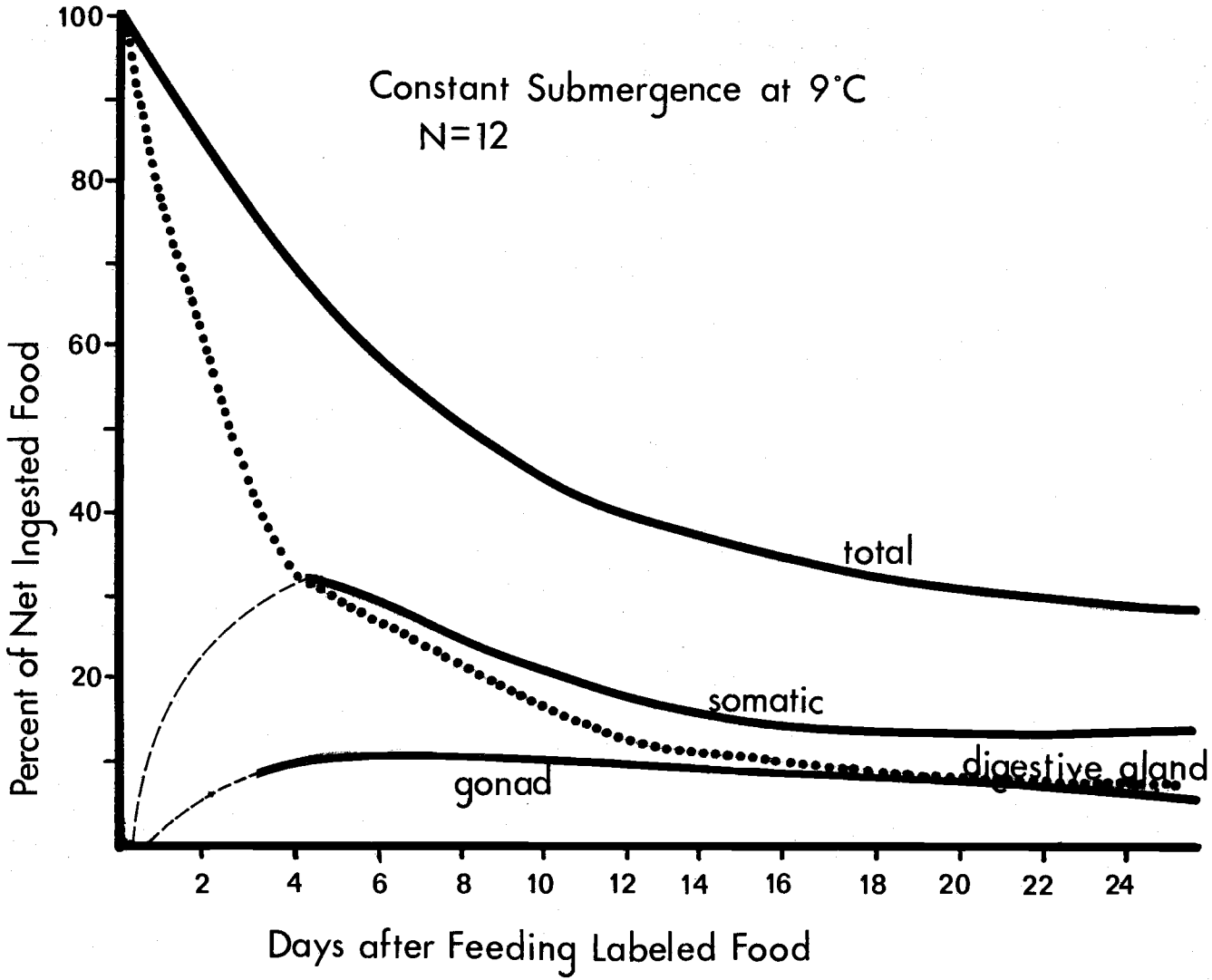


Figure 32. Amount of carbon-14 remaining in the mussel and in the three tissue categories following feeding at constant submergence at 9°C.

maximum carbon-14 in the gonadal tissue was attained within 3 days or at a rate of 3.3% of the total food retained per day. Based on the theoretical maximum food retention (Figure 21), one would expect the food available to the gonad to be 1.2 mg per day from February to March and 5.5 mg per day in May. These values agree well with the 3.9 mg food per day obtained from histological and biochemical methods.

Table 19 presents the allocation of the carbon-14 remaining in the tissue of mussels experiencing the four experimental conditions. The data suffer from small sample size when they are broken down into categories, and no significant differences were found for the two thermal or exposure regimes. It should be noted that animals with developing gonads incorporated a greater amount of label but that all of the increase was in the somatic muscles.

Analyses of carbon-14 incorporation into gonadal tissues were broken down into the major biochemical classes. The results are a combination of anabolic and catabolic processes and thus represent a picture of the net flow in a compartment. Figure 33 gives the amount of assimilated label in the major biochemical fractions.

The weight of a biochemical component was calculated by obtaining the total dpm in the fraction, dividing the activity by the specific activity of the component in the food. The result was then normalized

Table 19. Distribution of carbon-14 in the three tissue categories.

Conditions	N	Days from incorporation	Percentage of net incorporation			Remarks
			Average	90% Confidence interval	Range	
				Total incorporation		
9°C submerged	4	11-14	42	28-55	32-61	mature; partial spawn
9/9°C exposed	5	9-14	52	39-66	29-69	developing
15°C submerged	4	7-8	38	32-44	32-45	partial spawn; resorbing
9/20°C exposed	4	7-10	45	35-56	39-61	partial spawn
all mature	12		42	36-47	32-61	
				Somatic Tissue		
9°C submerged	4	11-14	19.1	15.6-22.4	15.9-22.6	mature; partial spawn
9/9°C exposed	5	9-14	27.3	18.2-36.5	12.8-39.5	developing
15°C submerged	4	7-8	15.9	12.7-19.0	13.1-19.9	partial spawn; resorbing
9/20°C exposed	4	7-10	17.3	11.0-26.0	13.2-29.6	partial spawn
all mature	12		17.8	15.0-20.5	13.1-29.6	
				Digestive Gland		
9° submerged	4	11-14	11.4	7.9-14.9	8.9-16.4	mature; partial spawn
9/9°C exposed	5	9-14	18.5	13.6-23.3	10.7-22.7	developing
15°C submerged	4	7-8	12.6	9.1-16.1	8.5-16.9	partial spawn, resorbing
9/20°C exposed	4	7-10	17.4	14.9-22.1	14.0-22.5	partial spawn
all mature	12		14.1	11.5-16.8	8.5-22.5	
				Gonadal Tissue		
9°C submerged	4	11-14	12.1	4.8-9.5	6.0-22.2	mature; partial spawn
9/9°C exposed	5	9-14	6.5	5.4-7.6	5.2-8.4	developing
15°C submerged	4	7-8	9.2	7.0-11.4	6.7-11.5	partial spawn; resorbing
9/20°C exposed	5	7-10	8.3	6.2-10.4	5.5-11.8	partial spawn
all mature	13		9.8	7.3-12.2	5.2-22.2	

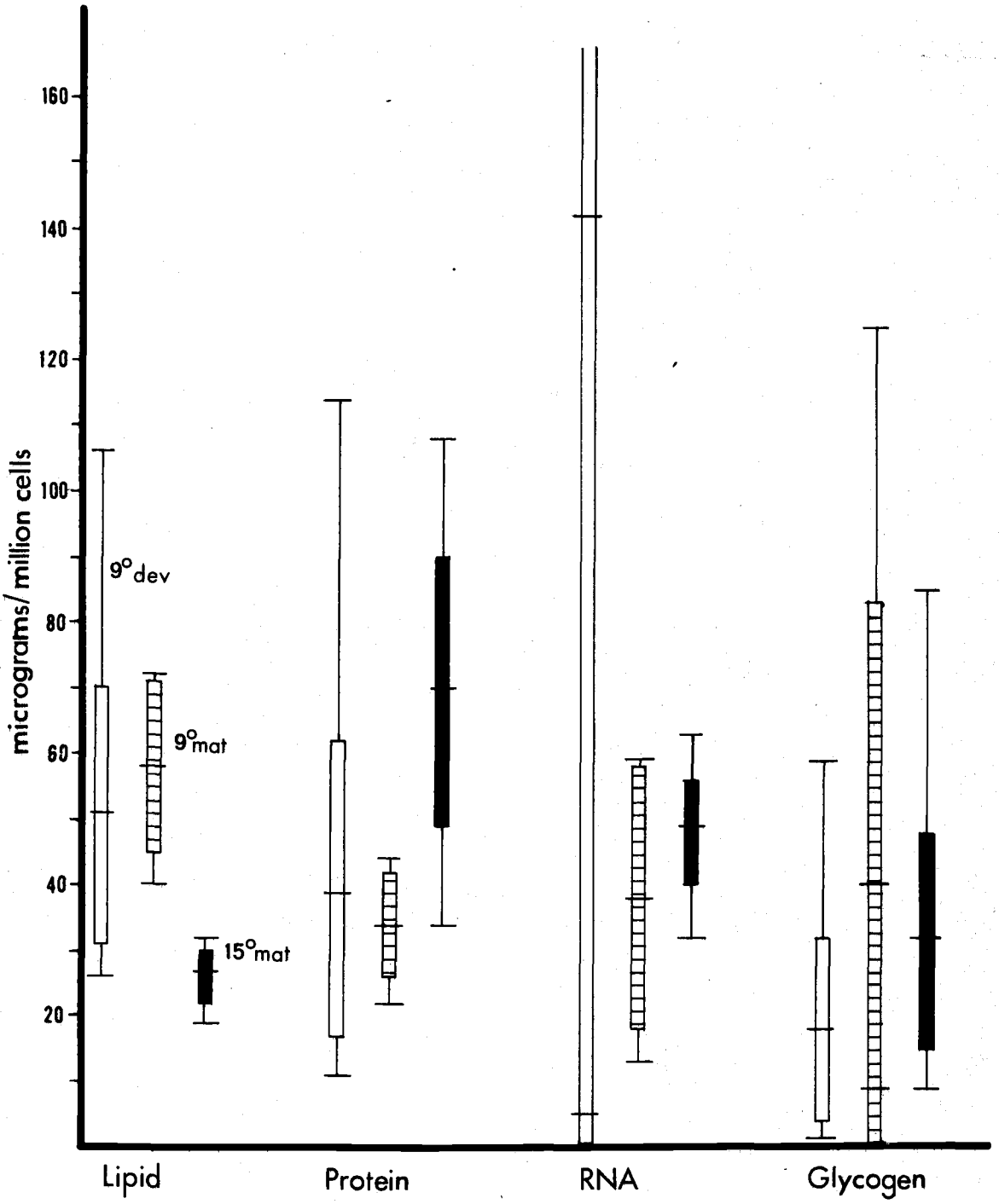


Figure 33. Amount of material accumulated in the biochemical fractions per million cells ingested. 9° Dev = developing, N = 8; 9° Mat = mature and partially spawned, N = 5; 15° Mat = mature, N = 8.

to a net incorporation of 10^6 cells since the proportion of the label accumulated will depend on the amount of food incorporated.

There is a tendency for temperature to influence the rate of accumulation of the larger molecules. However, the variations are large and the confidence intervals of the means are correspondingly increased. Since no difference was found between the submerged and exposed groups, they were combined for comparative purposes. Comparison of values in Figure 33 shows that in mature of partially spawned gonads, protein and RNA in the 15°C group contained greater amounts of carbon-14 than the 9°C group, while the lipid and glycogen were reduced. At 9°C, developing gonads had more label in the protein and RNA fractions than mature gonads but the variation is too great to draw a conclusion.

The radioactivity per mg of material, the specific activity, is given in Figure 34. This parameter gives an indication of either the turnover of material or the degree of recent synthesis. The carbon-14 specific activity of all components was higher in developing gonads than in mature tissue. Carbon-14 specific activity of all four components was the same or less in 15°C animals compared to 9°C animals. RNA is 10 times the value of the other components.

In summary, the radiotracer work shows different effects of temperature on oogenesis at the assimilation, allocation, and biochemical distribution levels. At high food levels, net incorporation

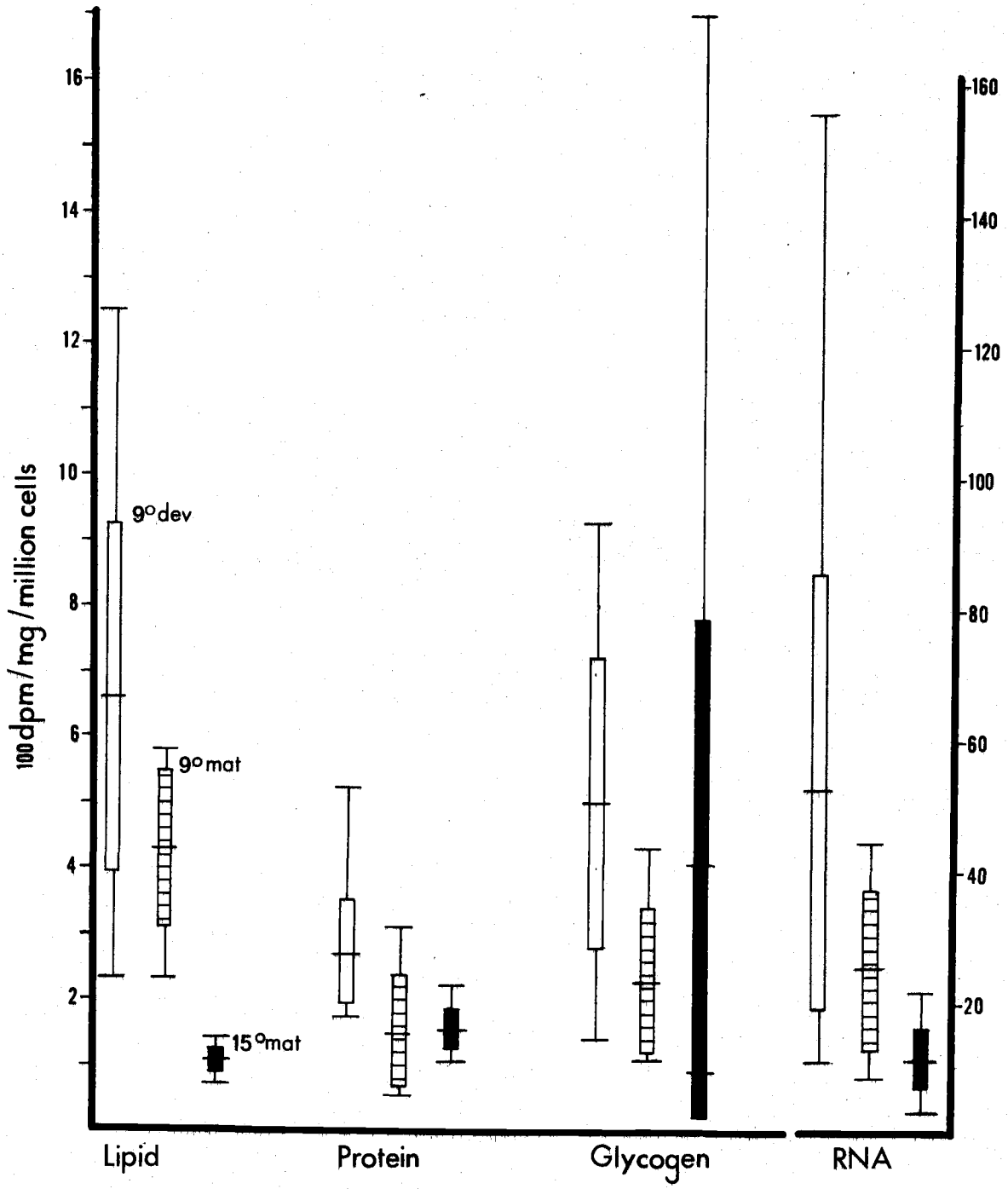


Figure 34. Specific activity of carbon-14 in the biochemical fractions per million cells ingested.

is higher at 15°C than at 9°C, but this amount of assimilation can also be attained with 6-hour periods of exposure at either temperature. Nitrogenous compounds are initially removed from food in greater amounts than other substances. Thermal and exposure regimes appear to have no effect on allocation of food. Temperature has a qualitative effect on the biochemical distribution since net protein and RNA accumulate in greater amounts in mature specimens at 15° than at 9°C while the amount into lipid decreased at higher temperatures.

IV. DISCUSSION

This study of mussel oogenesis involved the timing of events relative to the environment, quantity of eggs produced, and egg quality. Examination of oogenesis in M. californianus has resulted in the development of a hypothetical scheme for the mechanisms linking the environment to the reproductive sequence in the individual mussel. The results of the 2-year field study permitted an evaluation of environmental and physiological control of flow rates of both oocyte numbers and biochemical constituents.

Relationships between Environment and Oogenesis

The timing and duration of oogenesis is determined by both genetic and environmental factors. Reproductive systems of lamellibranchs have the capacity to undergo a complete reproductive cycle, but the appearance and duration of each stage is the result of environmental factors rather than a species characteristic. M. galloprovincialis and M. edulis from oceanic stations in France showed annual reproductive cycles with storage phases occurring during the summer. In contrast no annual cycle was found for M. californianus. The population of M. californianus examined demonstrated neither a distinct storage period nor a distinct mature period followed by complete spawning. The near complete reduction of the storage stage is

consistent with Lubet's (1959) findings that the duration of the mature stages in M. edulis and M. galloprovincialis is increased at the expense of the storage and developing stages in oceanic habitats compared to those of bays and estuaries. The oceanic environment has a more stable physical and nutritive regime relative to that of the bay. Water temperatures in Yaquina Bay range from 0° to 17°C while those at Yaquina Head range from 7° to 17°C. The annual range of salinity at Agate Beach, Oregon is only $20.4\,^{\circ}/_{oo}$ to $35.5\,^{\circ}/_{oo}$ and is small compared to the range from $0\,^{\circ}/_{oo}$ to $35.0\,^{\circ}/_{oo}$ found in Yaquina Bay, Oregon, the habitat of M. edulis (Thum, 1972).

Reproduction in the population was fairly synchronous, but gametogenesis and oocyte maturation are not necessarily annual events. Partial spawning and resorption of unused eggs with concurrent development of new oocytes appear to be the predominant reproductive phenomena. Both partial spawning and resorption are well known phenomena in the pelecypods (Loosanoff, 1951; Seed, 1969a).

M. californianus is a temperate species whose geographical and submerged thermal ranges extend from Mexico (20° to 26°C) to the Aleutian Islands (2.5° to 10°C). The population of mussels examined was located in the center of the geographical range with a temperature range from 8° to 14°C. Although the population had histologically mature gonads throughout an entire year, mussels were

most receptive to spawning stimuli before September 29. Histological samples indicated partial spawning during fall and, to a lesser extent, during early spring. Fall is a slightly earlier time for spawning than the times indicated by other authors dealing with more southern populations (Figure 1). The difference may be a reflection of environmental factors associated with latitude. In Europe the mature period for M. edulis has been found to decrease in duration and to shift towards summer for more northern populations. The "reproductive period" for M. edulis was found to be January to May in France, April to June in England, and June to July in Holland and Denmark (Lubet, 1959). Pearce (1968) has found that the duration and number of reproductive cycles per year decreased in populations of Indo-Pacific sea urchins in temperate regions compared to those in tropical regions. In higher latitudes the reproductive sequences take longer and environmental conditions are favorable only at certain times of the year. In tropical regions environmental conditions are favorable throughout the year resulting in shorter stages and continuous reproduction. Thus both the absolute environmental conditions associated with different latitudes and the varying conditions of local habitats can influence the sequence of reproductive events.

In order to insure a stable population it is advantageous, particularly in temperate latitudes, for oogenesis and spawning to be coordinated with the environmental features. The nearshore

environment off Oregon has been shown to have less annual variability than the bay habitat but also to be less predictable from year to year. In such a situation mussel reproduction will be most successful if efficient use of the accumulated gonadal material in the adult is made and if the gametes are spawned at a time when the larvae will survive.

During periods of less than optimal conditions, there seems to be protection against loss of eggs by a fine quantitative control on spawning. Material not used can be resorbed and does not have to be reaccumulated for the next set of oocytes. Survival of the larvae is a function of time in the planktonic stage. For M. edulis this period is usually 3 to 4 weeks but can be delayed for 2.5 months (Seed, 1969b). The duration of the larval stage is a function of salinity, temperature, and nutrition. Mortality of M. californianus larvae increases below 29.6 $^{o}/_{oo}$ (Young, 1941), but this condition occurs in the nearshore waters only from December to February. If a successful spawn takes place early enough in the fall, there may be sufficient time for larvae to develop and settle as plantigrades before the low salinity associated with winter occurs. During spring, released gametes develop into larvae able to make use of the increased concentration of particulate food and the adults are also ready to use the same increased food concentration. The influence of temperature on larval survival is not known for this species.

Annual Tissue Temperature

The features of the physical and chemical environment were found to be variable from 1971 to 1973 with little repetition between the 3 years. The thermal regime of M. californianus has been studied in detail in order to obtain an accurate picture of tissue temperature and to have good environmental data with which to correlate reproductive changes. When the details of tissue temperature are studied, it is clear that sea water temperatures obtained once a month are not adequate for the description of the thermal regime of this animal. Seasonal trends in incident insolation and time and duration of exposure are similar from year to year, and therefore the solar component of the tissue temperature equation is predictable. Any difference in the thermal regime between two years will depend on sea water and air temperatures. Inshore sea water temperature variations result from surface cooling and heating and summer upwelling. Records are often kept of air and sea water temperatures, and as a result, the thermal regimes of different populations along the Pacific Coast can be estimated by a formula easily adapted to computer techniques.

Three major thermal features of the environment are the absolute temperature, integrated heat in terms of degree-time, and the rate of temperature change. Absolute temperatures have an annual

range of 0° to 25°C. This range is greater than would be expected on the basis of sea water temperature measurements alone. Tissue temperature during exposure is particularly important during the spring and early summer when the major reproductive events may occur. The higher amounts of integrated heat (Figure 11b) and faster rates of heating (Figure 10) are a consequence of the low tide period passing through midday, resulting in heating by solar insolation during this season. There is also a possibility of heating in the fall. Although the absolute value of shade air temperatures may change from year to year, Gislen (1943) and Glynn (1965) have shown that the seasonal maximum occurs between August and September, and the daily maximum occurs between 1400 and 1800 hours. The years 1970 and 1971 were different in this respect and imply that heating in the fall during tidal exposure may differ from year to year. In Oregon, exposure occurs in the late afternoon during this period.

Rates of heating appear higher during spring and early summer, but may also increase during September to October. Weekly or bi-monthly estimates of tissue temperature are sufficient to follow long term trends. However, if change in temperature from submerged to exposed conditions is important, only daily data will suffice.

Annual Changes in Particulate Food

M. californianus is a ciliary mucoid feeder and would be

expected to be adapted to collecting small particulate food. Both the quantity and the chemical composition of the particulate food changes throughout the year. The absolute amount of oxidizable particulate carbon is fairly constant (2 $\pm$ 1 mg per liter) and is comparable to the value of 5 mg per liter found for the waters off La Jolla, California (Fox and Coe, 1943). It is low compared to values for Departure Bay, British Columbia, which average 15 mg per liter and which have a nine-fold annual variation (Bernard, 1974). The lower size limit of the particles measured was determined by the 10 μm filter which has been shown to have an effective retention size of 1 μm (Sheldon, 1972). Reiswig (1971) suggests that Mytilus species demonstrate high retention in the bacterial size category of 1 μm. He also notes that in Jamaican waters diatoms account for only 2% of the particulate organic carbon, flagellates 8%, detrital particles 0.5%, and microscopically unresolvable particles with a mean size of 1 μm constitute about 90% of the particulate fraction. Therefore, the filtered sea water samples in this study may slightly underestimate the true food concentrations but are a better indication of nutrition than the diatom counts.

Although the three-fold variation in the spring is associated with a diatom bloom, there is a major contribution by detritus or microscopically unresolvable material. This increased detritus concentration is correlated with increased inorganic particulates which are in turn associated with bottom mixing by storm waves. The

winter peak is nearly all detritus. Cell counts during the spring bloom may not be directly related to nutrition of the mussel. Coe and Fox (1944) found M. californianus to selectively remove dinoflagellates from the water, but also noted that although large blooms of the diatom Chaetocerous sp. were present, the diatoms were rarely represented in mussel stomach contents.

The chemical composition of the particulates also changes throughout the year. The dichromate procedure measures chemically oxidizable carbon and does not necessarily represent changes in physiologically available carbon or changes in nitrogeneous compounds. In Departure Bay, British Columbia, Stephens et al. (1967) observed that during May to July the carbon:nitrogen ratio was 6 which is comparable with that of living phytoplankton, and from October to December the ratio was 10. Thus, the spring and winter particulate organic peaks are qualitatively different, and more nitrogen would be available during the spring. The organic constituents of the particulate fraction may also change throughout the year. Campbell (1969) demonstrated a considerable increase in carotenoids in M. edulis during March and April associated with the diatom bloom.

Although correlations between oogenesis and the concentration of particulate organics may be made, the nutritional state of the sea water does not appear to be adequately explained by this parameter. The diatoms and dinoflagellates may only be indicative of seasonal

changes in dissolved organics. Dissolved organic carbon forms a considerable percentage of the organic carbon in sea water, and may act either as a nutritional source or as a seasonal cue. Thus dissolved organics may be in physio-chemical equilibrium with colloidal particles and thus contribute to the microscopically unresolvable fraction (Reiswig, 1971). In the North Sea a considerable increase in dissolved organic carbon is correlated with the production of new organic material by photosynthesis (Duursma, 1963).

Hypothetical Scheme for Environmental Control of Oogenesis

Figure 35 presents a hypothetical scheme, similar to that given by Olive (1970) for sperm production, for the connection between environmental events and the processes in the gonadal follicles. The product of the reproductive system is determined by many sub-lethal features of the environment and physiological state. Changes in the static picture seen in the bimonthly histological sections and biochemical samples are a result of the relative changes in the rates between compartments of oocyte categories. An increase in the number of previtellogenic oocytes could be due to an increase in the rate of previtellogenic processes or to a decrease in vitellogenesis. If the proper conditions for oogenesis are not met, a particular process may be delayed while the reproductive sequence is continued

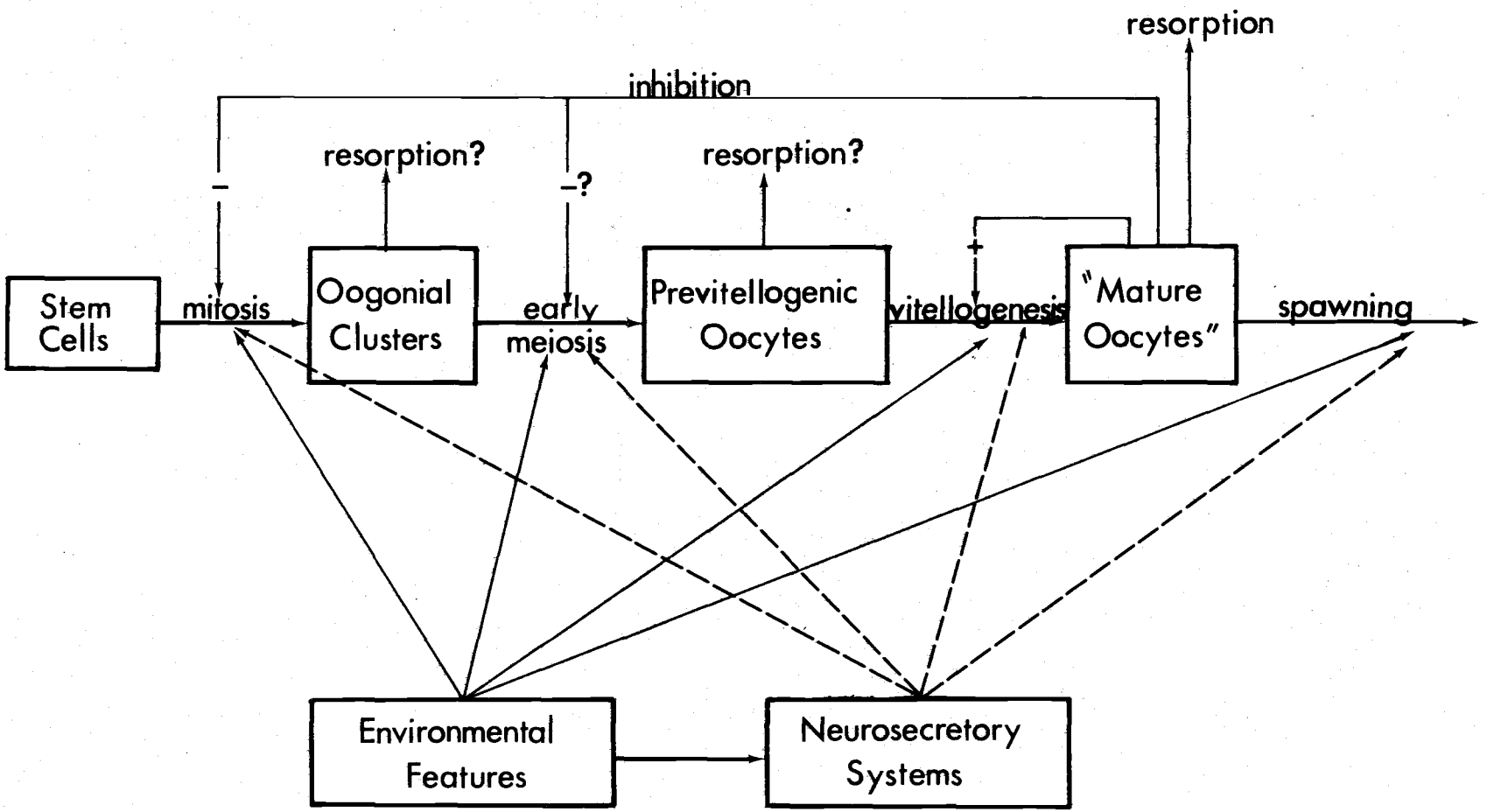


Figure 35. Hypothetical scheme for environmental and internal control of oogenesis.

with the available material. Thus the interval timer hypothesis for endogenous annual rhythms (Enright, 1970) does not apply in this case.

Hypothetical controlling features can be inserted into the diagram based on the available data. The response to any of the environmental parameters depends on the state of the system receiving the stimuli. The problem is complicated by possible influences of one environmental feature upon the response of the organism towards a second (Enright, 1970). For instance, the response of the mussel towards temperature may be determined by the less variable environmental feature of light. The cellular processes may also be indirectly influenced by the environment through neurosecretory activity (Lubet, 1959).

Four types of environmental influences on each process can exist. Cues initiate activity in a system and their presence is not required continuously. Controlling parameters determine rate of formation of individual eggs, while quantitative parameters influence the amount of eggs produced. Qualitative parameters affect the proportion of biochemical components in the eggs but may overlap with rate determining factors which are also known to influence the ratio of biochemical components.

Each of the four processes in Figure 35 appears to have different controls. The histological data give circumstantial evidence that mitotic activity leading to the production of oogonia is inhibited by

mature oocytes. An increase in the number of oogonial clusters followed a major spawning in 1971, and no increase was seen in the fall of 1972 when the amount of mature oocytes remained high. However, mitotic activity occurred in January, 1973, but it was asynchronous within the Mytilus population. Mature oocytes might secrete substances which inhibit mitosis, but the possibility that mitosis may also be under neurosecretory control is not excluded. Olive (1973) reports that ripe coelomic oocytes inhibit previtellogenic development of immature oocytes in the polychaete, Cirratulus cirratus, and Loosanoff (1973) states that in the clam, Venus mercenaria, "discharge of ripe eggs evidently removes the factors inhibiting production and growth of a new crop." Lower temperatures may be favorable to mitotic activity of the stem cells. Since the increase of oogonial clusters did not continue into the following February, it is assumed that mitotic activity had ceased. The percentage of oogonial clusters was still high in May after the peak development of the previtellogenic oocytes had occurred, and did not decrease until June. Because the number of oogonia in the clusters was not estimated, oocytes could have continued to develop without changing the number of clusters. The production of oogonia does not appear to be an intrinsically fixed annual event.

A 5-month lag period exists between the production of oogonia and the initiation of meiotic activity leading to previtellogenic

development in the spring. Thus previtellogenic processes do not automatically follow production of oogonia. Within the study population, there was synchronous previtellogenic development during spring of 1972 and asynchronous development in the spring of 1973. Quantity of sunlight, particulate food high in nitrogenous compounds and carotenoids, tissue temperature, and the rate of heating all change in the spring and could serve as cues.

Increasing solar radiation in the spring results not only in increased temperatures but also in greater amounts of visible light. Visible light should not be neglected as an environmental parameter. Known light sensitive organs in M. edulis are directional eyes at the base of the palps connected to the cerebral ganglion by a nerve and a photosensitive area on the edge of the mantle (Field, 1924).

Barnes (1963) demonstrated an interaction between the inhibitory influence of light and a cold temperature period of conditioning which serve as cues in the breeding of the barnacle, Balanus balanoides.

The best correlation with respect to the timing of previtellogenesis is with the increase in food concentration. It is difficult to see how food levels would initiate meiosis directly unless a critical substance suddenly becomes available or unless chemoreception of dissolved organics in the environment occurs. Once the process has been initiated it is under the influence of controlling parameters which affect rate. Temperature is an obvious example of such an

environmental feature. However, there appears to be little correlation between temperature and the rate of previtellogenic oocyte formation. It is possible that a thermal effect could be found if the growth of individual oocytes were followed.

Vitellogenesis may not need a cue for initiation but simply reflects an increase in the quantitative parameters of temperature and availability of nutrients. The period of yolk formation is associated with further increases in temperature up to 13°C, but again there is no correlation between mature oocyte development and temperature. Although the greatest rate of mature oocyte production occurred in late spring, oocytes were still being produced from November to February when the temperature was 9°C. There is a better correlation between the estimate of ingested food and the rate of mature oocyte formation. However, mature oocyte loss at spawning introduces error into the estimate. Sastry (1970) suggests that in the bay scallop, Aequipectin irradians, oocytes once stimulated cannot be stopped from further development.

The relationship between spawning and environmental features also appears to be complex. Complete spawning was observed in only three specimens during April, 1972, at a time when other specimens were undergoing partial spawning or resorption. Partial spawning was detected during fall, 1971, spring, 1972, and to a considerably lesser extent during fall, 1972 and spring, 1973. Spawning periods

were not related to increased periods of exposure as suggested by Campbell (1969), decreased salinity as suggested by Lubet (1959), or wave action which peaks during November and December (Creech, 1973) as suggested by Young (1945).

There is some relationship between spawning and temperature. Three criteria may have to be satisfied for spawning to occur. They are maturation of the gonad, synthesis and accumulation of neurosecretory material in the cerebral ganglion as a result of environmental conditions extending over days or weeks, and release of neurosecretory material due to rapid temperature change over a tide cycle (Lubet, 1959). Spring, summer, and fall satisfy the condition for rapid thermal change by having days in which tissue temperature increases from 6 to 12 degrees per hour. Chipperfield (1953) found the initial rate of spawning in M. edulis to be directly related to the rate of increase in submerged temperature. However, the absolute temperatures at the times of detected spawnings are maximum ($13^{o}C$) during fall and low ($9^{o}C$) but increasing during the spring. Clearly the long term "conditioning" leading to the production of neurosecretory material is determined by other factors. If the quantity of eggs spawned is a direct result of the amount of neurosecretory material released, partial spawning could result either from low amounts of neurosecretory synthesis or from a weak, short term stimulus. Spawning could be synchronized in the population by the

most sensitive individuals spawning and thereby releasing substances which increase the sensitivity of the remaining population toward environmental stimuli (Roberts, 1972).

Lysis and subsequent resorption of mature oocytes are indirectly related to environmental features. After development, the life of a mature oocyte may be short, and if spawning does not take place, terminates in lysis. However, the estimated residence time of mature oocytes during 1972 was about 200 days, and therefore under certain conditions, lysis may be delayed. The whole sequence occurs continuously, but the proportion of resorbed eggs at any time is usually small. However, if eggs are not spawned then the process may become a major phenomenon.

Quantitation of Oogenesis: Germinal Gonad Index

Gonad indices have been used to normalize the amount of gonad growth in echinoderms and some molluscs so that the effect of individual size will not influence the relative quantity of gonadal material (Giese, 1959; Giese and Pearse, 1974). Generally the gonad index is defined as the weight of the gonad divided by the total weight. In this study the gonad index was defined as the weight of gonadal material over the weight of the remaining somatic tissue. The gonad weight is then not divided by itself, and the gonad index is not minimized at maturity. In addition, the ratio of gonad to somatic tissue is a more

meaningful parameter since the somatic tissue represents the collecting and processing mechanisms supplying nutrients to the gonad. The assumption is made that a particular amount of somatic tissue is capable under optimal environmental conditions of producing a specific maximum amount of gonadal material. The index then measures to what extent this theoretical maximum is achieved. Gonor (1972) has shown that the assumption does not hold for small-sized mature sea urchins. This problem was avoided in this study by using only individuals from the largest size class in the study area.

Somatic weight is not necessarily constant throughout the year. An increase in the ratio of the gonad to somatic tissue in the fall could be due to weight loss of somatic tissue and not increased gonad weight. The estimated somatic weight of a 70 mm specimen (Table 4) was found to be too variable to determine if seasonal differences exist. A better measure of animal size might be the inner shell volume (Moon, 1969).

In addition to the above considerations, a correction had to be applied for the tissue in the "gonad" which is not directly involved in the reproductive processes, the Leydig tissue. For this reason the germinal gonad index was used instead of a normal gonad index in this animal. This index averages about 500 mg dry weight of gonad per gram dry weight of somatic tissue. Since the maximum index found was 800 mg per gram, the mussel is capable of producing and

accumulating nearly as much reproductive tissue as its somatic weight. However, the population was usually found to achieve only 60% of its capacity. Fox and Coe (1943) report gonad indices up to 1200 mg per gram of somatic tissue in M. californianus. The values for M. californianus can be compared with 620 mg per gram for M. edulis, 60 to 100 mg per gram for the chitons, Katherina tunicata and Cryptochiton stelleri respectively, and 670 mg per gram for the sea urchin Strongylocentrotus purpuratus (Giese, 1966, 1969).

The gonad index has ecological meaning since it represents the potential and realized fecundity per unit biomass. The index also gives an indication of the allocation of energy and material resources to somatic growth and to reproduction. The index allows the fecundities of different populations or subdivisions of a population to be compared. If 2 or 3 years are required to produce the maximum amount of gonad in M. californianus, the value of the index represents a considerably smaller percentage of food incorporation into gonad than it would if maximum amount of gonad were produced in 1 year.

Quantitation of Oogenesis: Rates of Oocyte Formation

The estimates of the total numbers of previtellogenic and mature oocytes allow rates to be established for the processes shown

in Figure 35. Previtellogenesis increased significantly to 13.5×10^4 oocytes per day per gram animal during the second week of April, 1972, and the rate then dropped to a steady state of 2×10^4 oocytes per day for the next 12 months. Olive (1970) calculated the length of time cells remained in a category at steady state by dividing the total amount by the rate of input or removal. This calculation for mussels yields a value of 50 days for the cells in the previtellogenic category. The negative rates in May and December could be due to the development of previtellogenic oocytes into the mature oocyte category faster than the conversion of oogonia into previtellogenic oocytes. Oogonial clusters were present in small amounts throughout the steady state period. It is not known whether mitosis was continuing at a slow rate or whether all the oogonia for the 12 month period were produced during the fall and winter of 1971. If the latter is the case, there would be a point at which all the oogonia were consumed, but a lag of several weeks would follow before this effect would be felt at the level of mature oocytes. Resorption of previtellogenic oocytes was not observed.

The rate of mature oocyte formation is more difficult to estimate since both partial spawning and resorption remove oocytes to a category which cannot be sampled. The decrease in mature oocytes is associated with partial spawning and during February to April, 1972, considerable resorption was also occurring. The maximum

number of mature oocytes found during the study was 10.5 million per gram animal. Of an average total of 6.3 million eggs per animal produced from April, 1972, to April, 1973, only 2.6 million or 40.5% were released by spawning. The mature oocyte category did not reach steady state but continued to increase at a rate of 1.2×10^4 oocytes per day. Production of mature oocytes was about 2.4×10^4 oocytes per day, and a minimum spawning rate of 0.75×10^4 eggs per day was found. The missing 0.45×10^4 oocytes per day probably represent those undergoing lysis and resorption. The value of 19% resorption corresponds to the 10% to 20% seen in the histological samples. Although the mature oocyte category never reached a steady state, a residence time of 200 days was calculated for cells in this compartment. Either the amount produced in 1972 was mostly resorbed or completely lost by spawning during fall, 1973.

The predominance of partial spawning is not necessarily less favorable than complete spawning. A complete spawn would require a recovery period and redevelopment of the follicles, while a gonad nearly in steady state undergoing a series of partial spawns would have no recovery period and conceivably could release more eggs per unit time. It is possible that the range of spawning behavior of the mussel permits an amount of eggs to be released which is appropriate to the quality of the environment in terms of fertilization and larval success.

At Yaquina Head, Oregon the mean density of the mussels of 60 to 70 mm in length in the intertidal beds is 1,120 animals per square meter. Therefore, during the year 1972-1973, 2.9×10^9 eggs per square meter per year were released. For an average 8.25 meter band width of M. californianus along the shore, this value is then 2.4×10^{10} eggs per linear meter. If the dry weight of one million eggs is 50 mg, then 1.2 kg per linear meter is released per year. This value is comparable to that of 0.352 kg carbon per square meter per year (Curl and Small, 1968) or 1.24 kg dry weight per square meter per year for primary productivity in Oregon coastal waters. Fox and Coe (1943) suggest that 2-year-old mussels in Southern California "enrich the environment with about three grams of gametes." As Gonor (1972) has suggested, this contribution to nearshore production cannot be neglected.

Biochemical Changes

Another quantitative aspect of oogenesis is the amount of material accumulated and synthesized for reproduction. The macromolecular biochemical constituents can be divided into proteins, lipids, carbohydrates, and nucleic acids. These categories reflect the partitioning of energy and of the elements, carbon, nitrogen, and phosphorus. Quantitative analysis of the above chemical groups allows an estimate of the material requirements relative to the

resources of the environment, and establishes which metabolic pathways are relevant to reproductive systems. The biochemical composition of the gonad is very dependent upon the reproductive state of the individual.

Samples from populations of asynchronous individuals have increased variation which tends to mask environmental and reproductive state effects. Previous investigations in which the authors have combined samples from different individuals for convenience have limited use for this reason. The seasonal rate of synthesis and accumulation during development can only be determined if the specimens are separated by reproductive states. The biochemical results are often presented in terms of per cent dry weight, but the meaningful parameter is total amount per tissue, sometimes called the component level. Otherwise, a rise in one component may only reflect a decrease in a second. Furthermore, a true increase in a component can take place without the percentage changing. At times when the population is synchronous, rates of accumulation can be determined from the differences in bimonthly samples.

Large sample variation seems to be common for ovarian material. Giese (1966) found coefficients of variation of 19%, 30%, 60%, and 65% in the per cent dry weight of protein, lipid, glycogen, and non-protein nitrogen respectively in the starfish, Pisaster ochraceus. The coefficients of variation found in this study for

M. californianus (Tables 9-12) are of the same order of magnitude as those given by Giese. Based on the variation in the sampled population, a sample size of 40 to 100 animals would be required to obtain a mean with a 10% confidence interval. Because of this large variation, seasonal comparison of biochemical levels in a population is limited to large differences.

RNA is found in relatively small amounts, 2% to 5% dry weight. Histological examination reveals that previtellogenic oocytes have a basophilic cytoplasm associated with relatively high nucleic acid content. As oocyte development continues the staining reaction changes due to the addition of protein but large basophilic bodies are still seen in the cytoplasm. RNA was measured by the orcinol method for pentose sugars which is quite sensitive to the amounts present, but only 70% recovery results from the isolation procedure (Munro and Fleck, 1966). RNA increases 2.5 times between the storage (2 mg/gram animal) and the previtellogenic stages and three to six times with the formation of the mature stages (38 mg/gram animal).

Although not enough analyses were made to follow the rate of RNA increase directly, a value of 0.6 mg per day was estimated from the above component levels. RNA synthesis during oogenesis is mainly associated with ribosome production. The relatively high amount of RNA in the mature oocytes may indicate that in Mytilus

as in Ilyanassa, the egg contains all the ribosomes necessary for early development through gastrulation (Collier, 1966). The nutritional requirements for RNA synthesis include phosphate and the nitrogen containing amino acids used for purine and pyrimidine synthesis as well as carbohydrates.

Protein increases are associated with membrane development, enzyme and histone production during previtellogenesis, and yolk protein production during vitellogenesis. The large increase in cytoplasmic yolk protein is responsible for the acidophilic aspect of the cytoplasm at maturity in the histological samples. The biuret procedure measures peptide bonds by forming a purple colored compound which can be measured spectrophotometrically and is sensitive to amounts of 0.5 to 10 mg. Although there was 99% recovery of standards, loss occurs from lipoproteins going into the chloroform-methanol fraction.

The per cent dry weight of protein ranges from 22% (storage stage) to 63% (mature stage) and is similar to the 51% to 59% given by Giese (1966). Gabbott and Bayne (1973) recorded seasonal changes in the mantle of 90 to 140 mg of protein per gram in M. edulis. The maximum amount found for M. californianus was 470 mg per gram animal. The calculated rate of protein accumulation was 1.7 mg per day during previtellogenesis and 3.6 mg per day during yolk formation. This material is clearly not stored in the Leydig tissue and

probably comes directly from the ingested food. The period of protein increase is correlated with the suspected increase in the nitrogen to carbon ratio of the particulate food in the water in spring, as was documented by Russell-Hunter (1970).

Lipid is found in structural components which include the phospholipids and non-saponifiable sterols and the energy storing components such as the triglycerides in yolk. As Giese (1966) points out, lipids such as fatty acids and triglycerides are an ideal storage material because they liberate twice as much energy (9.3 kcal/g) as an equal weight of carbohydrate or protein (4.1 kcal/g), and water is not deposited in the tissues along with the fat as it is with glycogen. Minor lipids such as carotenoids may have specific functions. Campbell (1969) has shown that in M. edulis carotenoids are concentrated in the gonad and released with the eggs during spawning, but he does not suggest their function.

Lipid removal from the wet tissue was initiated with acetone to remove water and salts, and followed by chloroform-methanol extractions. The procedure results in 8% greater values in percentage dry weight lipid than ethyl ether extractions which are often used since it removes more of the polar lipids (Giese, 1966). The chloroform-methanol method removes a small amount of protein associated with the lipoproteins which is then weighed as lipid. Little loss due to

volatilization occurs during drying if the temperature is kept below 70°C.

The percentage dry weight of lipid was found to range from 2% (storage stage) to 23% (mature stage) and again is comparable to an average value of 7% given by Giese (1966) for this species. The lipid content increases three-fold from the storage stage to the previtellogenic stage at a rate of 0.59 mg per day per animal. This lipid can be considered essentially structural. An increase of three to four times at a rate of 1.8 mg per day per animal is found during yolk formation. The lipid level of a mature gonad was found to go as high as 138 mg per gram, and again M. californianus levels are higher than the 25 mg per gram reported by Gabbott and Bayne (1973) for M. edulis.

Most of the carbohydrate can be accounted for as glycogen although other carbohydrates are present. The vitelline coat surrounding the oocytes is composed mainly of mucopolysaccharides (Lunetta, 1969). The term glycogen is a functional definition for a group of carbohydrates that precipitate in 60% ethanol. Barnes (1963) distinguishes a TCA soluble glycogen and a TCA insoluble glycogen which includes glycoproteins. Little glycogen, however, was found associated with the TCA precipitated protein. Glycogen is correlated with Leydig tissue and in particular with the storage stage which can have 50% to 60% glycogen by dry weight. Zwann and Zandee (1972)

report a maximum of 48% glycogen in M. edulis. The range found in the Oregon population of M. californianus was 0 to 140 mg per gram. Gabbott and Bayne (1973) found in M. edulis a range of 0 to 150 mg per gram for mantle tissue.

During February 1973, gonads undergoing resorption were found to have 79 to 142 mg per gram glycogen, but little Leydig tissue was present. It is possible that the granular amoebocytes were temporarily storing the material while the gonadal follicles were still full. Throughout most of the year the values for total carbohydrate were mostly below 40 mg per gram. The population of M. californianus at Yaquina Head does not appear to have a major glycogen storage period. Zwann and Zandee (1972) suggest that the large reserve of glycogen in M. edulis is used in glycolysis to withstand anaerobic conditions during winter dormancy. M. californianus does not have a winter dormancy period in Oregon and is functioning at all times. The continual low levels of glycogen throughout the year may reflect the lack of extended anaerobic periods in this population. Giese (1969) suggests that nutrient storage in molluscs occurs generally in body components rather than in a localized organ, and Zwann and Zandee (1972) found at the mid-summer peak that only 39% of the glycogen was found in the mantle while 61% was located in the food, muscles, hepatopancreas, gills, and residue in M. edulis.

Rate and Percentage of Food Ingestion

Rates of food intake, transfer, and accumulation in the gonad were determined directly using pulse feeding of carbon-14 labeled Isochrysis galba. The composition of this flagellate differs from the diatoms and detritus containing chitin and cellulose consumed by the mussels in the field. Another limitation occurs because the label is distributed throughout all of the biochemical components of the alga rather than on a specific molecule. However, under uniform growth conditions using flagellates cultured for the same number of generations, the food and label composition were taken as constant.

The rate of clearance of particulate food from the water was found to be about 400 ml per hour per gram wet weight and is higher than the 100 to 150 ml per hour per gram wet weight given by Rao (1953) who used a graphite suspension. However, the values found in this study are consistent with those given for other bivalves by Winter (1969). He determined filtration rates on a continuous basis using another flagellate, Chlamydamonas, and found a mean rate of 200 ml per hour per gram. Winter has shown that there can be as much as a four-fold daily variation in the filtration rate of mussels. The data in this study indicate a seasonal change in the maximum daily filtration rate under the same laboratory conditions. The seasonal change is observed from calculations based on either the rate

per animal or the rate per gram wet weight. Since the filtered sea water used during the experiments was obtained fresh each month, there is a possibility that dissolved substances in the water influenced clearance rates. Changes in the feeding rates with season were also noted in the sea urchin, Strongylocentrotus intermedius, by Fuji (1967). In any event, there is a three-fold increase in food ingestion in the months of April to June compared to the rest of the year. The maximum value of 167 mg particulate carbon ingested per day is comparable to 140 to 400 mg per day found by Fox and Coe (1943).

Although the object of the radiotracer study was to estimate only the amount of ingested food transferred to the gonad, some observations can be made concerning the net food intake. True net assimilation results from the combined activity of rate of food flow through the gut, rate of digestion in the stomach, rate of absorption and further digestion by the digestive gland, and excretion of metabolites. The pulse feeding with carbon-14 does not give an estimate of the true net assimilation. As Sorokin (1966) points out, fecal composition of food passing directly through the gut does not equal food composition since organic material from mucus and intestinal secretions is added. He suggests that the carbon-14 method is unsatisfactory due to this exchange of food in the gut with unlabeled compounds in the gut wall, and the observed net flux is only a small fraction of the total flux. When specific activity of the carbon in the

food and the feces was compared, the lower specific activity in the feces indicated that considerable unlabeled carbon is being added. Furthermore, there may be leakage of dissolved organic molecules. Jacobs (1965) found that in the shrimp, Palaemonetes, excreted dissolved organic carbon was 33% of the amount ingested. However, within the first two days following food intake, neither the mucus nor the waste water showed any evidence of carbon-14 activity. It is possible that nitrogenous compounds are initially selectively absorbed from the food since the first fecal ribbons show a decrease in nitrogen relative to the food source. Thus instead of measuring net assimilation, net food intake, defined as the amount of particulate food cleared from the water less the amount of the initial feces, was considered. The percentage of net food intake was influenced by the number of cells ingested. Ingestion of 1.16 mg food per hour gave 95% net intake, and 5.8 mg food per hour gave 80% to 90% net intake. Kuenzler (1961), feeding phosphorus-32 labeled algae to Modiolus demissus, found an average of 11% in the feces. Using the constant feeding technique, Winter (1969) found at the same cell concentrations used in this study only 72% "utilization" by M. edulis. Widdows and Bayne (1971) who employed the ash-free gravimetric method on M. edulis report only 70% to 80% "assimilation" at one-tenth the food concentrations employed in this study. They also used a different food, Tetraselmis suecica, and food levels of 9 to 31 mg per animal

per day. In this study total daily ingestion by M. californianus was higher (12 to 58 mg per animal per day), and net intake was 10% higher than that of M. edulis. However, application of these values of net food intake to behavior in the field must be made with caution. The flagellate diet in the laboratory may be retained in greater amounts than the diatom and detritus diet in the field.

At high food levels net food intake was found to vary under different thermal and exposure conditions. The lowest net intake was found for 9°C submerged specimens. Increased intake at 15°C could be due to more rapid digestion, while increased intake with exposure could result from holding food in the gut longer. Any phenomenon which prolongs the time the food remains in the gut such as a low rate of feeding or low temperatures tends to increase incorporation (Fuji, 1967). It is not clear whether or not the lower efficiency at submerged 9°C conditions is compensated for by longer feeding times. Dehnel (1956) has shown that growth rate is greater for mussels submerged for longer periods, but Rao (1954) found evidence for possible tidal rhythms in feeding rate which affect continuously submerged mussels, as well as mussels undergoing tidal exposure.

The implication of the above is that M. californianus, an intertidal animal at +3 feet above MLLW, can achieve high food intake when food is available under temperature conditions which lower feeding rate. At low sea water temperatures an exposure at low tide shuts

off feeding and fecal production mechanisms which results in higher net food intake than under conditions of continuous submergence. This tidal effect reduces any temperature influence on net intake and increases the dependence of net intake on food availability more than it would be for continuously submerged filter feeding species under a seasonally varying thermal regime.

Allocation of Ingested Food

Allocation of the ingested nutrients is the next factor in determining the amount of resources going to the gonad. The period of growth, May to September, and that of gonad development, October to April, in M. edulis can be shown to be mutually exclusive by comparing data on the same population (Seed, 1969a, b). Coe and Fox (1942) demonstrated that growth is of secondary importance to reproduction in M. californianus. During periods of gonad formation, growth ceases and the thickening of the shell is recorded as a growth ring. Therefore, allocation of resources to other tissues is determined by the reproductive state.

Digestion begins in the stomach within hours of ingestion (Fox, 1936). Digested food is transferred to the gonad by means of the haemocoelic fluid. The control of the rate of transfer to the gonad is probably the oocyte growth rate itself. As the rate of egg growth increases, the fluid is depleted of nutrients creating a gradient

between gonad and digestive gland. During the carbon-14 incorporation study in 1973, the mussels used were in the mature or advanced developmental stages. The values for food transfer therefore represent values for the steady state condition of a mature gonad in which most of the food resource is going to growth.

The label in all the tissues accounted for 38% to 52% of the net amount incorporated. The remainder presumably is lost by respiration and excretion of soluble substances. Observations on the partitioning of the labeled food into the three tissue categories showed only 7% to 12% of the incorporated food going into the gonad and even distribution of the remaining label between the somatic tissue and the digestive gland. The amount of carbon-14 in the gonad attained a maximum value within 3 days. These observations are consistent with the hypothesis that at times of little gonad growth, most of the food is going to the somatic tissues. Thus, the 7% to 12% for gonadal incorporation should be considered minimal. As a comparison, Fuji (1967) found from 6% to 8% of the annual food taken by sea urchins goes into gonad growth.

Comparison of the amounts remaining in the gonads of specimens under the two temperature and exposure regimes showed no significant differences. For a maximum spring intake of 160 mg of oxidizable particulate carbon per day, about 10% or 16 mg per day is theoretically available for the gonad in animals living at the level of

+3 feet above MLLW. This value is comparable to the 6 to 10 mg per day calculated from the histological and biochemical data for the gonad in the spring of 1972. The agreement is closer if one considers that not all the chemically oxidizable material in the water is available to the mussel. Thus the possibility arises that gonad growth is limited by food availability. It should be recalled that the rate of vitellogenesis leading to mature oocyte formation can be correlated with environmental food levels.

The rate of loss of carbon-14 is faster from some mussel tissues than from others. For constantly submerged animals at 9°C, the loss of incorporated activity during the third to twenty-third day was 2.5% per day per gram wet weight for the digestive gland, 0.2% per day per gram for somatic tissue, and 0.08% per day per gram for gonadal tissue. These values give a qualitative estimate of the metabolic activity of the various tissues. The loss from the gonad was clearly minimal. The loss from the digestive gland including the stomach and its contents occurs as respiration, transfer of digested materials, and fecal material.

Distribution of Carbon-14 between Chemical Groups

Distribution of the carbon-14 in the chemical groups gives some indication of the compounds retained or synthesized by the mussel

gonad and allows comparison with the rates derived from the seasonal biochemical data. The lack of specificity limits the use of the carbon-14 at the biochemical level. While the broad classes of biochemical constituents were sufficiently fractionated for analysis, they are not as clean for radiotracer analysis. The TCA precipitate contains DNA in addition to protein. The glycogen fraction may contain some amino acids (Hassid and Abraham, 1955), and the RNA fraction contains some protein breakdown products (Shibko et al., 1967). There may also be interconversion of compounds through intermediate metabolism. In spite of these limitations, some implications can be drawn from the distribution of carbon-14 in the biochemical species.

The amount of material accumulated in any biochemical compartment at any time will be the net sum of incorporation and loss by interconversion of compounds through intermediary metabolism. The weight increase of each component was estimated by dividing the dpm in that fraction by the specific carbon activity of the same fraction in the food (Appendix II). This method does not distinguish between the transfer of a material and the production of a new substance by synthesis or interconversion. Nevertheless, it gives the order of magnitude of the quantities involved. About 50 μg of each component was formed within 3 days to give an approximate rate of 17 μg per day. In general the amounts found are about 1% of those found during times of rapid oocyte growth by the biochemical methods. One million

dpm is equivalent to 1.87 mg dry weight. Since a normal day's intake is 50 mg during most of the year and 160 mg during the spring, the observed rates would have to be multiplied by 27 and 86 to give between 460 and 1,460 μg per day respectively. The lower amount probably represents a steady state replacement incorporation since little oocyte development occurred during the spring of 1973 when the work was carried out. The larger value corresponds to those found by long-term biochemical techniques during the spring of 1972 and adds support to the validity of the calculated rates.

In mature animals at 15°C lipids were accumulated in only half the amount of either of the 9°C groups and may have been used as an energy source for the increased respiration at 15°C. The protein in the 15°C mature gonads was significantly higher than in those at 9°C, and the increased temperature appears to favor protein incorporation. Sastry and Blake (1971)followed gonad uptake of carbon-14 leucine in scallops and concluded that in addition to an increase in protein accumulation at higher temperatures as found here, nutrient transfer may also be regulated by the developing oocytes themselves. The variation in RNA incorporation into developing gonads was large, but with a greater sample size it would undoubtedly be found to be significantly higher than that of mature gonads. From the carbon-14 data there appears to be a tendency at the higher temperature for

compounds containing nitrogen to increase and the lipids and carbohydrates which store energy to decrease.

The biochemical picture becomes clearer when one considers the carbon-14 specific activity in dpm per mg. This parameter gives an indication of either the turnover rate of a component in the tissue or the relative amount of new material present and therefore implies which biochemical pathways might be active. In all cases the specific carbon-14 activity of the compounds in the developing gonads was slightly higher than that in mature or partially spawned gonads. The specific carbon-14 activity of RNA was 10 times that for the other components, and therefore the metabolic pathways for this material are quite active at all stages. The lower specific carbon-14 activities of protein relative to other components result from the lower percentage of carbon (33%) in proteins compared to 40%, 75%, and 42% for carbohydrates, lipids, and RNA respectively. Although protein is incorporated at the higher temperature, the effect is decreased by the large, and possibly inactive protein reservoir. Glycogen accumulation seems to be a very individual phenomenon, and large ranges occurred in this category.

Qualitative Differences in Eggs

The final topic for discussion is the qualitative differences between oocytes produced under different conditions. Based on the

rates determined from the radiotracer study, increasing temperature might decrease lipid content relative to the other components, but the absolute differences measured are not significant.

Analyses of spawned eggs showed a significant decrease in protein per egg from the fall of 1972 into the late winter, February, 1973. This trend is supported by the values calculated from the histological and biochemical samples. Although production of oocytes and partial spawning appeared to be continuous from the spring of 1972 into the spring of 1973, the eggs at the end of this period contained only half as much protein. M. californianus continued to make more eggs with less material. Russell-Hunter (1970) noted that in the salt marsh snail, Melampus, the ratio of nitrogen to carbon decreased 2.4 times between the first and third egg laying. He suggests that gonadal protein turnover was not keeping up with egg output. It will be recalled that the estimated residence time for mature oocytes in M. californianus was 200 days.

Collyer (1959) found no correlation between spawning success and glycogen content of oyster eggs. On the other hand, lower amounts of protein may have two possible results. First, the decrease in protein may be in the category of yolk protein and lead to smaller larvae. Fertilization of spawned eggs showed that eggs of all sizes developed into normal veligers. However, Bayne (1972) remarks that the greatest reliance on stored food reserves occurs during the

development of the foot and primary gill filaments. Larvae which are smaller than normal may be at a slight disadvantage in their natural environment.

The decrease in protein may also result in morphological changes. Bayne (1972) found that adults subjected to nutritive and thermal stress produced eggs giving rise to abnormal trochophore larvae. The parent mussels had a considerable loss of protein from their mantles. Changes might also occur in the amino acid composition as well as in the quantity of proteins. Jeffries (1972) has shown that seasonal stresses to the clam, Mercenaria mercenaria, produce a biochemical syndrome. Under stress taurine increases while glycine and alanine decrease.

Oogenesis in an Oregon population of M. californianus has been shown to differ from that of M. edulis in several respects. The glycogen storage stage is reduced, and the amounts of net food ingestion and allocation to gonad are greater in M. californianus. Reproductive events are not entrained to an annual cycle, but when conditions are favorable the reproductive response occurs within weeks. Partial spawning and egg resorption appear to predominate over complete spawning followed by a storage stage. The tidal effect on food utilization and the control of spawning could be a physiological adaptation for living in an environment with considerable thermal variation.

V. SUMMARY

1. Oogenesis was studied in a population of Mytilus californianus on a semi-protected shore at Yaquina Head on the Central Oregon Coast. This animal is a sessile filter feeder and produces large amounts of eggs.

2. An equation was developed for determining tissue temperature from continuously recorded physical data in order to determine the annual temperature trends and the daily rate of heating. Heating during exposure is an important contribution to mussel temperature during the spring.

3. Oxidizable particulate carbon was measured throughout the year and was found to range from 1 to 3 mg per liter sea water. During the spring, increases in particulate organics are associated with diatom blooms and possible disintegration products of gametes spawned from other animals. The rest of the year, detritus associated with wave action contributes a significant portion of the suspended oxidizable particulate carbon.

4. A germinal gonad index was developed, and the largest amount of gonadal tissue was 800 mg per gram dry weight of somatic tissue. Generally the population maintained a germinal gonadal index of about 60% of its potential maximum. The storage stage was found to be greatly reduced in this population.

5. Reproductive progress was studied by quantitatively following the categories of oogonial clusters as an indicator of mitosis, previtellogenic oocytes as an indicator of early meiotic activity, and mature oocytes as an index of vitellogenesis. Oogonia are not produced when mature oocytes are present. Synchronous previtellogenic processes were initiated in the spring when tissue temperature reaches 11 ^{o}C and food level is high; however, continual renewal occurs throughout the year. Mature oocyte formation is correlated with food availability. Spawning is related to periods of high daily heating rates. Partial spawning, oocyte lysis, and subsequent resorption of egg material appear to be the predominant fate of gonads.

6. The dynamic aspect of oogenesis was followed using the flow model of Olive (1971). Maximum rates of previtellogenic oocyte production reach 1.4×10^{5} oocytes per day and that for mature oocytes reached 2.5×10^{4} oocytes per day. Only 40% of the total oocytes produced throughout the year were lost by spawning.

7. Gonadal material was fractionated into the broad biochemical categories of protein, lipid, glycogen, and RNA to determine rates of accumulation and important pathways. During the spring, rates of accumulation of 0.6, 3.6, and 1.8 mg per day

were found for RNA, protein, and lipid respectively. Glycogen was rarely found in large amounts.

8. The flagellate, Isochrysis galba, uniformly labeled with carbon-14 was fed to mussels to directly determine the rate of incorporation and the percentage of food allocated to the gonad. In mature gonads, 7% to 12% of the food was found in the gonadal tissue within 3 days of feeding. Pathways associated with protein and RNA accumulation appear to be the most active. The rates for all components found using the radiotracer confirm those found indirectly by histological and biochemical methods.

9. The amount of protein per egg directly and indirectly determined was found to decrease as the reproductive year progressed.

10. The oogenic sequence was not repeated during the 2-year interval of observation. As a result of the timing of events and the observed variation in the population, elucidation of the relationship between reproductive phenomena and the environment by conventional techniques would require analysis of 50 to 70 specimens every 2 weeks for a period of at least 2 years.

BIBLIOGRAPHY

Barnes, H. 1963. Light, temperature, and the breeding of Balanus balanoides. Journal of the Marine Biological Association of the United Kingdom 43:717-727.

Barnes, H. and M. Barnes. 1968. Egg numbers, metabolic efficiency of egg production, and fecundity: Local and regional variations in a number of common cirripedes. Journal of Experimental Marine Biology and Ecology 2:135-153.

Bartlett, B. 1972. Reproductive ecology of the California sea mussel, Mytilus californianus Conrad. M.S. Thesis. University of the Pacific, Dept. of Zoology. 58 numb. leaves.

Bayne, B. L. 1972. Some effects of stress in the adult on the larval development of Mytilus edulis. Nature 237:459.

Bayne, B. L. and R. J. Thompson. 1970. Some physiological consequences of keeping Mytilus edulis in the laboratory. Helgolander wissenshaft Meeresunters 20:526-552.

Bernard, F. R. 1974. Annual biodeposition and gross energy budget of mature Pacific oysters, Crassostrea gigas. Journal of the Fisheries Research Board of Canada 31:185-190.

Berry, S. S. 1954. On the supposed stenobenthic habitat of the California sea mussel. California Fish and Game 40:69-73.

Bourke, R. H., B. Glenne, and B. W. Adams. 1971. The nearshore physical oceanographic environment of the Pacific Northwest coast. Reference 71-45. Oregon State University, Dept. of Oceanography. 127 p.

Bush, E. T. 1964. How to determine efficiency automatically in liquid scintillation counting. In: A collection of comprehensive scientific papers on the application and measurement of radioactivity. Nuclear-Chicago Corporation. p. 53-55.

Campbell, S. A. 1969. Seasonal cycles in the carotenoid content in Mytilus edulis. Marine Biology 4:227-232.

Chipperfield, P. N. J. 1953. Observations on the breeding and settlement of Mytilus edulis in British waters. Journal of the Marine Biological Association of the United Kingdom 32:449-476.

Coe, W. R. and D. L. Fox. 1942. Biology of the California sea mussel, Mytilus californianus. I. Influence of temperature, food supply, sex, and age on the rate of growth. Journal of Experimental Zoology 90:1-30.

Coe, W. R. and D. L. Fox. 1944. Biology of the California sea mussel (Mytilus californianus). III. Environmental conditions and rate of growth. Biological Bulletin 87:59-72.

Collier, J. R. 1966. The transcription of genetic information in the spiralian embryo. In: Current topics in developmental biology, Vol. I, ed. by A. A. Moscona and A. Monroy. New York, Academic Press. p. 39-59.

Collyer, D. M. 1957. Viability and glycogen reserves in the newly liberated larvae of Ostrea edulis. Journal of the Marine Biological Association of the United Kingdom 36:335-337.

Creech, C. 1973. Wave climatology of the central Oregon coast. Informal Report, School of Oceanography, Oregon State University.

Curl, H. and L. Small. 1968. Marine phytoplankton ecology lecture notes. Oceanography 544. Oregon State University.

Daniel, R. J. 1921. Seasonal changes in Mytilus edulis. Proceedings and Transactions of the Liverpool Biological Society (Session 1920-1921) 35:146-156.

Daniel, R. J. 1922. Seasonal changes in the chemistry of the mussel Mytilus edulis (continued). Proceedings and Transactions of the Liverpool Biological Society (Session 1921-1922) 36:269-285.

Daniel, R. J. 1923. Seasonal changes in the chemical composition of the mussel (Mytilus edulis) (concluded). Proceedings and Transactions of the Liverpool Biological Society (Session 1921-1922) 37:85-108.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs 41:351-389.

Dehnel, P. A. 1956. Growth rate in latitudinally and vertically separated populations of Mytilus californianus. Biological Bulletin 110:43-53.

Duursma, E. K. 1963. The production of dissolved organic matter in the sea, as related to the primary gross production of organic matter. Netherlands Journal of Sea Research 2:85-94.

Enright, J. T. 1970. Ecological aspects of endogenous rhythmicity. Annual Review of Ecology and Systematics 1:221-238.

Field, I. A. 1924. Biology and economic value of the sea mussel, Mytilus edulis. Bulletin U. S. Bureau of Fisheries 38:127-260.

Folch, J., I. Ascoli, M. Lees, J. A. Meath, and F. N. LeBaron. 1951. Preparation of lipid extracts from brain tissue. Journal of Biological Chemistry 191:833-841.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497-509.

Fox, D. L. 1936. The habitat and food of the California sea mussel. Bulletin of the Scripps Institution of Oceanography of the University of California. Technical series 4:1-64.

Fox, D. L. and W. R. Coe. 1943. Biology of the California sea mussel (Mytilus californianus). II. Nutrition, metablism, growth, and calcium deposition. Journal of Experimental Zoology 93:205-249.

Fraga, F. and M. Lopez-Capont. 1958. Variación estacional de la composición química del mejillón (Mytilus edulis). II. Hidratos de carbano. Investigación Pesquarea, Barcelona 11:33-37.

Fuji, A. 1967. Ecological studies on the growth and food consumption of Japanese common littoral sea urchin, Strongylocentrotus intermedius. Memoirs of the Faculty of Fisheries, Hokkaido University 15:83-160.

Gabe, M. 1955. Particularités histologiques des cellules neuro-sécrétrices chez quelques lamellibranches. Comptes Rendues Academy of Sciences, Paris 240:1810-1812.

Gabbott, P. A. and B. L. Bayne. 1973. Biochemical effects of temperature and nutritive stress on Mytilus edulis L. Journal of the Marine Biological Association of the United Kingdom 53:269-286.

Giese, A. C. 1959. Comparative physiology: annual reproductive cycles in marine invertebrates. Annual Review of Physiology 21:547-576.

Giese, A. C. 1966. Lipids in the economy of marine invertebrates. Physiological Review 46:244-298.

Giese, A. C. 1967. Some methods for study of the biochemical constituents of marine invertebrates. Oceanography and Marine Biology Review 5:159-186.

Giese, A. C. 1969. A new approach to the biochemical composition of the mollusc body. In: Oceanography and marine biology annual review, ed. by H. Barnes. London, George Allen and Unwin Ltd. 7:175-229.

Giese, A. C. and J. S. Pearse. 1974. Reproduction of marine invertebrates. I. Acoelomate and pseudocoelomate metazoans. New York, Academic Press. 546 p.

Gislen, T. 1943. Physiological and ecological investigations concerning the littoral of the Northern Pacific. Lunds Universitits Arsskrift, N. F. Avd 2. Bd. 39. Nr. 5. Kungl. Fysiografiska Sallskapets Handligar N. F. Bd. 54. Nr. 5. P. 1-63.

Glynn, P. W. 1965. Community composition, structure, and interrelationships in the marine intertidal Endocladia muricata-Balanus glandula association in Monterey Bay, California. Beaufortia, University of Amsterdam 12:1-198.

Gonor, J. J. 1968. Temperature relations of central Oregon marine intertidal invertebrates: A pre-publication technical report to the Office of Naval Research. Data Report No. 34. Oregon State University, Dept. of Oceanography. 49 p.

Gonor, J. J. 1972. Gonad growth in the sea urchin, Strongylocentrotus purpuratus (Stimpson) (Echinodermata: Echinoidea) and the assumptions of gonad index methods. Journal of Experimental Marine Biology and Ecology 10:89-103.

Gonor, J. J. and A. B. Thum. 1970. Sea surface temperature and salinity conditions in 1969 at Agate Beach and Yaquina Bay, Oregon. Data Report 39. Oregon State University, Dept. of Oceanography. 26 p.

Gonor, J. J., A. B. Thum, and D. W. Elvin. 1970. Inshore sea surface temperature and salinity conditions at Agate Beach, Yaquina Bay, and Whale Cove, Oregon in 1970. Data Report 45. Oregon State University, Dept. of Oceanography. 30 p.

Good, C. A., H. Kramer, and M. Somogyi. 1933. The determination of glycogen. Journal of Biological Chemistry 100:485-491.

Hassid, W. Z. and S. Abraham. 1955. Chemical procedures for analysis of polysaccharides. In: Methods in enzymology, Vol. III, ed. by S. P. Colowick and N. O. Kaplan. New York, Academic Press. p. 447-454.

Hutchinson, G. E. 1957. A treatise on limnology. I. Geography, physics, and chemistry. New York, John Wiley and Sons, Inc. 1015 p.

Jacobs, F. A. 1965. Bidirectional flux of amino acids across the intestinal mucosa. Federation Proceedings 24:946-952.

Jeffries, H. P. 1972. A stress syndrome in the hard clam, Mercenaria mercenaria. Journal of Invertebrate Pathology 20:242-251.

Jorgensen, C. B. 1949. The rate of feeding by Mytilus in different kinds of suspension. Journal of the Marine Biological Association of the United Kingdom 28:337-344.

Kinne, O. 1970. Temperature-invertebrates. In: Marine ecology, ed. by O. Kinne. London, Wiley-Interscience. p. 407-514.

Kuenzler, E. J. 1961. Phosphorus budget of a mussel population. Limnology and Oceanography 6:400-415.

Layne, E. 1955. Spectrophotometric and turbidimetric methods for measuring proteins. In: Methods in enzymology, Vol. III, ed. by S. P. Colowick and N. O. Kaplan. New York, Academic Press. p. 447-454.

Loomis, W. D. 1972. Professor, Oregon State University, Dept. of Biochemistry and Biophysics. Personal communication.

Loosanoff, V. L. 1937. Seasonal gonadal changes of adult clams, Venus mercenaria (L.). Biological Bulletin 72:406-416.

Loosanoff, V. L. 1951. Delaying spawning of the lamellibranchs by low temperatures. Journal of Marine Research 10:197-202.

Loosanoff, V. L. and H. C. Davis. 1950. Conditioning of Venus mercenaria for spawning in winter and breeding its larvae in the laboratory. Biological Bulletin 98:60-65.

Loosanoff, V. L. and H. C. Davis. 1952. Temperature requirements for maturation of gonads of northern oysters. Biological Bulletin 103:80-96.

Lowry, W. P. 1969. Weather and life, an introduction to biometeorology. New York, Academic Press. 305 p.

Lubet, P. 1959. Recherches sur le cycle sexuel et l'emission des gametes chez les mytilides et les pectinides. Revue des Travaux de l'Institut des Pêches Maritimes 23:395-547.

Lubet, P. 1969. Données expérimentales sur les effets des ablations des ganglions nerveux chez les mollusques pélécypodes. In: Cultures d'orgones d'invertébrés, ed. by H. Lutz. New York, Gordon and Breach. p. 161-173.

Lunetta, J. E. 1969. Fisiologia da reprodução dos mexilhões (Mytilus perna-mollusca lamellibranchia). Zoologia e Biologia Marinha, Sao Paulo no. 26:33-111.

Malouf, R. E. 1971. Food consumption and growth of the larvae of the Pacific oyster, Crassostrea gigas. M. S. Thesis. Oregon State University, Dept. of Fisheries and Wildlife. 82 numb. leaves.

Mason, J. 1972. The cultivation of the European mussel, Mytilus edulis Linneas. In: Oceanography and marine biology, an annual review, ed. by H. Barnes. London, George Allen and Unwin Ltd. 10:437-460.

Medcof, J. C. and C. J. Kerswill. 1965. Effects of light on the growth of oysters, mussels, and quahaugs. Journal of the Fisheries Research Board of Canada 22:281-288.

Moon, T. W. 1969. Aspects of respiration in vertically separated Mytilus californianus (California sea mussel). M. S. Thesis. Oregon State University, Dept. of Zoology. 62 numb. leaves.

Moore, H. B., T. Jutare, I. A. Jones, B. F. McPherson, and C. F. E. Roper. 1963. A contribution to the biology of Tripneustes esculentus. Bulletin of Marine Science of the Gulf and Caribbean 13:267-281.

Munro, H. V. and A. Fleck. 1966. The determination of nucleic acids. In: Methods of biochemical analysis, Vol. XIV, ed. by D. Glick. New York, Interscience Publishers. p. 113-176.

Nagabhushanam, R. 1962. Physiological experiments on the neurosecretion in the oyster, Crassostrea virginica. American Zoologist 2:543.

Nagabhushanam, R. 1963. Some observations on the neurosecretion in the bivalve mollusc, Branchidontes recurvus. Journal of the Anatomical Society of India 12:54-56.

Newell, R. C. 1970. Biology of intertidal animals. Great Britain, Logos Press Limited. 555 p.

Olive, P. J. W. 1970. Reproduction of a Northumberland population of the polychaete, Cirratulus cirratus. Marine Biology 5:259-273.

Olive, P. J. W. 1973. The regulation of ovary function in Cirratulus cirratus (Polychaeta). General and Comparative Endocrinology 20:1-15.

Paine, R. T. 1964. Ash and caloric determinations of sponge and opisthobranch tissues. Ecology 45:384-387.

Panshin, D. A. 1967. Sea level, winds, and upwelling along the Oregon coast. M. S. Thesis. Oregon State University, Dept. of Oceanography. 71 numb. leaves.

Pearse, J. S. 1968. Patterns of reproductive periodicities in four species of Indo-Pacific echinoderms. Proceedings of the Indian Academy of Sciences 68:247-275.

Price, K. S. and D. Maurer. 1971. Temperature requirements of gonads of oysters. Proceedings of the National Shellfish Association 61:29-34.

Radin, N. S. 1972. How to prepare samples for liquid scintillation counting. In: A collection of comprehensive scientific papers on the application and measurement of radioactivity. Nuclear-Chicago Corporation. p. 45-47.

Rankin, E. P. 1918. The mussels of the Pacific coast. California Fish and Game 4:113-117.

Rao, K. P. 1954. Tidal rhythmicity of the rate of water propulsion in Mytilus and its modification by transplantation. Biological Bulletin 106:353-359.

Reiswig, H. M. 1971. Particle feeding in natural populations of three marine demosponges. Biological Bulletin 141:568-591.

Reverberi, G. 1967. Some observations on the ultrastructure of the ovarian Mytilus egg. Acta Embryologiae et Morphologiae Experimentalis 10:1-14.

Roberts, D. 1972. The assimilation and chronic effects of sub-lethal concentrations of endosulfan on condition and spawning in the common mussel, Mytilus edulis. Marine Biology 16:119-125.

Roberts, R. B., P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten. 1957. Studies of biosynthesis in E. coli. Carnegie Institution of Washington Publication 607. 521 p.

Russell-Hunter, W. D. 1970. Aquatic productivity, an introduction to some basic aspects of biological oceanography and limnology. New York, Macmillan. 306 p.

Sastry, A. N. 1968. The relationships among food, temperature, and gonad development in the bay scallop, Aequipecten irradians Lamarck. Physiological Zoology 41:444-453.

Sastry, A. N. 1970. Environmental regulation of oocyte growth in the bay scallop Aequipecten irradians Lamarck. Experientia 26:1371-1372.

Sastry, A. N. and N. J. Blake. 1971. Regulation of gonad development in the bay scallop, Aequipecten irradians Lamarck. Biological Bulletin 140:274-283.

Sattler, L. and F. W. Zerban. 1950. Limitations of the anthrone test for carbohydrates. Journal of the American Chemical Society 72:3814.

Savage, R. E. 1956. The great spatfall of mussels in the R. Conway Estuary in the spring of 1940. Fishery Investigations (London) Ser. II 20:1-21.

Schneider, W. C. 1955. Determination of nucleic acids in tissues by pentose analysis. In: Methods in enzymology, Vol. III, ed. by S. P. Colowick and N. O. Kaplan. New York, Academic Press. p. 680-684.

Seed, R. 1969a. The ecology of Mytilus edulis on exposed rocky shores. I. Breeding and settlement. Oecologia (Berlin) 3:277-316.

Seed, R. 1969b. The ecology of Mytilus edulis on exposed rocky shores. II. Growth and mortality. Oecologia (Berlin) 3:317-350.

Sheldon, R. W. 1972. Size separation of marine seston by membrane and glass fiber filters. Limnology and Oceanography 17:494-498.

Shibko, S., P. Koivistoinen, C. A. Tratnyek, A. R. Newhall and L. Friedman. 1967. A method for sequential quantitative separation and determination of protein, RNA, DNA, lipid, and glycogen from a single rat liver homogenate or from a subcellular fraction. Analytical Biochemistry 19:514-528.

Soot-Ryen, T. 1955. A report on the family Mytilidae (Pelecypoda). Allan Hancock Pacific Expeditions 20:1-174.

Sorokin, J. I. 1966. Carbon-14 method in the study of the nutrition of aquatic animals. Internationale Revue der Gesanten Hydrobiologie 51:209-224.

Stephens, K., R. W. Sheldon, and T. R. Parsons. 1967. Seasonal variations in the availability of food for benthos in a coastal environment. Ecology 48:852-855.

Stohler, R. 1930. Beitrag zur Kenntnis des Geschlecthszyklus von Mytilus californianus. Zoologia Anzeiger 90:263-268.

Strickland, J. D. H. and T. R. Parsons. 1968. A practical handbook of seawater analysis. Fisheries Research Board of Canada Bulletin 167. 311 p.

Takatsuki, S. I. 1934. On the nature and function of the amoebocytes of Ostrea edulis. Quarterly Journal of Microscopical Science 76:379-431.

Thorson, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates with special reference to the planktonic larvae of the sound (Øresund). Kommissionen for Dansmark Fiskeri. Havunders. Ser. Plankton 4. 523 p.

Thum, A. B. 1972. An ecological study of Diatomovora amoena, an interstitial acoel flatworm, in an estuarine mudflat on the central coast of Oregon. Ph. D. Thesis. Oregon State University, Dept. of Zoology. 185 numb. leaves.

Tranter, D. J. 1958. Reproduction in Australian pearl oysters. II. Pinctada albina: gametogenesis. Australian Journal of Marine and Freshwater Research 9:144-158.

Umiji, S. 1969. Neurosecreção em Mytilus perna. Zoologia e Marinha, Sao Paulo no. 26 :181-254.

Van Handle, E. 1965. Microseparation of glycogen, sugars, and lipids. Analytical Biochemistry 11:266-271.

Wang, C. H. and D. L. Willis. 1965. Radiotracer methodology in biological science. Englewood Cliffs, New Jersey, Prentice Hall, Inc. 382 p.

Whedon, W. F. 1936. Spawning habits of the mussel Mytilus californianus with notes on the possible relation to mussel poison. University of California Publications in Zoology 41:35-44.

White, K. M. 1937. Mytilus. Liverpool Marine Biological Commission 31:1-117.

Widdows, J. and B. L. Bayne. 1971. Temperature acclimation of Mytilus edulis with reference to its energy budget. Journal of the Marine Biological Association of the United Kingdom 51:827-843.

Williams, C. S. 1969. The effect of Mytilicola intestinalis on the biochemical composition of mussels. Journal of the Marine Biological Association of the United Kingdom 49:161-173.

Wilson, B. R. and E. P. Hodgkin. A comparative account of the reproductive cycles of five species of marine mussels in the vicinity of Fremantle, Western Australia. Australian Journal of Marine and Freshwater Research 18:175-203.

Winter, J. E. 1969. Uber den Einfluss der Nahrungskonzentration und anderer Faktoren aur Filtrierleistung and Nahrungsausnutzung der Muscheln Artica islandica and Modiolus modiolus. Marine Biology 4:87-135.

Young, R. T. 1941. The distribution of the mussel, Mytilus californianus, in relation to the salinity of its environment. Ecology 22:379-386.

Young, R. T. 1942. Spawning season of the California mussel, Mytilus californianus. Ecology 23:490-492.

Young, R. T. 1945. Stimulation of spawning in the mussel, Mytilus californianus. Ecology 26:58-69.

Young, R. T. 1946. Spawning and setting season of the mussel, Mytilus californianus. Ecology 27:354-363.

Zwaan, A. De and D. I. Zandee. 1972. Body distribution and seasonal changes in the glycogen content of the common sea mussel, Mytilus edulis. Comparative Biochemistry and Physiology 43A:53-58.

APPENDIX I

CULTURE METHOD FOR LABELED ISOCHRYSIS GALBA

The culturing technique developed for this flagellate was based on methods described by Malouf (1970). The formula was as follows:

600 ml of U. V. filtered sea water ($32^{o}/_{oo}$, $23^{o}C$)
5 ml NaH_2PO_4 (4 mg/ml P)
5 ml KNO_3 (8 mg/ml N)
1 ml Vitamin B_{12} (5.1 µg/l)
1 ml of trace metal mix:
- 8.0 ml $CuSO_4$ (1.96 g/l)
- 0.5 ml $ZnSO_4$ (4.40 g/l)
- 5.0 ml $CoCl_2$ (2.00 g/l)
- 10.0 ml $NaMO_4$ (1.26 g/l)
- 1.0 ml Fe-EDTA (10.0 g/l)

0.5 ml $MnCl_2$ (36 g/l)
0.2 ml Thiamine (1.05 mg/l)
0.2 ml Biotin (7.4 µg/ml)

For culturing the above media was made fresh, and 100 ml of cells in log phase were added giving a concentration of about 20 to 50 x 10^4 cells/ml. The culture was constantly aerated and placed 6 inches from fluorescent lamps. After the cells had grown for at least one generation and log growth was in progress, 10 ml of $Na_2C^{14}O_3$ (2.5 µCi/ml; 67 µg Na_2CO_3/ml) was added. This gave a final solution of 42 µCi/liter but a low specific activity of carbonate. The culture was grown for 2 to 3 days to get label evenly distributed and consistently showed growth constants of $K_{10^oC} = 0.014$ and $K_{23^oC} = 0.023$. At this time 36% of the label was incorporated into the flagellates giving an approximate activity of 6.2 x 10^3 dpm/million cells.

APPENDIX II

CHEMICAL CHARACTERISTICS OF CULTURED ISOCHRYSIS GALBA

Dry weight of cells: 11.6 μg/million cells

8.62 x 10^{9} cells/mg dry weight

Caloric value of cells: 1.48 x 10^{-2} glucose units/million cells

5.53 x 10^{-2} calories/million cells

Weight of carbon and nitrogen: 1.48 x 10^{-3} mg carbon/million cells

2.44 x 10^{-4} mg nitrogen/million cells

Chemical and Label Composition of Cultured Cells

Component	Dry Weight (μg/million cells)	Biochemical Percentage	Label Percentage	Specific Activity (10^{5} dpm/mg)
Protein	6.06	45.0	9.5	4.5
Lipid	5.25	39.0	21.8	4.5
RNA	1.76	13.0	20.5	4.6
Glycogen	0.30	2.0	13.4	4.0
Free sugars	0.02	0.2	-	-
Residual	-	-	34.7	-
Total	13.39	99.2	99.9	

APPENDIX III

FIELD DATA FOR TISSUE TEMPERATURE EQUATIONS

Date	Insolation (ly/min)	Air Temp. (°C)	Substrate Temp. (°C)	Vapor Pressure Diff. (mm Hg)	T_{mussel} Obs.	T_{mussel} Calc.
16 June 70	1.00	17.7	-	16.0	24.9	22.8
	1.16	18.2	-	10.0	22.4	24.1
10 June 71	0.24	11.9	-	1.0	12.5	13.1
14 June 71	1.30	20.0	-	20.4	28.8	26.6
6 Aug. 71	0.36	17.4	14.0	3.0	20.3	19.2
	0.44	14.8	15.0	2.7	16.8	17.0
7 Aug. 71	0.36	17.7	14.0	0	18.7	19.5

Statistical Characteristics of Temperature Values in the Field Population

Date	N	Average	t_{90} (SE)	Range
16 June 70	3	24.9	6.5	20.4 - 27.2
	3	22.4	2.6	20.6 - 23.3
14 June 71	6	28.8	1.0	26.6 - 30.0
4 Aug. 71	4	18.7	1.8	17.2 - 20.2

APPENDIX IV

DEVELOPMENT OF THE GERMINAL GONAD INDEX

It was required that the total dry weight of the gonad be partitioned into its components. However, the conversion from area to weight cannot be made directly since the water contents are not the same. Tissue samples of pure Leydig cells have 13% dry weight while that of the mantle with complete germinal tissue is about 24%. The density of mantle tissue is 1.13 g/ml.

The wet weight volume percentage of each component is proportional to the percentage area seen on the microscope section.

$$\%V_{Leydig} = \%A_{Leydig}$$

$$\%V_{germinal} = \%A_{germinal}$$

where V and A are the volumes and areas respectively.

The dry weight present in each volume is

Dry weight = volume (% dry weight) (density)

The percentage dry weight of the germinal tissue is then

$$\% \text{ Germinal dry weight} = \frac{\% A_{germinal}(0.24)1.13}{\% A_{Leydig}(0.13)1.13 + \% A_{germ}(0.24)1.13}.$$

Since $\% A_{Leydig} = 1 - \% A_{germinal}$ and since the densities cancel we can say:

$$\% \text{ Germinal dry weight} = \frac{\% A_{germ}\,(0.24)}{(1 - \% A_{germ})(0.13) + \% A_{germ}(0.24)}$$

or

$$= \frac{0.24\,(\% A_{germ})}{0.13 + 0.11\,(\% A_{germ})} .$$

This factor times the gonad index gives total dry weight of the germinal tissue per gram somatic tissue:

$$\text{G. G. I.} = \text{G. I.} \times \left[\frac{0.24\,(\% A_{germ})}{0.13 + 0.11\,(\% A_{germ})} \right]$$

APPENDIX V

DERIVATION OF THE FORMULA FOR COMPUTING TOTAL NUMBER OF EGGS PER ANIMAL FROM CELL DENSITIES

Number of previtellogenic oocytes (N_{pvo}) = D x K x F

where

D = density of oocytes, number per follicle cross section

K = counting correction factor for size (1/1.2)

F = number of follicles/mm^2 of germinal tissue which is equivalent to number/10^6 μ^2

Since 1 mm^2 of a 7 μ section is equal to 7×10^{-6} ml, then the number of oocytes per ml is found by multiplying by 1.43×10^5 or

$$N_{pvo}/cc = D \times F \times 1.19 \times 10^5$$

The volume of the germinal tissue is found by

$$\frac{\text{Germinal gonad index}}{\text{density}}$$

Since the density of the tissue is 1.13 g/ml and the germinal gonad index (G.G.I.) is given in per cent, the formula reduces to:

$$N_{pvo}/\text{animal} = D \times F \times (G.G.I.) \times 1.06 \times 10^3$$

The number of mature oocytes is estimated by:

$$N_{mo}/cc = D_{mo} \times C \times (G.G.I.) \times \text{density}^{-1} \times (1.43 \times 10^5 \text{ mm}^2/cc)$$

where

D_{mo} = density of mature oocytes, as number/$\mu^2 \times 10^6$ or number per square mm

C = a counting correction factor for size of (1/2).

Density is again taken as 1.13 g/ml giving

$$N_{mo}/\text{animal} = D_{mo} \times (G.G.I.) \times (6.36 \times 10^{2}).$$

APPENDIX VI

DERIVATION OF THE FORMULA FOR ESTIMATING BIOCHEMICAL COMPOSITION OF EGGS IN THE OVARY

The total amount of a component present is due to four groups of tissues: previtellogenic oocytes, mature oocytes, storage tissue, and residual tissue. The total amount is then given by:

$$A_p(N_{pvo}) + A_m(N_{mo}) + A_s(\text{storage}) + R = \text{total amount}$$

where N_{pvo} and N_{mo} are the total numbers of previtellogenic oocytes and mature oocytes respectively; A_p and A_m are the amounts of the component per egg; A_s is the amount associated with storage or Leydig tissue, and R is the residual contribution.

For gonads of mature animals storage and residual contributions were considered negligible, and the equation reduces to

$$A_p(N_{pvo}) + A_m(N_{mo}) = \text{Total}$$

The constant, A_p, was found by solving two representative equations simultaneously. The equation was then rearranged to allow calculation of the amount of biochemical component/mature oocyte as follows:

$$A_m = \frac{\text{Total} - A_p(N_{pvo})}{N_{mo}} .$$

It is realized that this is a rough approximation and as such is used only to support other data. A_p was taken to equal 5.18 mg/million eggs.

APPENDIX VII

THEORETICAL DEVELOPMENT OF THE TISSUE TEMPERATURE EQUATION

The mussel bed was considered to be a two dimensional interface with a heat budget at equilibrium (Lowry, 1965) represented by:

$$Q_s + Q_L + Q_b + Q_{cv} + Q_{evap} + Q_{met} = 0$$

where each component is in units of cal/min/cm^2. Q_s is the rate of absorption of short wave solar radiation and is given by:

$$Q_s = K_1 \text{ (langleys/minute)}.$$

The constant K_1 combines the coefficient of absorption and a coefficient for reflectance which is modified by encrusting barnacles. Q_L is the long wave radiation. For a cloudless day, Hutchinson (1957) has estimated the value of this component to be

$$Q_L = 8.26 \times 10^{-11} (T_{air})^4 (0.820 - 0.025 \times 10^{-0.126\ vp})$$

where the temperature is in degrees Kelvin and the vp is the vapor pressure of water in mm of mercury. Q_b is the long wave back radiation from the mussel bed itself and is equivalent to

$$Q_b = (e) \times (8.17 \times 10^{-11}) \times (T_{mussel})^4$$

where e stands for the emissivity and T_m is again in degrees Kelvin. The <u>net</u> long wave radiation would then be

$$Q_b - Q_L = 8.17 \times 10^{-11} (e_m T_m^4 - e_{air} T_{air}^4).$$

Hutchinson (1957) suggests a close approximation for this net long wave energy to be

$$Q_b - Q_L = 7.6 \times 10^{-3} (T_{mussel} - T_{air}).$$

This simplifies the calculations considerably. Q_{cv} is the convective heat loss to the air above the mussel bed. For free convection (in contrast to forced convection)

$$Q_{cv} = h (T_m - T_{air}).$$

Strictly speaking, the coefficient of convection, h, is a function of the temperature differences

$$h = 3.6 \times 10^{-3} (T_m - T_{air})^{1/4}.$$

For a first approximation this change in the coefficient with temperature was neglected. The net long wave radiation loss could then be combined into one expression:

$$Q_L - Q_b - Q_{cv} = K_2 (T_{mussel} - T_{air}).$$

Q_{cd} is the heat loss by conduction to or from the substrate and is given by

$$Q_{cd} = C_3 \frac{(T_{mussel} - T_{substrate})}{\text{distance}}.$$

If the distance is measured in the substrate the same way then the expression could be given by:

$$Q_{cd} = K_3 (T_{mussel} - T_{substrate}).$$

There is also conduction to the air but it is two orders of magnitude less than that due to convection. Q_{evap} is the heat loss due to evaporation. It takes the form

$$Q_{evap} = K_4 (vp_{mussel} - vp_{air}) W^b (540 \text{ cal/g}) .$$

The vapor pressure of the mussel bed is a function of temperature and W^b is the wind speed to some power. Relative humidity at Yaquina Head is generally rather high, but can drop to 50%. Laboratory experiments demonstrated that at high temperatures and low humidities, mussels closed and did not lose water. However, in the mussel bed there is water between the mussels which can evaporate. Q_{met} is the metabolic heat which is very small for this animal.

When the above expressions and modifications are substituted into the original equation one obtains:

$$K_1 (\text{langleys/min}) = K_2 (T_{mussel} - T_{air}) + Q_{cd} - Q_{evap} .$$

Conduction to the substrate and heat loss by evaporation were an order of magnitude smaller than that of the other components and were neglected for a first approximation. The remaining parameters give

$$K_1 (\text{langleys/min}) = K_2 (T_m - T_{air})$$

or

$$T_{mussel} = T_{air} + K_{12} (\text{langleys/min}) .$$

The single constant was evaluated from three types of easily obtainable data and found from even sets of field observations (Appendix III) to average 5.07 ± 1.7 (range 2.5 to 8.1). When it is substituted into the formula one gets

$$T_{mussel} = T_{air} + 5.07 \text{ (langleys/min)} .$$