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Title: SEASONAL GAMETOGENESIS AND EGG LAYING IN THE PROSOBRANCH GASTROPODS NUCELLA LAMELLOSA, NUCELLA EMARGINATA, SEARLESIA DIRA AND AMPHISSA COLUMBIANA ON THE OREGON COAST

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J. J. Gonor

A 15-month field study was made on the reproductive cycles of the prosobranch gastropods Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana on the Oregon coast. Amphissa columbiana and Searlesia dira were collected from Boiler Bay, Nucella emarginata from Seal Rock and Nucella lamellosa from inside Yaquina Bay.

All of these species are carnivores and produce relatively few large eggs which are deposited in capsules. Each species produces a non-pelagic crawling larva whose developmental time is highly temperature dependent.

The seasonal gametogenic process in females was studied by measuring oocytes in histological sections. Since these oocytes are elliptical in cross section due to packing in the ovary, the long and short axis of each oocyte was measured and converted to the diameter

of a circle of equivalent area.

The time required to produce an egg is approximately 20 months in Nucella lamellosa, 16 months in Searlesia dira and Amphissa columbiana and 14 months in Nucella emarginata. Daily relative growth rates for oocytes are initially low in all four species, approximately 1.6% per day, increasing two or three months prior to spawning to 5.6% in Nucella emarginata, 4.9% in Searlesia dira and 5.4% in Amphissa columbiana, but only 2.0% in Nucella lamellosa. Previtellogenic oocytes begin to appear when middle-sized oocytes begin to grow rapidly to larger sizes. This rapid increase in oocyte growth rates begins in July for Amphissa columbiana and Searlesia dira and in September for Nucella emarginata and Nucella lamellosa.

In males of all four species spermatogenesis continues throughout the year except for a brief period immediately after spawning. Spermatogenesis is not well correlated with environmental temperature or salinity changes, but the percentage of sperm in tubules of the testis does decrease during the spawning season.

Amphissa columbiana, Searlesia dira and Nucella emarginata begin spawning in October and November at the time coastal upwelling ceases. Nucella lamellosa, found inside Yaquina Bay, does not begin spawning until June, well past the period of extremely low salinities of December and January.

The oogenic cycles of Nucella lamellosa, Searlesia dira and

Amphissa columbiana are adapted to maximize fecundity through the use of a restricted breeding season and a fixed-sized juvenile emerging at the time of the year when food availability for juveniles is at a maximum. Fecundity is maximized by Nucella emarginata in response to an increased food supply by repeated spawnings over a long period of time. Juvenile size at hatching varies in this species due to a constant number of food eggs and a variable number of embryos placed in each capsule.

Seasonal Gametogenesis and Egg Laying in the Prosobranch Gastropods *Nucella lamellosa*, *Nucella emarginata*, *Searlesia dira* and *Amphissa columbiana* on the Oregon Coast

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INTRODUCTION

Purpose of this Study

Quantitative studies of reproductive cycles in Prosobranch snails of the Order Neogastropoda are few. Similarities and differences among species with internal fertilization and direct development in egg capsules have not been well explored. Correlations with envivonmental parameters which might help in understanding the adaptive significance of observed reproductive patterns have been made in only a very few species. This study compares several species from the same habitat type which: 1) overlap extensively in their latitudinal distributions; 2) are gonochoristic and have internal fertilization; 3) deposit large eggs in capsules in the intertidal region and 4) have direct development.

The neogastropod species examined in this study are *Nucella lamellosa* (Gmelin, 1791) and *Nucella emarginata* (Deshayes, 1839) (Superfamily Muricacea, Family Thaisidae), *Searlesia dira* (Reeve, 1846) (Superfamily Buccinacea, Family Buccinidae) and *Amphissa columbiana* Dall, 1916 (Superfamily Buccinacea, Family Columbellidae) according to the classification of Keen and Coan (1974). These

are intertidal species whose vertical ranges overlap but do not completely coincide. Nucella lamellosa, Searlesia dira and Amphissa columbiana all deposit their egg capsules in the lower portion of their vertical ranges, while Nucella emarginata deposits its egg capsules in the high intertidal at the upper edge of its vertical range. Development in all four species is direct and crawling juveniles emerge from the capsules. Kitching (1976) found Nucella emarginata to be very well adapted to high intertidal wave-exposed situations on the basis of its shell morphology. Using the same shell morphology criteria Nucella lamellosa was found to be specialized for low intertidal levels in sheltered situations.

These species also overlap extensively in their latitudinal distributions on the west coast of North America. Nucella lamellosa occurs on the west coast of North America from the Bering Straits to Santa Cruz, California; Nucella emarginata from the Bering Sea to Mexico; Searlesia dira from Alaska to Monterey, California; and Amphissa columbiana from Alaska to San Pedro, California (Abbott, 1974). The overlap of the geographical ranges of these four species extends from approximately 37° N to approximately 57° N. Yaquina Bay (44° 37' N) represents a point well within the sympatric portion of the ranges of these species.

Reproductive synchrony and regulation of the timing of the events of gametogenesis are central to understanding the adaptive

significance of observed reproductive patterns. Environmental parameters such as temperature, salinity, photoperiod and nutrition may all be interrelated in the control of reproduction. Cycles in the levels of several biochemical constituents of various body organs are known for many invertebrates (e. g. Giese, 1966, 1969). Metabolic shifts change the proportion of incoming or stored energy channeled into somatic or gonadal growth. Neuroendocrine mechanisms, known for many vertebrates and a few invertebrates, probably control reproduction in most animals. The fact that most species reproduce during discrete breeding seasons implies that changes in environmental factors serve as cues to regulate reproduction. These cues are proximate factors which may insure that reproduction will take place at a time most suitable for survival of the young. Temperature, salinity, day length and food availability, separately or in concert, have each been shown to synchronize reproductive activities, either gametogenesis or spawning, in a few marine invertebrates.

Several methods are useful in determining which environmental cues may be important in synchronizing reproductive processes in a species. Determination of breeding times at several points within the geographical range of a species, coupled with good physical and biotic data, may suggest the importance of some factors over others. If the reproductive cycle of a species is synchronized over a wide geographical area, then local fluctuations in measured parameters may not be

important. If local fluctuations are not important, then reproductive rhythm encountered in the field should be maintained when the animals are brought into the laboratory. While an environmental cue may be correlated with a given reproductive process, that same cue may not directly initiate the process. Experiments which bracket the suspect parameter should be performed to test any hypothetical cue. An attempt was made in this study to experimentally study temperature as a controlling factor.

Among the few quantitative histological studies that have been done on the reproductive cycles of gastropods are those by Webber and Giese (1969), Kudinskii (1972) and Underwood (1972) on Archaeogastropods and the studies by Feare (1970a), Houston (1971), Manzi, Calabrese and Rawlins (1972) and Lambert and Dehnel (1974) on Neogastropods.

The reproductive cycle in both males and females of Nucella lapillus in Great Britain was quantified by Feare (1970a). He examined external features of the gonad immediately after removing the animals from their shells and measured the proportions of the constituent stages of spermatogenesis and oogenesis. In males, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperm were recognized by nuclear characteristics and by the size of the nuclei of the individual cells. He then calculated the percentage of apparently ripe sperm occupying the central tubules of

the testis. In females, oocytes were characterized as pre-vitellogenic, intermediate or post-vitellogenic and their numbers per unit area of ovary tissue were counted. Feare (1970a) concluded that the spermatogenic cycle of Nucella lapillus required 14-15 months, with an overlap of two to three months between successive cycles since the species breeds every year. In female Nucella lapillus he found only a slight overlap of cycles similar to that described for males, with the main production of oocytes occurring immediately after spawning, rather than before (Feare, 1970a).

Qualitative observations of the sectioned gonads of both males and females of Nucella emarginata were made by Houston (1971). He concluded that spermatozoa were present in all of the males during all months of the year and that females were capable of spawning throughout the year.

In Nucella canaliculata males, ripe sperm were present throughout the year, but females showed definite maturation and spawning seasons (Houston, 1971).

Manzi, Calabrese and Rawlins (1972) concluded on the basis of microscopic examination of histological sections that spermatogenesis in Urosalpinx cinerea and Eupleura caudata appeared to be a continuous process with no seasonally progressive stages and no clearly defined inactive period. They also found oogenesis in both species to be continuous with no clearly defined inactive stage, but

no quantitative measurements were made in either males or females.

The reproductive cycle of Nucella lamellosa was quantified by Lambert and Dehnel (1974) on the basis of mean diameter measurements of 25 oocytes in each of five females per month for a 12 month period. On the basis of these measurements, they concluded that proliferation took place during the three months following the end of the spawning season and that the growth of an oocyte to spawnable size took less than one year (Lambert and Dehnel, 1974). They made no attempt to quantify reproduction in males of this species.

This paper reports on work related to the following objectives: 1) to compare the timing of the events of gametogenesis among Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana, 2) to quantitatively analyze gametogenesis in a large-egged invertebrate, 3) to examine in detail the suggestion of Lambert and Dehnel (1974) that gametogenesis in Nucella lamellosa begins four months after the spawning peak and that oocytes take less than one year to grow to a spawnable size, 4) to compare the length of time it takes these snails to make their large eggs to the approximate two year period necessary for the brooding seastar Leptasterias hexactis to make its large egg (Chia, 1968), 5) to add to the information regarding the spawning times of these species and the possible effect of seasonally reduced salinity on the spawning time of Nucella lamellosa in Yaquina Bay, 6) to indicate possible points where synchrony could

take place in the protracted gametogenic cycle of gastropods which take longer than one year to make an egg.

The relevance of these objectives to understanding cyclic reproduction in marine invertebrates is established in the following review of current knowledge. Since so few studies have been conducted on the reproductive cycles of Neogastropods and the timing and control of events in these cycles, it is helpful to also examine the relevant work that has been done on other groups, e. g. polychaetes, echinoderms and other molluscs.

Definition of Reproductive Cycle

Many marine invertebrates undergo annual reproductive cycles. These cycles usually include: 1) an accumulation of nutrients to be utilized during gametogenesis, 2) proliferation of gonial cells and their differentiation into gametes, 3) accumulation of ripe gametes, 4) release of the gametes, and 5) a reproductively quiescent period when gametes which were not spawned are removed (Giese and Pearse, 1974). Variations of the timing of these events from year to year or geographically within the range of a species indicate that some mechanism exists to synchronize the events of a reproductive cycle within a population. When more than one year is required for egg production, synchronization becomes more long-range and complex and may involve clear separation of the above phases and

their cues.

Methods of Determining Reproductive Cycles

There are a number of methods that may be used to establish the annual reproductive cycle of a population. Among them are:

1) Spawning can be used as a criterion of ripeness. Using this method, one may plot the percentage of ripe animals versus time. However, one should keep in mind that the factors which induce spawning may be quite different from those that induce the gametogenic cycle.

2) Determining the histological condition of the gonads may be used to stage the timing of the events of gametogenesis. This method will yield a precise definition of the stages of the gametogenic cycle. The results may be quantified by the measurement of egg diameters or the tallying of the number of eggs in a given stage of oogenesis per unit area of gonad tissue.

3) A gonad index may be determined. In this method the volume of gonad-displaced fluid in a graduated cylinder or the wet weight of the gonad is divided by the wet weight of the animal and multiplied by 100. This assumes that large gonads contain mature gametes and small gonads contain immature gametes or contain no gametes at all and are in a resting phase. If nutrients are stored in the gonad and are converted to gametes in situ,

this will not be detected. Neither is there any indication of when gametogenesis is initiated after spawning.

4) Plankton may be analyzed for the presence of larvae. This method requires systematic sampling of the plankton and is useful only for species with pelagic larval stages.

Types of Reproductive Cycles

Perhaps the most common kind of invertebrate reproductive cycle in temperate regions is one in which a population has a single, well-defined breeding season. Such a restricted breeding season has been described for the polychaetes Caulleriella caput-esocis (August to October), Tharyx marioni (late October to early November) and Cirriformia tentaculata (late June to early July) at Plymouth, England on the basis of field observations and measurements of the diameters of mature oocytes (Gibbs, 1971). A very short annual breeding season in late autumn has been established for Arenicola marina on the basis of the annual variation in the mitotic index of the testis after 48 hour treatment with colchicine (Olive, 1972). A life span of about one year and a restricted breeding season from mid-April to early July has been shown for Nicolea zostericola from Nanhant, Massachusetts on the basis of a light and electron microscope study of gametogenesis in this species. However, an individual may breed from two to five times over a period of two weeks at the end of its life span

(Eckelbarger, 1975). A two week period at the end of December and the beginning of January is the restricted breeding season for the subtidal Melinna cristata from the Northumberland, England area (Hutchins, 1973).

A peak spawning period in the month of August with partial spawning from June to October was described for the clam Mercenaria mercenaria from Delaware Bay, Delaware on the basis of the greatest difference between egg size (large) and egg number (small) measured and counted from monthly samples collected over a period of three years (Keck, Maurer and Lind, 1975). On the basis of the analysis of histological sections and the presence of larvae in plankton samples, Calabrese (1970) established a spawning period from July to September for Mulinia lateralis.

Among gastropods, Fritchman (1961b) observed a peak reproductive season in March and April for Acmaea persona at Rockaway Beach, San Mateo County, California. For Acmaea mitra, he found a single spawning period in January and February at Moss Beach, California and in December and January at Pigeon Point, California (Fritchman, 1961a). On the basis of gonad indices, Webber and Giese (1969) established a six week spawning period during August and September for the black abalone Haliotis cracheroidii at Pacific Grove, California.

Paine (1971) has shown by direct observation that Tegula

funebralis spawns once a year in May and June at Mukkaw Bay, Washington. Feare (1970a) described for Nucella lapillus a normal spawning season during April and May at Robin Hood's Bay, Yorkshire, England on the basis of the number of "post-vitellogenic oocytes" per unit area of ovary tissue. On the western side of the Atlantic Ocean, Nucella lapillus was found to breed once a year in October (Hughes, 1972). Observations of egg capsules made with SCUBA gear by Stickle and Mrozek (1973) at Edwards reef off the west coast of San Juan Island, Washington revealed a spawning period during July for a subtidal population of Fusitriton oregonensis.

A yearly spawning with a peak in March was established for Nucella lamellosa by Lambert and Dehnel (1974), who measured oocytes in sections of ovaries. Individuals were first observed to deposit egg capsules in the field in January at Brockton Point, Stanley Park, Vancouver, British Columbia, Canada. On the basis of the first observation of egg capsules in the field, spawning times for Nucella lamellosa have been recorded as November at Golden Gardens, Seattle and Cattle Point, San Juan Island (Spight, 1972); November and December at Port Townsend (Emlen, 1966); January at Turn Island, San Juan County (Stickle, 1973); and April at Shady Cove, San Juan Island (Spight, 1972), all localities within the state of Washington.

Nucella emarginata has been described as having a spawning

season restricted to March at Monterey Bay, California by Glynn (1965), as indicated by the presence of egg capsules. A restricted spawning period from the end of May through July has been recorded for the columbellid Anachis avara from Beaufort, North Carolina on the basis of field observations and the timing of egg capsule deposition in the laboratory (Scheltema, 1969).

Many echinoderms exhibit restricted breeding seasons. Chia (1968) described a December spawning season for the brooding seastar Leptasterias hexactis from Friday Harbor, Washington. These results were based on field observations and the measurement of oocytes from both fresh and sectioned materials which also indicated that the development of an oocyte from premeiotic stage to the time of spawning requires about two years. Well-defined spawning periodicities in the echinoid Echinometra mathaei were found by Pearse (1969) in populations near the head of the Gulf of Suez. He found no synchronized periodicity within populations of these same species near the mouth of the Gulf of Suez. On the basis of staging and counting oocytes in section, a single annual spawning period was described by Gonor (1973a) for the echinoid Strongylocentrotus purpuratus from the Boiler Bay, Oregon area. Samples taken at 15 day intervals or less indicated an annual spawning period of 30 days maximum duration. Data taken over a four year period indicated that the maximum spawning period might take place any time between late December and

March (Gonor, 1973a). Using measurements of sectioned oocytes which contained a nucleolus, fluctuations in the size-frequency structure of the post-pachytene oocytes indicated a single spawning period for the unstalked crinoid Comanthus japonica from Koaziro Bay, Kanagawa Prefecture, Japan (Holland, Grimmer and Kubota, 1975).

In contrast to those animals which have only a single spawning period there are some temperate species and many tropical species which have either an extended breeding period (up to six months) or have portions of their populations breeding at all times of the year.

Among the Annelids the polychaete Cirratulus cirratus has no distinct breeding season. Small numbers of histologically ripe or spent females may be found throughout the year (Olive, 1970).

Individuals of the bivalve Adula californiensis capable of shedding mature gametes were collected at Newport, Oregon from June through October (Lough and Gonor, 1971). They were observed to spawn more than once during the summer when kept without burrows in the laboratory in running seawater.

In Britain the limpet Patella vulgata has been shown to have a peak spawning season from October to March, based on the color of the gonad as the live animal was removed from the shell (mature oocytes are green; ripe testis are white) and microscopic examination of gonad tissue sections (Orton, Southward and Dodd, 1956). Evidence from gonad indices and histological examinations of ovary

sections indicated that the red abalone Haliotis rufescens may be a potential spawner throughout the year in the Fort Bragg, California area (Young and DeMartini, 1970). Multiple spawnings in the spring for a high intertidal population and multiple spawnings in the spring and summer for a low intertidal population of Acmaea scabra at Bodega Head, Sonoma County, California have been described by Sutherland (1970) on examination of the gonads as the animals were removed from their shells. Using the same technique, Fritchman (1961c) described Acmaea scabra from Rockaway Breakwater, San Mateo County, California as a nearly continuous spawner with distinct spawning periods in the fall, summer and winter.

Three lines of evidence have been used by Edwards (1968) to establish that Olivella biplicata breeds and spawns throughout the year. One is the observation of courting pairs in every month of the year. The second is that the youngest snails taken by sieving (4-5 mm) could be found in the beach at Coos Bay, Oregon throughout the year. The third is that size-frequency distributions showed no breaks that would indicate year classes. At Dillon Beach, California qualitative observations showed that Nucella emarginata spawned throughout the year with a peak of spawning activity from late November through February (Houston, 1971). No spawning season could be detected for Bembicium nanum on the basis of the presence of egg capsules in the field near Sidney, Australia. Rather, the time of

spawning appeared to be independent of the time of year and different for each individual (Bedford, 1965).

Using data obtained from the measurement of oocytes and ova in histological sections, the echinoids, Diadema setosum and Echinometra mathaei, the asteroid Linkia laevigata and the holothuroid Holothuria atra have been shown to be reproductively active and spawn more or less continuously when they occur near the equator (Pearse, 1968).

Control of the Timing of the Events in Reproductive Cycles

If, under normally occurring environmental conditions, the events of sexual reproduction are repetitive and if the sequence of those events may be defined and their duration measured, then some proximate factor such as the rate of change in temperature, or salinity, or day length, or the quality or quantity of food available should be subject to correlation with the advent of one or more of the events in the reproductive cycle. Laboratory experiments under controlled conditions are very useful in detecting which environmental parameters may be involved. Care must be exercised when making inferences to what the species does under natural field conditions if the laboratory experiments exceed the range normally encountered by the species in the field.

Among the parameters that have been measured in an attempt

to elucidate the cause of seasonally synchronized reproductive cycles are temperature, salinity, photoperiod and nutrition.

The Effect of Temperature

All intertidal habitats and most shallow water habitats exhibit a distinct annual temperature cycle. A rise in temperature in the spring has been shown to induce gametogenesis in the oyster Ostrea edulis whose gonads are inactive during the winter when the temperature is lower (Korringa, 1957). Orton (1920) described spawning in Ostrea edulis when the temperature reached a critical level of 15-16° C, but Korringa (1957) could not reproduce these results.

Attempts to ripen lamellibranchs out of season by exposing them to elevated sea water temperatures for a period of time have been reported by Loosanoff and Davis (1963). Of 19 species tested, most did ripen out of season, but only about half of the species could be induced to spawn by a sudden rise in temperature of 5-10° C and the addition of gonadal tissue to the water. Gonad growth and oocyte cytoplasmic growh were induced in the bay scallop Aequipecten irradians held at 15° C and provided with a constant food source. When held at 5° C and provided with a constant food source, only oogonia developed (Sastry and Blake, 1971). Gonadal development started earlier in animals transferred to California than in the parental population in Maine for the oyster Ostrea edulis observed by

Leonard (1969).

The echinoid Strongylocentrotus purpuratus in southern California has been shown to lose its ability to produce gametes when the temperature rises above 17° C (Cochran and Engelman, 1975). A period of several weeks at a critical minimal temperature is necessary before the gonads of some boreo-arctic barnacles will mature (Crisp and Patel, 1969). The gonad index of Haliotis cracheroidii showed a well-defined reproductive cycle, but no correlation between the onset of gametogenesis and the average sea surface temperatures over a period of ten years could be demonstrated (Webber and Giese, 1969). Barnes (1972) was unable to induce spawning in Tonicella lineata by either raising or lowering the temperature of the water the animals were held in.

A temperature requirement for spawning may not benefit the adults but may be necessary for the normal development of embryos and larvae. The lower temperature for cleavage in Aequipecten irradians corresponds to the lower temperature limit for spawning (Sastry, 1966). Work on zygotes of the mussel Mytilus edulis demonstrated that normal cleavage took place in the temperature range from 8-18° C in populations from Denmark and North Wales, and that the larvae from Denmark had an increased growth rate at temperatures above 18° C (Bayne, 1965).

The Effect of Salinity

The body fluids of most marine invertebrates are isotonic with the surrounding sea water. When the salinity changes they may either absorb water from dilute salinities with a resulting increase in their total volume or expend some energy to remove the excess water. Many invertebrate eggs simply swell when exposed to lowered salinities. Increased oxygen consumption usually accompanies exposure of marine organisms to lowered salinities (Beadle, 1931). Nucella lapillus has been observed to become totally inactive at salinities less than 8‰ (Arnold, 1972). Comparing populations of the sea star Asterias rubens from the North Sea and the western Baltic, Schlieper (1957) found that gametogenesis and spawning occurred earlier in the North Sea (March and April) at higher salinities (30‰) than in the western Baltic (June and July) at lower salinities (15‰). Gametogenesis and spawning have been correlated with salinity fluctuations experienced by different populations of the estuarine mussel Xenostrobus securus (Wilson, 1969). However, Houston (1971) concluded there was no relationship between salinity and gametogenesis in Nucella emarginata or Nucella canaliculata. As with temperature, the major effect of salinity changes may be on the embryo or larva of a species, which is less able to compensate for environmental fluctuations, rather than the adult. The lower salinity limit for normal

cleavage in Adula californiensis was found to be near 26‰ (Lough and Gonor, 1971).

The Effect of Photoperiod

Rapid changes in the effective day length occur in the northern hemisphere during the summer and winter solstices. During June and July the days start to become measurably shorter while during December and January the days start to become measurably longer. The control of reproduction by changes in photoperiod has been described for many vertebrates (Bullough, 1961). Light receptor organs are connected by nervous pathways to parts of the brain which secrete hormones that control reproductive organs.

By periodically sampling the testis of a single Strongylocentrotus purpuratus kept under a constant light regime, Boolootian (1963) found the production of spermatogonia predominated when the cycle was 14 hr light:10 hr dark and that the production of spermatids and sperm predominated when the cycle was changed to 6 hr light:18 hr dark. No changes in gonad weight were recorded in several populations of Strongylocentrotus purpuratus from the Yaquina Head, Oregon area from March through June by Gonor (1973a). He recorded an instantaneous growth rate of about 1% per day for the period of July through November for these same populations which was not triggered by the summer solstice and decreasing day length. Cochran and Engelmann

(1975) found no correlation between photoperiod and either the initiation or termination of reproductive activity in a subtidal southern California population of Strongylocentrotus purpuratus.

Spawning has been induced in the hydroids Hydractinia and Pennaria by holding them in complete darkness for a period and then subjecting them to light (Ballard, 1942). Thus, in this group of animals light would synchronize the release of gametes early in the morning during the breeding season. Lambert and Brant (1967) suggested that spawning in the tunicate Ciona intestinalis may be initiated by the effect of light on heme-proteins which stimulate a nervous pathway. An increase in gametogenic activity soon after the day length began to increase was noted by Houston (1971) for Nucella canaliculata.

The Effect of Nutrition

Gametes, particularly large eggs, may be very expensive to make in terms of the amount of energy required. Organisms may either convert food directly into gametes at the time the food is eaten, or they may store nutrients for conversion into gametes later.

The Antarctic seastar Odontaster validus, which produces large eggs, stores nutrients during the summer period of phytoplankton abundance (Pearse, 1965, 1966). These nutrients are converted to gametes after the phytoplankton bloom has passed, synchronizing the

seastar population at that time of the year. The seastar Pisaster ochraceus feeds during the summer, presumably when its feeding efficiency is highest, and converts the stored nutrients to gametes during the winter (Farmanfarmaian, Giese, Boolootian and Bennett, 1958; Greenfield, Giese, Farmanfarmaian and Boolootian, 1958; Mauzey, 1966; Nimitz, 1976), so that seasonally synchronized reproductive activity results.

The copepod Calanus finmarchicus spawns repeatedly during the summer in temperate waters. Regeneration of its gonads after spawning depends on food availability since food is converted directly to gametes (Marshall and Orr, 1955). The rates of increase of gonad weight calculated by Gonor (1973a) for Oregon intertidal populations of the echinoid Strongylocentrotus purpuratus were suggested to be dependent on the seasonal (summer) increase in the amount of algal drift available. Given the proper temperature, Sastry and Blake (1971) determined nutrients are transferred directly to the gonad for the production of gametes rather than being stored in the digestive gland in the bay scallop Aequipecten irradians. Lambert and Dehnel (1974) suggested that glycogen is converted to either lipid or protein and stored in the digestive gland of Nucella lamellosa or is converted directly to gametes in the gonad. Individuals starved for 120 days sacrificed the gonad and removed protein even though they contained maximum reserves in the digestive gland.

The chiton Katherina tunicata has been shown from histological observations to use both nutrients stored in the gonad itself and currently ingested food to make gametes during the period when gametes are actively growing (Nimitz and Giese, 1964).

These examples show that a consequence of producing large quantities of gametic material is a requirement for large amounts of food resources to be channeled into gamete growth and that seasonal limits may prevent this requirement from being met continuously.

This study examines how the duration and rate of egg growth in species producing large eggs is adapted to a seasonally fluctuating environment.

The Strategy of Egg Numbers and Egg Size

The rate of egg production for a given species may depend on both the maximum size of the egg and the size of the individual producing the eggs. Under constant conditions more small eggs can be produced per unit time than large eggs. However, the larvae developing from small eggs must have an environmental food source sooner than larvae developing from larger eggs. Usually, small eggs result in planktotrophic larvae. These larvae give the species wide distribution, good genetic mixing and possible exposure to physical conditions which may result in the expression of genetic material not previously expressed phenotypically in the species. The cost to the species is high in terms of the number of larvae that serve as food for

associated predators (Fox and Coe, 1943).

Thorson (1950) states that in order to survive in the high arctic areas, a planktotrophic larva must complete its development, from hatching to metamorphosis, at a temperature below 4.5° C within one to one and one-half months. This period includes the very short arctic season of phytoplankton production. The problem of synchrony of the spawning period with such a short period of phytoplankton production may be overcome by producing very large eggs, or by placing smaller eggs in capsules along with food eggs for the developing embryos. The number of prosobranchs with pelagic larvae decreases with increasing latitude. No pelagic larva has been found in the life history of any arctic prosobranch and 95% of all marine species of benthic invertebrates living in the arctic have direct development (Thorson, 1936).

Larger eggs, either brooded as in the seastar Leptasterias hexactis or placed in capsules as in the snails Nucella lamellosa and Nucella emarginata, may be fewer in number than smaller eggs and still provide the species with successful reproduction (Chia, 1968; Emlen, 1966; Spight, 1972). A species is then freed from having its spawning season synchronized to the time of the spring phytoplankton bloom. However, a large snail may have difficulty in acquiring sufficient food to both produce eggs and keep up its basal metabolic rate (subtidal Nucella lamellosa with a maximum length of 112 mm,

Emlen, 1966). An added cost of direct larval development may be an increased rate of speciation and the extinction of local populations that have no genetic exchange with neighboring populations.

Among the prosobranchs, the entire archaeogastropod group, with the exception of the Neritacea, produce small eggs that are spawned free into the sea in large numbers. By contrast, all mesogastropods and neograstopods have internal fertilization and fewer eggs that are either deposited in gelatinous masses or in distinctive capsules. In the neogastropods in particular, these eggs are much fewer in number and very large compared to the freely spawned eggs of archaeogastropods.

The smaller eggs of lower prosobranchs would be expected to be produced in the same annual breeding cycle in which they are spawned. Entrainment of annual cycle events, such as proliferation and spawning, to environmental cues would take place within one annual cycle. Himmelman (1975) was able to correlate spawning in the field with the spring phytoplankton bloom for the chitons Tonicella lineata and Tonicella insignis and the echinoid Strongylocentrotus droebachiensis. He was repeatedly able to induce spawning of chitons and urchins in the laboratory using both fresh and frozen phytoplankton. Spawning occurred when the amount of chlorophyll-a in the water was changed from one to eight to ten mg/m^3. Snail species with larger eggs may require longer periods of egg growth as in

large-egged echinoderms. In these forms, any environmental cue for proliferation must be well separated in time from those related to spawning. This study attempts to determine the duration of egg growth, its seasonality and correlated factors in some examples of these snails.

The strategy of egg numbers versus egg size must depend on the survival characteristics of the hatchling, i. e. the fitness of individual offspring, which must be optimized (Smith and Fretwell, 1974). Spight (1975) calculated the chance of a newly hatched Nucella lamellosa surviving its first three months as 1-2%. Once it reaches three months of age it has a 35% chance of becoming one year old, and after one year, a 40-60% chance of surviving through the following years. Therefore, the larger an embryo at hatching the greater its chances of survival (Spight, 1976a). Among the Muricidae that have food eggs in the capsules, embryos reach the same size in crowded capsules but variable sizes in uncrowded capsules (Spight, 1976b). An unrestricted breeding season requires variable embryo size since environmental conditions may vary drastically over a long hatching period. Therefore, having a constant number of food eggs and a variable number of embryos per capsule would be an optimum strategy for a species with an unrestricted breeding season, e. g. Nucella emarginata. A species with a restricted breeding season could optimize embryo size and hence maximize fecundity without using food eggs to feed the

embryos once they were in the capsule, e. g. Nucella lamellosa.

However, a flexibility in the time of energy accumulation would be necessary for the production of large eggs which could optimize embryo size during a restricted breeding season. Logically one would expect that it would either take a species longer to produce large eggs than it would to produce small ones, or that the growth rate of large eggs would be greater than that of small ones.

This review clearly indicates that the duration and timing of production of the large yolky neograstopod egg has not been rigorously examined on a comparative basis. The studies on Nucella lapillus and Nucella lamellosa cited concluded that the large oocytes in these species were produced within one cycle, implying either very rapid seasonal growth or sustained growth at a moderate level throughout the year. The possibility that the large egg strategy used in neogastropods requires a two year oocyte growth process was re-examined in this study.

MATERIALS AND METHODS

Description of Study Areas

Yaquina Bay is an estuary located 98 nautical miles south of the Columbia River at latitude 44° 37' N, longitude 124° 02' W (Figure 1). The Yaquina estuary (Figure 2) was formed from the drowned river mouth of the Yaquina River. It receives the surface run-off from 1036 square kilometers (400 square miles) of the Coastal Mountain Range and has a surface area of 1093 hectares (2700 acres) at Mean High Water (MHW) and 449 hectates (1109 acres) at Mean Lower Low Water (MLLW). Its tidal flats consist of approximately 678 hectares (1650 acres) (Goodwin, Emmett and Glenne, 1970, Wick, 1970).

Coquille Point is located on the northeast shore of Yaquina Bay three kilometers (1.9 miles) upstream from the Oregon State University Marine Science Center dock (Figure 2). Nucella lamellosa samples were collected at Coquille Point from the bay side of an abandoned artificial dike which had been constructed from dredge tailings. The snails were taken from the -0.3 to +0.6 m (-1 to +2 ft) tidal levels on large boulders (approximately one m in diameter) partially buried in a substrate of soft mud.

Boiler Bay, latitude 44° 50' N, longitude 124° 03' W, is 22.5 kilometers (14 miles) north of Yaquina Bay (Figure 1). It is a small indentation in the coast line which is completely exposed to the open

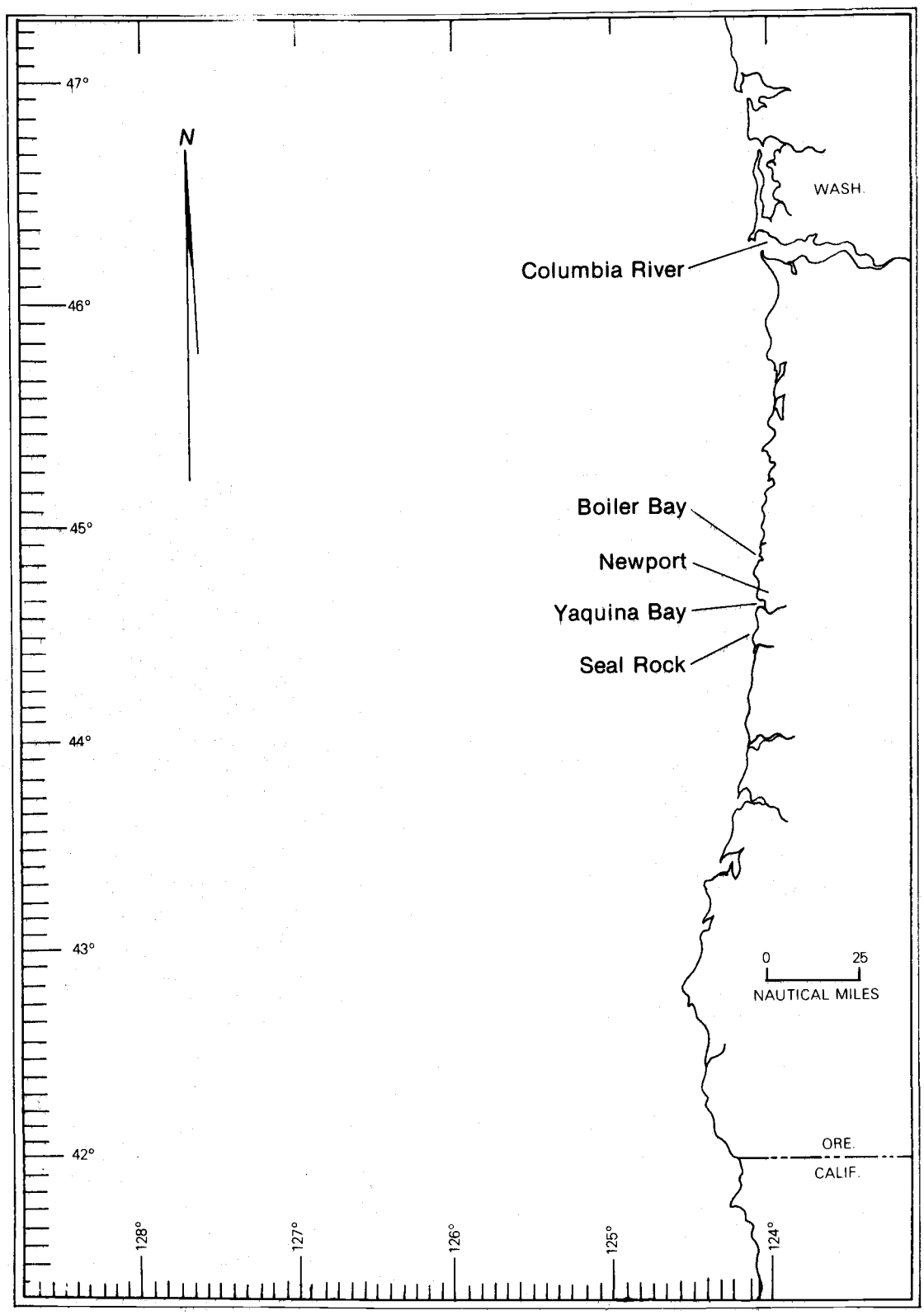


Figure 1. The location of Boiler Bay, Yaquina Bay and Seal Rock in relation to the state of Oregon.

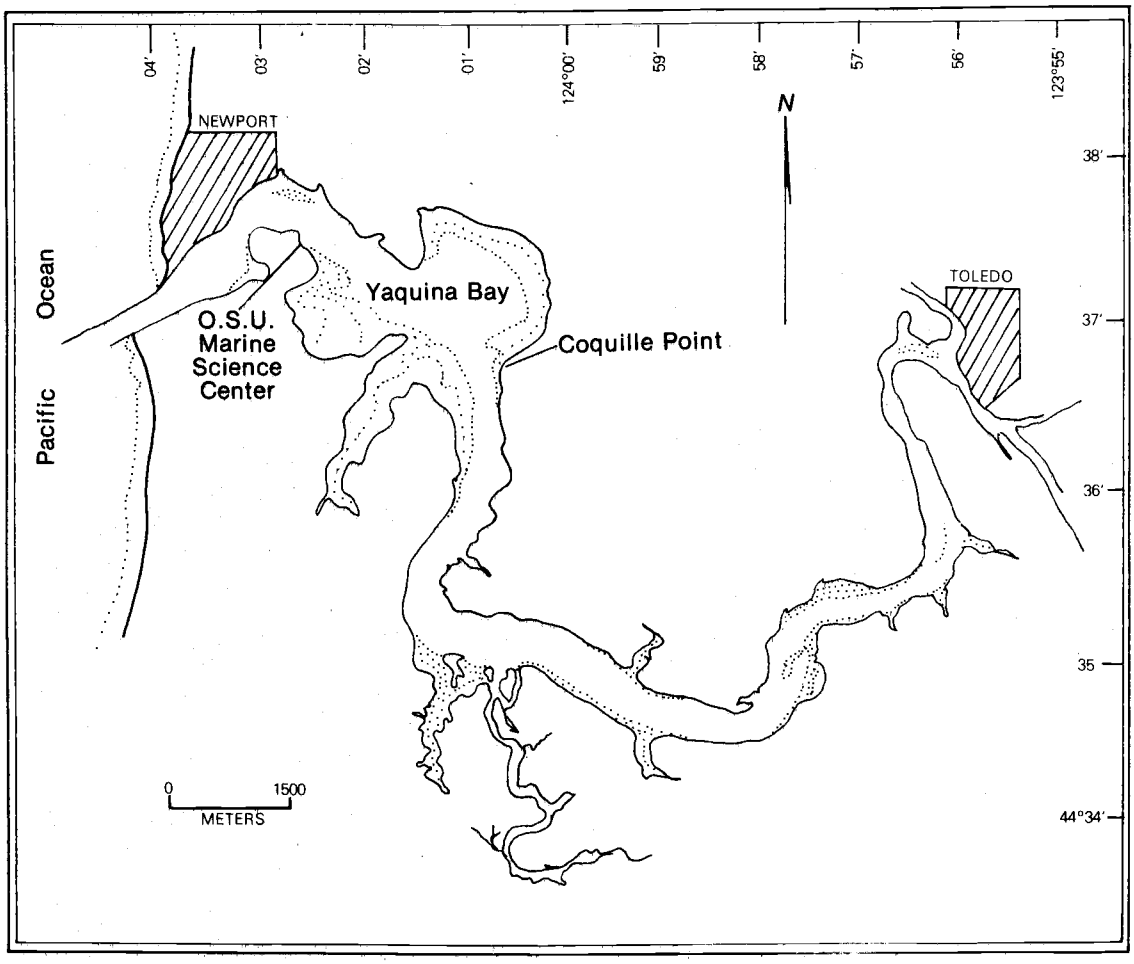


Figure 2. Yaquina Bay, Oregon with the Coquille Point sampling site.

ocean. The cliffs above the beach are composed of sand stone which is eroding onto the lower basalt bench. Amphissa columbiana and Searlesia dira were collected from the northern edge of Boiler Bay from the -0. 3 to +0. 3 m (-1 to +1 ft) levels.

Seal Rock, latitude 44° 29' N, longitude 124° 05' W, is 16. 1 kilometers (10 miles) south of Yaquina Bay (Figure 1). Nucella emarginata was collected from the eastern facing wall of a sand stone outcrop overlying basalt. This site was semi-protected, being exposed only to waves coming directly from the south. Snails were taken from the +0. 9 to +1, 5 m (+3 to +5 ft) tidal levels.

Tidal levels were measured relative to the sea surface at the predicted time of low tide. The time was noted and the tide gauge at the Marine Science Center dock was used as a reference. The diurnal tidal range in Yaquina Bay has been established at 2. 68 m (8. 8 ft) with the mean tidal level at +1. 4 m (+4. 58 ft) above Mean Lower Low Water (Thum, 1972).

These sites were chosen not because of uniform or similar physical characteristics but because they supported large populations of the snails under consideration.

The physical characteristics of the study sites in terms of water temperature, salinity and exposure were defined for the period from May, 1970 through July, 1971 from the following sources. For the open coast, monthly range and mean surface temperatures and

salinities were taken from Wyatt and Gilbert (1971, 1972) for the Columbia River Lightship located five miles south of the Columbia River south jetty (latitude 46° 11. 2' N, longitude 124° 11' W), and from data collected at Whale Cove (latitude 44° 47. 3 N) by Gonor, Thum and Elvin (1970). For Yaquina Bay, monthly range and mean temperatures and salinities were taken at the pump outlet which supplies the Oregon State University Marine Science Center (Wyatt and Gilbert, 1971, 1972). This water comes from the bottom of Yaquina Bay.

The temperature of the surface water in the Yaquina Bay estuary at the times of daily higher high water and lower low water as recoreded at the Marine Science Center floating dock from May, 1970 through May, 1971 was taken from Thum (1972). The salinity of the bottom water in the Yaquina Bay estuary as recorded in the Marine Science Center sea water system at the times of daily higher high water and lower low water from May, 1970 through May, 1971 was taken from Thum (1972).

Histology

A sample of 25 individuals of each species representing the entire available size range was collected monthly from May, 1970 through July, 1971. To avoid a two month interval between samples, samples were taken during the first part of January, 1971, and again

during the last part of January, 1971, because the time of the lower low tide was changing from the first part of the month to the last part of the month. The length and width of the shell and the length and width of the shell aperture of each individual were measured. With the help of a small vice, the snails were removed from their shells and sexed on the basis of the presence or absence of a penis. Each was fixed in the dark for 24 hours in Smith's fixative (Smith, 1912; Galigher and Kozloff, 1964). They were then rinsed in 2% formaldehyde as many times as needed to remove all traces of the fixative as determined by the clear color of the rinse. Following fixation, the apical third of the gonad-digestive gland complex was dissected off, dehydrated in ethanol, cleared in toluene and embedded in parapast (melting point 56-57° C).

For light microscopy sections of females were cut 10-12 μm thick and sections of males were cut 7 μm thick. Sections were stained with alum hematoxylin and counter stained with eosin-Y.

Measurement of Gametes

The very large, yolky oocytes of all four species are very tightly packed in the ovary. Serial sections of ovaries showed that the oocytes varied from spherical to slightly elliptical in section. All oocytes were treated as though they were elliptical in shape. By setting the formula for the area of an ellipse equal to the formula for

the area of a circle and combining terms, it was possible to derive a formula for converting an ellipse to a circle of equivalent area. The long and short axis of each oocyte was measured in section and the square root of the product of these was multiplied by a calibration factor which resulted in the diameter of an equivalent circle. This gave a single number for each oocyte.

If a wide size-range of oocytes is present in a given sample, their measurement from sections has the disadvantage of increasing the percentage of small oocytes in the measured sample because the absolute number of large oocytes is fewer in the total sample. It has the advantage of displaying the entire size range available in a sample and showing the timing of the various stages of gametogenesis.

Each month, five female Nucella emarginata, Searlesia dira and Amphissa columbiana were selected and 50 oocytes were measured from each. The oocytes of Nucella lamellosa are large and relatively scarce so that only 25 per female could be measured per month. The oocytes chosen to be measured were not selected in a random manner, but rather as a section of gonad was systematically scanned under the microscope, only those oocytes with a nucleolus present in the section were measured. An exception to this was very early primary oocytes in which the nucleolus could not be distinguished. This insured that an oocyte would not be measured twice from the same sample since examination of serial sections of oocytes of various sizes indicated

that the nucleolus occupied the same relative position in each oocyte. These measurements were arranged into size classes and plotted for each month.

Estimates of daily relative growth rates were calculated for primary oocytes using the method described by Brody (1945). Oocytes were assumed to be adequately represented as spheres and their volumes were calculated from nominal diameters. Since the physiological significance of a unit of time, such as a day, may change rapidly with age, daily relative growth rates were determined between the points in time where significant changes in the mean size or probable proliferation took place.

Sections of the male gonad were scanned for the time of appearance of sperm. Three males per month were selected for each of the four species and the percent of sperm in the tubules was measured and plotted for each month. If spermiogenesis was seasonal and cued in on the same stimulus as oogenesis it might be possibe to more finely define the timing of events in the reproductive cycle in males than it would in females. However, if sperm were produced more or less continuously in small amounts and stored for later use one might suspect that gametogenesis in males and females is not synchronized by the same cue or cues.

Controlled Temperature Experiments

Thirty individuals of each species were kept for a one year period, from June, 1970 through June, 1971 under controlled temperature conditions. The temperatures chosen were 5°, 10°, 15° and 20° C. The 20° C water bath was cooled by flowing tap water and fluctuated slightly with the temperature of the cooling source. The 5°, 10°, and 15° C aquaria were maintained in cold rooms whose temperature did not fluctuate appreciably. Nucella lamellosa and Nucella emarginata were fed either barnacles (Balanus glandula) or mussels (Mytilus edulis). Searlesia dira and Amphissa columbiana were fed barnacles and Mytilus edulis which had been opened by removing one valve. Any opened Mytilus that was not eaten after one hour was removed.

Observations were made to determine if these individuals would eat, grow and lay egg capsules. Comparisons of the timing of egg capsule production of the laboratory specimens were made with the field populations to note any correlations. At the end of the one year period, these animals were measured, fixed, and embedded and sectioned in the same manner as the field samples.

Field Observations

Observations were made in the field to determine the vertical

distribution of the snails. Snails were tipped to see what they were feeding on. The time of appearance of egg capsules was determined as well as the vertical distribution of the egg capsules and the time at which they were empty.

Recently deposited egg capsules were collected and kept in sea water at about the temperature of the environment at the time of collection to determine how long the embryos took to hatch and the stage of development at the time of hatching. Eggs within a capsule were counted and measured. The mean diameter of capsule eggs was compared with the largest size measurable in the tissue sections to determine whether or not fully grown oocytes were being sampled in the sections. Egg capsules were collected for Nucella lamellosa on June 4, 1970, June 23, 1971 and July 6, 1976; for Nucella emarginata on October 16, 1970, November 12, 1970, January 10, 1971, April 24, 1971 and May 2, 1976; for Searlesia dira on January 26, 1971, April 24, 1971, March 19, 1972 and May 2, 1976; and for Amphissa columbiana on November 12, 1970, January 26, 1971 and February 17, 1971.

Statistical Treatment of Data

In the laboratory the individuals of each species were arranged according to decreasing size (shell length) prior to being measured and fixed, and a sample of 25 individuals which included

representatives of all sizes available in a given month was processed. It has been shown that gonad index or ratio methods are useful only for comparing animals of the same size through time within a population (Gonor, 1972). Therefore, measurements of oocyte diameters were restricted to five of the larger females of each species in each monthly sample.

Oocyte diameters were key punched and processed on an IBM 360 model 67 computer located at Washington State University, Pullman, Washington. A two way analysis of variance using species and month as fixed factors was performed (Winer, 1962). Means, standard errors of the means, standard deviations, variances and coefficients of variability were calculated as part of a statistical analysis system designed and implemented by Barr and Goodnight (1972). Size-frequency histograms of egg diameters were constructed using methods for setting class interval midpoints on the basis of sample size and range suggested by Strickberger (1968).

RESULTS

Environmental Temperatures and Salinities

The comparison of water temperatures between the open coast (Figure 3b) and Yaquina Bay (Figures 3a and 4a) indicate that maximum temperatures were reached during July and August, 1970, and minimum temperatures were reached during January and February, 1971. Monthly mean water temperature varied between 8° and 12° C throughout the entire year (Figures 3a, 3b). The Yaquina Bay surface water temperatures measured at the time of higher high water (HHW) and lower low water (LLW) had a range from 17° C in June, 1970 to 5° C in January, 1971 (Figure 4a). From May through October, 1970 the temperature of the surface water at the time of LLW was warmer than at the time of HHW, demonstrating the cooling influence of upwelled ocean water on Yaquina Bay (Figure 4a). From November, 1970 through February, 1971 the temperature of the surface water at the time of LLW was colder than the temperature of the surface water at the time of HHW in Yaquina Bay (Figure 4a). Decreased day length and consequent reduced insolation, and increased run-off in the Yaquina River during the winter period contributed to this cooling effect.

Comparison of salinities between the open coast and Yaquina Bay indicate a period of relatively high salinity, about 33‰-35‰, from

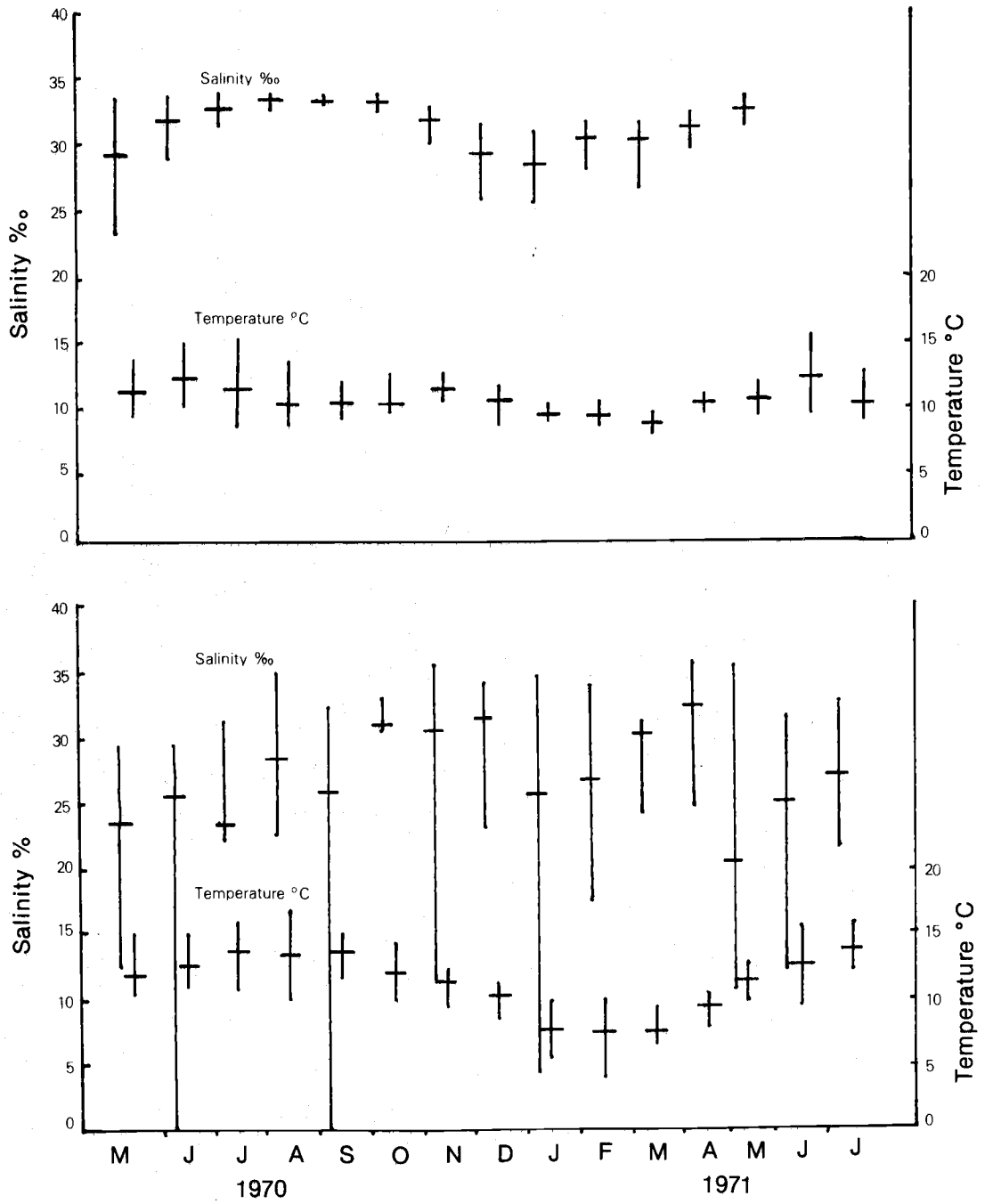


Figure 3a. The monthly range and mean of salinity and temperature in Yaquina Bay from May, 1970 through July, 1971 measured at the intake to the Marine Science Center sea water system. Graphed from the data of Wyatt and Gilbert, 1971, 1972.

Figure 3b. The monthly range and mean of surface water salinity and temperature measured at the Columbia River lightship from May, 1970 through July, 1971. Graphed from the data of Wyatt and Gilbert, 1971, 1972.

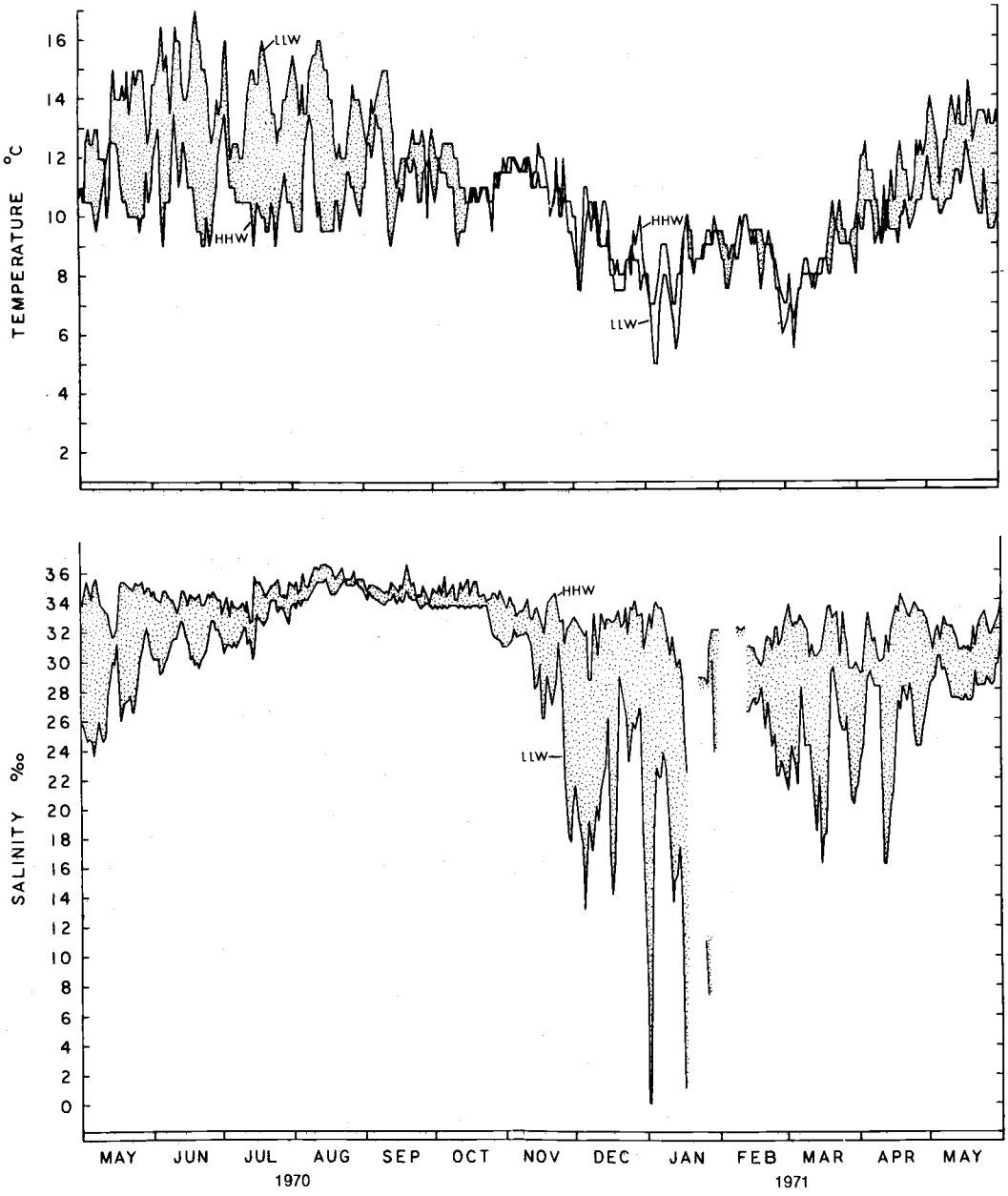


Figure 4a. Surface water temperature measured in Yaquina Bay at the Marine Science Center floating dock from May, 1970 through May, 1971. Temperatures were recorded twice daily at the times of higher high water (HHW) and lower low water (LLW). Stippling indicates the times when the LLW temperature exceeded the HHW temperature. Graph and data from Thum, 1972.

Figure 4b. Bottom water salinity in Yaquina Bay recorded from the Marine Science Center sea water system at the times of higher high water (HHW) and lower low water (LLW) from May, 1970 through May, 1971. The salinity range is stippled. Graph and data from Thum, 1972.

August through November, 1970 (Figures 3a, 3b, 4b). A slight decrease in the salinity of the bottom water of Yaquina Bay occurred during December, 1970 and January, 1971 (Figure 3a). However, during this same period salinities as low as 0‰ were recorded in the Marine Science Center sea water system (Figure 4b). The difference between the curves presented in Figure 3a and Figure 4b for Yaquina Bay lies in the periods of summary. Figure 3a presents averages while Figure 4b presents daily ranges. Over long time periods the mean value may be less important than the frequency content of the distribution from which the mean was formed. In terms of the salinity regime experienced by low intertidal animals at the time they are exposed to low tides, Figure 4b presents a clearer picture than Figure 3a. Bakum (1975), using the synoptic surface atmospheric pressure analyses produced by the Fleet Numerical Weather Central to estimate the sea surface stress, has shown that the upwelling period at latitude 45° North, longitude 125° West occurred from about April 1 to October 1 during the period of 1967 to 1973. Upwelling was based on six-hourly computations of the offshore component of Ekman transport.

Daily air and water temperatures from Whale Cove were used to describe the temperature regime at the 0.0 and +1.4 m (0.0 and +4.5 ft) tidal levels. For each level the temperature at the times of higher high water (HHW) and lower low water (LLW) were used

to determine a daily temperature range at that level. The temperature at the -0.6 m (-2.0 ft) level at the times of HHW and LLW were averaged for a daily baseline. This represented the temperature an individual would experience if it were covered by water 99% of the time. Percent exposure at selected tidal levels was taken from the percent exposure curve calculated by Thum (1972) and reproduced here as Figure 5. The temperature for the baseline ranged from a low of 7.5° C in September, 1970 to a high of 15° C in June, 1971 (Figures 6 and 7). Variable periods of upwelling caused fluctuations in the baseline temperatures of as much as 5° C over a period of four days in June, 1971. The effect of spring and neap tides was also observed in fluctuations of the baseline temperatures, spring tides resulting in decreased temperatures at approximately two-week intervals.

Temperatures experienced by individuals at the 0.0 m level (Figure 6) were colder than the baseline during most of the period from September, 1970 through May, 1971, reflecting the fact that the majority of the lower low tides occurred at night during this period. From June through August, 1971, temperatures at the 0.0 m tidal level fluctuated above the baseline, reflecting the fact that these tides occurred in the early morning to mid-day period.

Temperatures experienced by individuals at the +1.4 m (+4.5 ft) level (Figure 7) were much colder than the baseline for most of the

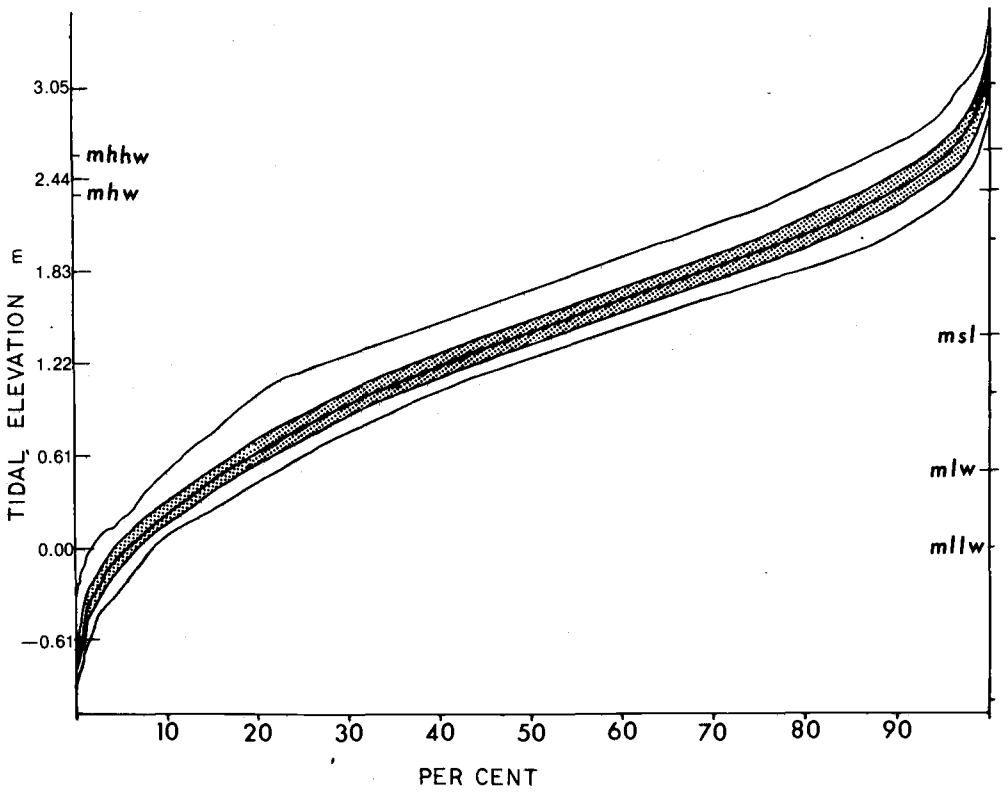
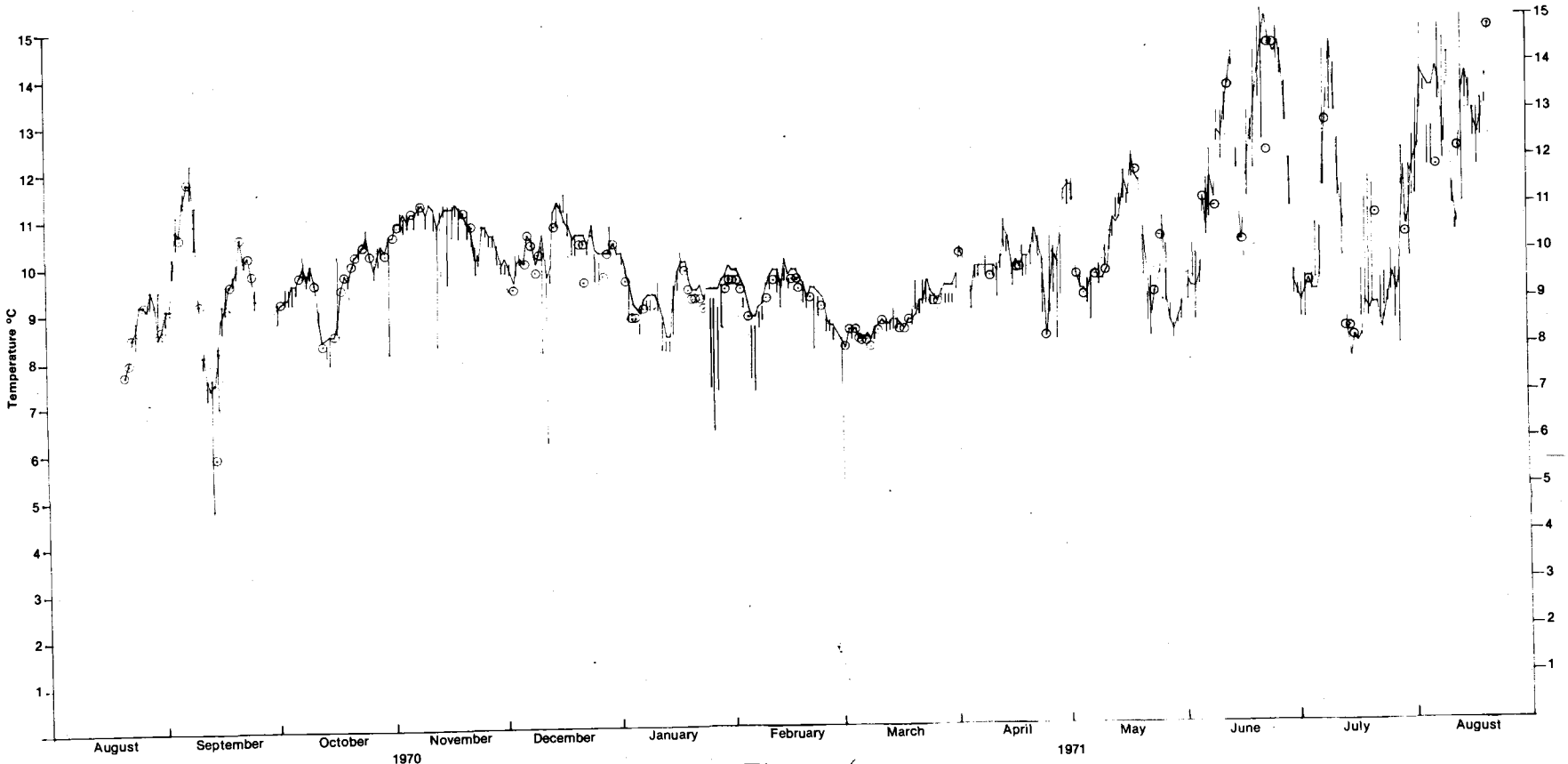


Figure 5. Graph of percent tidal exposure with elevation for 1968. Tide gauge data were taken at the Marine Science Center dock. The center line represents the mean percent exposure for one year, while the outer lines represent the annual range. The stippled area represents ±2x standard error for the year, based on monthly percent exposure values. mhhw = mean higher high water; mhw = mean high water; msl = mean sea level; mlw = mean low water; mllw = mean lower low water. Tidal elevation is in meters. The graph is adapted from Thum (1972).

Figure 6. Daily temperature range at the 0.0 m tidal level measured at Whale Cove from August 21, 1970 through August 20, 1971. Temperatures were recorded twice daily at the times of higher high water (HHW) and lower low water (LLW). The solid line indicates the water temperature at the -0.6 m (-2.0 ft) tidal level. Circled points indicate temperatures on days when the temperature at the time of HHW was the same as the temperature at the time of LLW. Data from Gonor, Thum and Elvin, 1970 and Elvin, pers. comm.


Temperature °C
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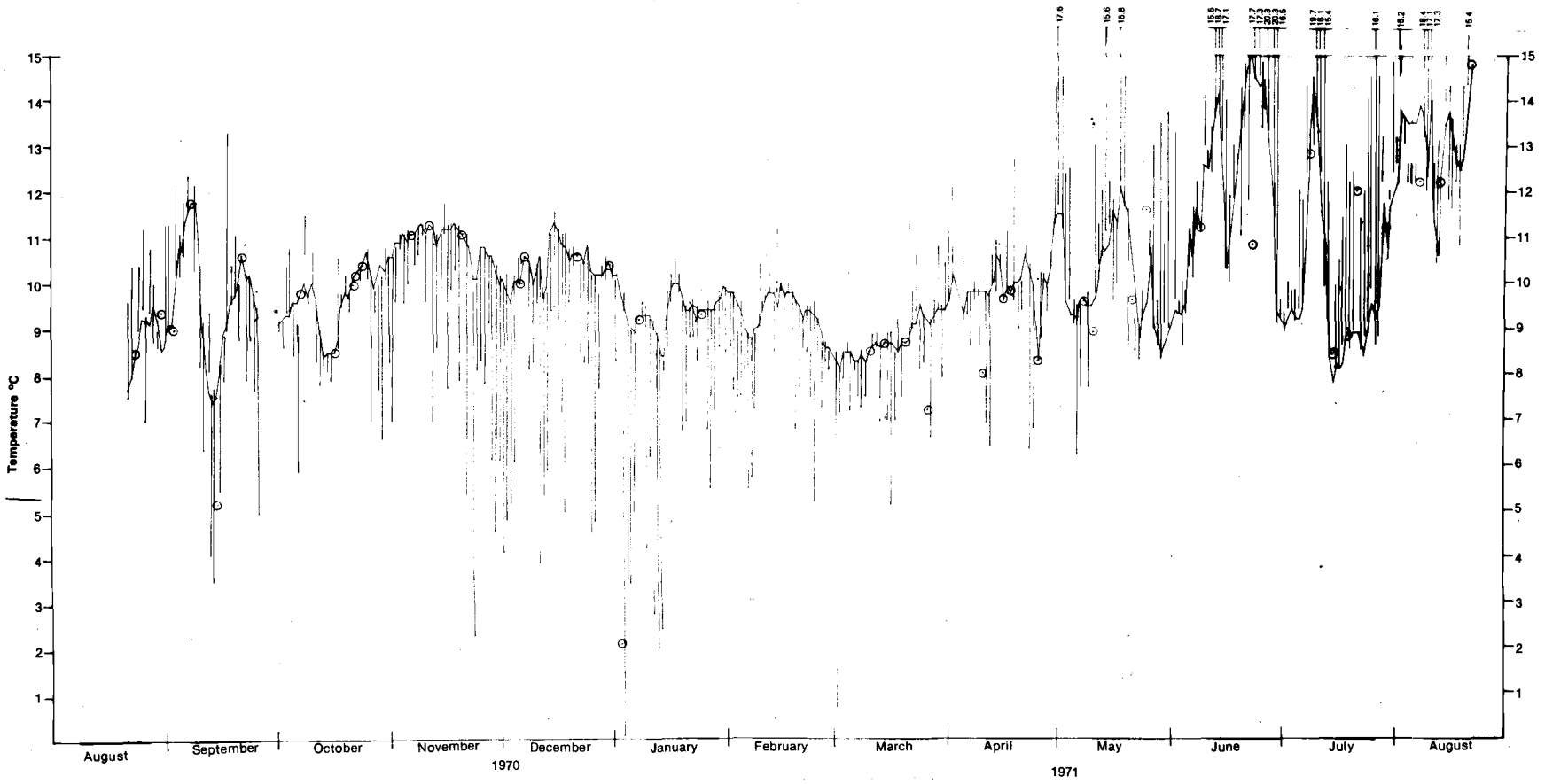
February
March
April
May
June
July
August
1970
1971

Figure 6

Figure 7. Daily temperature at the +1.4 m (+4.5 ft) tidal level measured at Whale Cove from August 20, 1970 through August 21, 1971. Temperatures were recorded twice daily at the times of higher high water (HHW) and lower low water (LLW). The solid line indicates the water temperature at the -0.6 m (-2.0 ft) tidal level. Circled points indicate temperatures on days when the temperature at the time of HHW was the same as the temperature at the time of LLW. Data from Gonor, Thum and Elvin, 1970 and Elvin, pers. comm.


Temperature °C
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May
June
July
August
1970
1971

Figure 7

period from September, 1970 through mid-March, 1971. From May through August, 1971 temperatures at the +1.4 m (+4.5 ft) level were consistently above baseline temperatures. During this period (May through August, 1971) temperatures at +1.4 m (+4.5 ft) at the time of LLW were higher than the baseline during the spring tide series, with a peak of 20.3° C reached in late June, 1971.

Baseline temperatures increased rather steadily during October and the first half of November, 1970. During this same time period Searlesia dira and Amphissa columbiana began spawning at Boiler Bay. A decrease in baseline temperatures occurred from mid-November, 1970 through February, 1971. Baseline temperatures increased rather steadily during March and April, 1971 and began to fluctuate with the onset of upwelling in late April, 1971.

Nucella lamellosa

Field Observations

At Coquille Point Nucella lamellosa occupies an intertidal range from about -0.3 to +1.83 m (-1 to +6 ft) MLLW. Smaller individuals are found in the upper part of the range, while the larger, sexually mature individuals occupy the middle to lower portion of the range. A similar size distribution for Nucella lamellosa on Puget Sound has been described by Bertness and Schneider (1976). On a yearly basis, the individuals at the lower end of their range are exposed about two

to three percent of the time, while the animals living at the upper end of the range are exposed about 75% of the time (Figure 5). Animals living at the upper end of the range may be exposed to near-freezing temperatures and lowered salinities (Figure 24) due to surface run-off during the winter since the low tides then occur at night. During the spring and summer the animals at the upper end of the range would be exposed to warming and possible desiccation since the low tides then occur in the early morning to mid-day period. Bertness and Schneider (1976) found higher thermal limits for small individuals. Both vertical and horizontal movements may be undertaken by individuals in search of food.

Extremely young individuals are very difficult to locate. Their shells are initially white and they blend in with the barnacles and clam shell beach material and also fit tightly into the crevices between barnacles and other organisms. Individuals less than 10 mm long were seldom encountered at the times of collection for this study, a period of 15 months. Similar findings have been reported for a San Juan Island, Washington population over a period of four years, 1968 through 1972 (Spight, 1974).

Nucella lamellosa become sexually mature at Coquille Point when they have attained a shell length of between 30 and 35 mm. Females with a shell length between 20 and 30 mm have very small primary oocytes in their ovaries, but all of these oocytes are

pre-vitellogenic. Spight (1972) classified as juveniles all individuals less than 29 mm long at San Juan Island, Washington.

During the breeding season sexually mature individuals (snails over 35 mm long) aggregate in clumps at the lower edge of the population vertical range, where the egg capsules are deposited. Breeding aggregations were first observed May 14, 1970. Egg capsules were not found until June 4, 1970. By October 10, 1970 the breeding aggregations had disbanded. It was not possible to sex these animals externally, but after removing them from their shells, the sex of individuals could be determined by the color of the gonad. The female gonad is a golden brown color. Lambert and Dehnel (1974) distinguished sex on the basis of the presence of a penis in the male. Fretter and Graham (1962) indicate that this is a good diagnostic feature for Nucella lapillus, an Atlantic species closely related to Nucella lamellosa. However, a penis-like outgrowth in females of Ocenebra erincea, a gonochoristic species, was observed by Feral (1976) in a Bassin d'Arcachon population. In one sample of a breeding aggregation of Nucella lamellosa used in this study, 99% of 162 individuals sampled possessed a penis (Appendix). The difference between the males and females was not the presence of a penis, but rather, the size of the penis. In mature males, the penis was longer than 6 mm, and in mature females it was shorter than 3 mm. In extremely large females a penis was lacking altogether. There was no evidence

of hermaphroditism in any of the individuals examined.

The time of deposition of egg capsules at Coquille Point is during June, July and August. Egg capsules were first observed on June 4, 1970 and June 23, 1971. This is considerably later than the capsule deposition periods of November to April at Port Townsend, Washington (Emlen, 1966), the January period at Brockton Point, Vancouver, British Columbia (Lambert and Dehnel, 1974), and the April period on San Juan Island, Washington (Spight, 1974).

Oogenesis

The staining characteristics and distinctive features of appearance in routine histological sections were used to define the stages in oocyte growth and development. According to Fretter and Graham (1962), meiosis in gastropods with internal fertilization takes place in the gonoduct. Therefore, any divisions seen in the ovary are proliferative and the observed changes in staining characteristics and distinctive features are the result of primary oocyte growth and nutrient accumulation. Oogonia could not be distinguished from primary oocytes, possibly because of the thickness of the sections (12 μm). Initially, the cytoplasm of a primary oocyte is basophilic and stains blue with hematoxylin and eosin. The shape is determined by the size and degree of packing and may be round to triangular. Each primary oocyte contains a large nucleus with a conspicuous

nucleolus (Figure 8). During the initial growth phase the cytoplasm appears dense and granular and is strongly basophilic. As the oocyte grows and vitellogenesis begins, the cytoplasm becomes slightly acidophilic and stains pink. These intermediate primary oocytes appear in many cases to be associated with nurse cells. In a fully grown oocyte the nucleus is large and has a conspicuous nucleolus. The cytoplasm is completely filled with yolk platelets and is either weakly acidophilic or does not stain at all, but appears yellow, the color of the yolk platelets.

Oocytes within an individual female gonad lack synchrony and instead, the ovary may contain oocytes in all stages of vitellogenesis throughout the year. Large oocytes that are not spawned are broken down and resorbed in situ. These oocytes are no longer membrane bounded (Lambert and Dehnel, 1974), and the yolk platelets left in the tubule are resorbed.

Determination of the Reproductive Cycle from Egg Diameter Data

Figure 9 depicts the range, mean and 95% confidence interval for the period of May, 1970 through July, 1971 for the oocytes measured in Nucella lamellosa at Coquille Point. The decrease of the mean of the samples for the period of May through July for each year corresponds to the time that egg capsule deposition was observed in

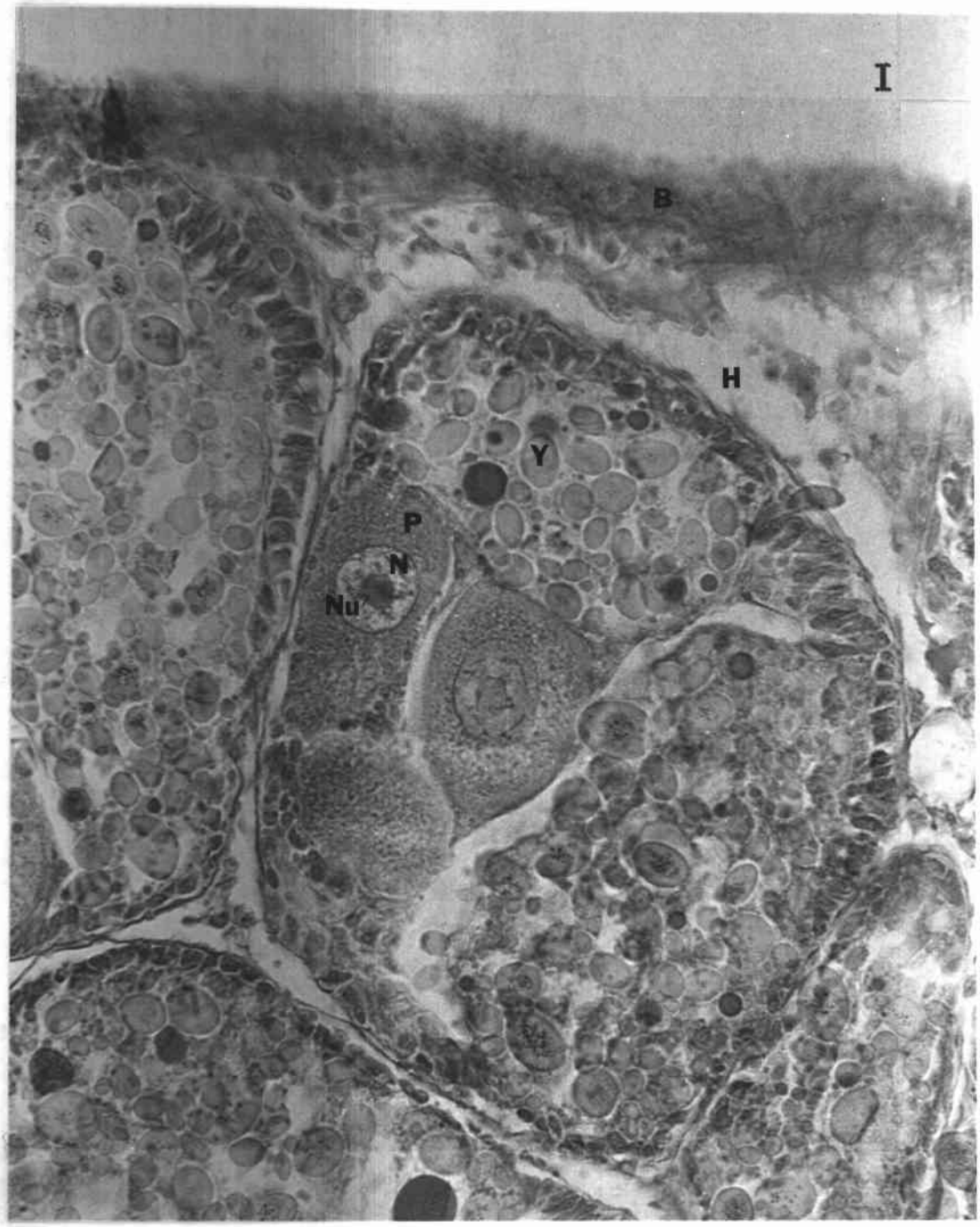

Figure 8. Cross section of an ovary tubule from Nucella lamellosa showing two pre-vitellogenic primary oocytes sectioned through the nucleus and nucleolus and yolk platelets of two vitellogenic oocytes not sectioned through the nucleus. The scale line represents ten microns. B = body wall; H = hemocoel; N = nucleus; Nu = nucleolus; p = pre-vitellogenic oocyte; y = yolk platelet.

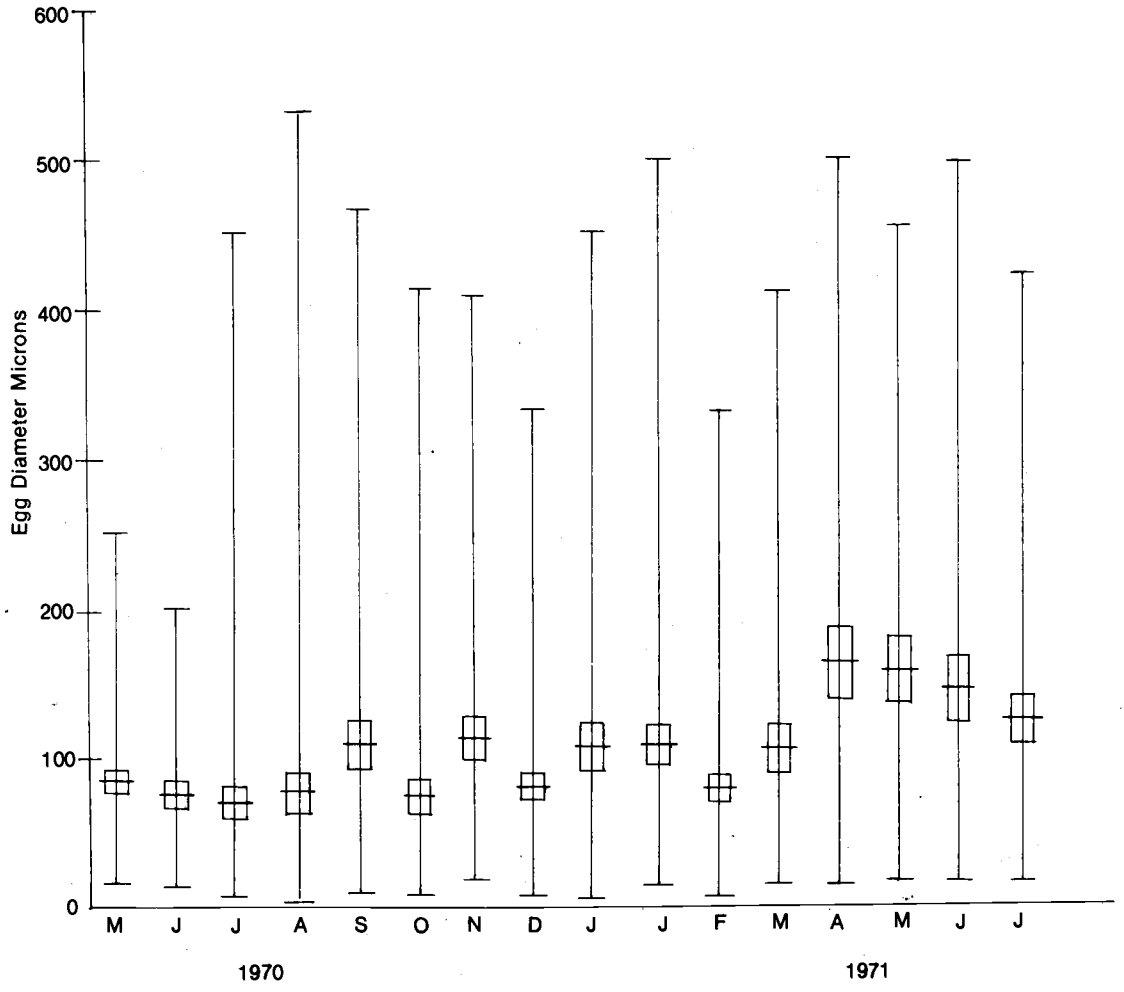

Figure 9. Range, mean and 95% confidence interval for the oocytes measured in Nucella lamellosa from May, 1970 through July, 1971. The total number of oocytes measured per month is 125. Two samples were collected during January, 1971.

the field. From February through April in 1971, both the maximum oocyte diameter measured and the mean increased, indicating rapid growth of the larger oocytes. The mean remains far below the size of mature ova, and the low end of the range is approximately constant throughout the year, indicating that new oocytes are continually being introduced into the system.

The oocyte size-frequency histograms presented in Figure 10 show that oocytes below 50 μm diameter (29 μm class interval mid-point) constituted a significant percentage of all oocytes measured in all months of the year. The smallest size classes dropped only in April to July, 1971 when the histograms clearly indicate a period of rapid growth to larger sizes by the middle-sized oocytes. By contrast, the frequency of occurrence of oocytes above 100 μm diameter changes dramatically with season. Since the supply of smaller oocytes within the gonad is not depleted by this seasonal growth to larger sizes, some proliferation of oogonia and their growth to small oocytes may take place throughout the year. It is possible that the size-frequency method obscures a seasonality in this rate of proliferation and hence the rate of appearance of young oocytes.

A high degree of variation between years in the synchrony of growth of oocytes is indicated by a bimodal size-frequency distribution of April to July, 1971 and a lack of a distinct larger size cohort in the same period of 1970.

Figure 10. Oocyte diameter frequency histograms for Nucella lamellosa for the period from May, 1970 through July, 1971. The total number of oocytes measured per month is 125. Two samples were collected during January, 1971.

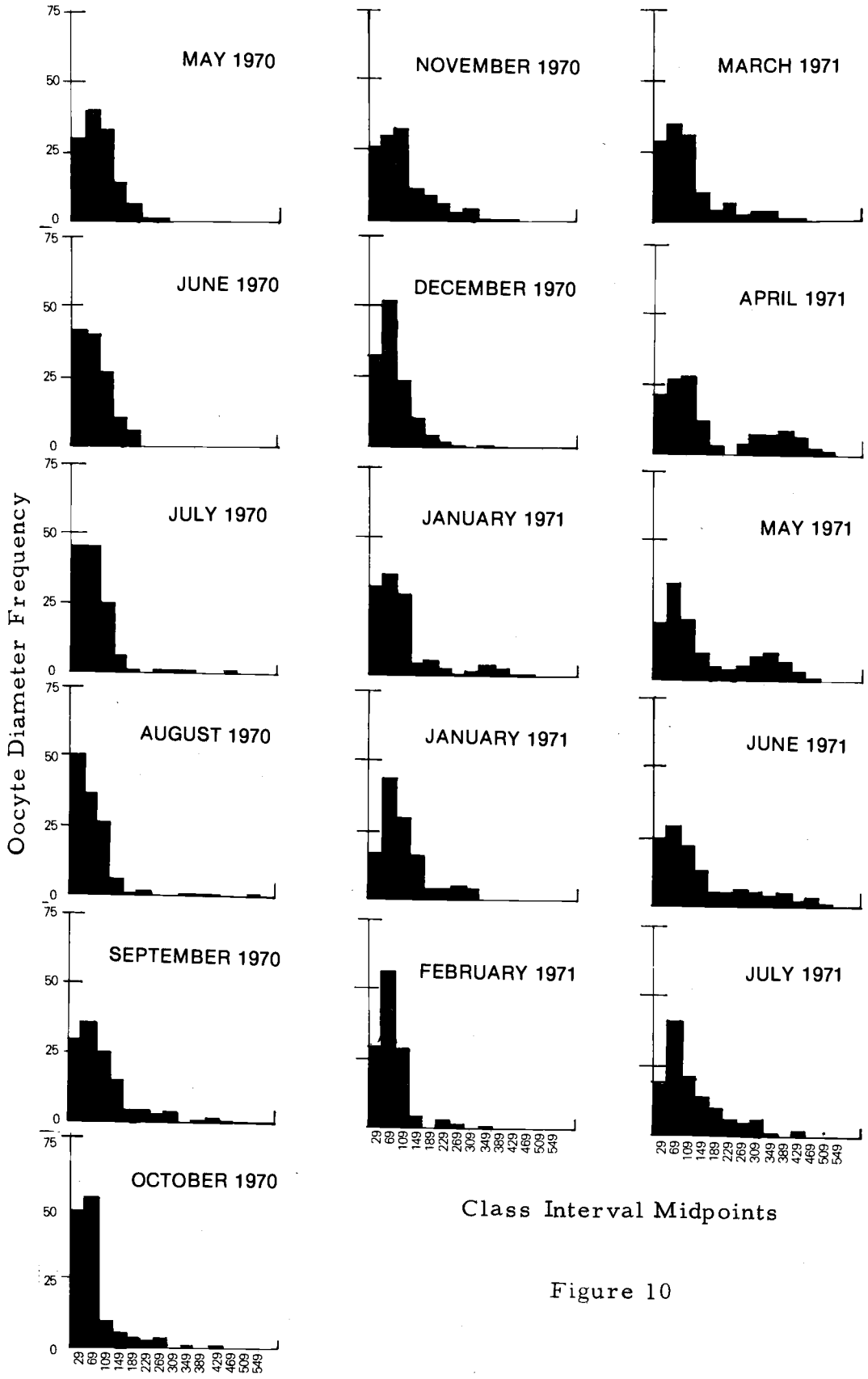
MAY 1970
NOVEMBER 1970
MARCH 1971
JUNE 1970
DECEMBER 1970
APRIL 1971
JULY 1970
JANUARY 1971
MAY 1971
AUGUST 1970
JANUARY 1971
JUNE 1971
SEPTEMBER 1970
FEBRUARY 1971
JULY 1971
OCTOBER 1970
75
50
25
0
29
69
109
149
189
229
269
309
349
389
429
469
509
549
Oocyte Diameter Frequency
Class Interval Midpoints

Figure 10

Lambert and Dehnel (1974) found that large oocytes occupied the ovaries of the females they studied most of the year in British Columbia except for about three months after spawning. This same phenomenon for the Coquille Point population of Nucella lamellosa can be seen in Figure 10 for the period of June through August, 1970, even though the period of egg laying is very different from that observed by Lambert and Dehnel (1974). Egg capsules were first observed on June 4, 1970 and June 23, 1971, much later than the January observation by Lambert and Dehnel (1974) in British Columbia. Apparently the physiology of the two populations is similar, but the cue which induces egg capsule deposition may either be different or perhaps occur at a different time in the two locations. A period of rapid growth is indicated by the increase in the number of oocytes in the larger size classes during the period of March through June, 1971 (Figure 10). The period of egg capsule deposition began slightly later in 1971 than in 1970. Aggregations were first observed in 1970 on May 14 and new egg capsules on June 4, while the first aggregations in 1971 were seen on May 25 and new egg capsules on June 23. Breeding aggregations had dispersed by October 10, 1970. No observations of breeding aggregation dispersal were made in 1971. New egg capsules were not observed after August 28, 1970 or August 19, 1971. This much variation in the time of egg laying is not unusual, however, since Nucella lamellosa

were observed to begin egg laying November 20, 1964, but not until December 10, 1965 at Port Townsend, Washington by Emlen (1966).

Nucella lamellosa at Coquille Point completed spawning by the end of August in both 1970 and 1971. At this time only small primary oocytes remained in the ovary (Figure 11). If September 1 is taken as a starting point for measuring growth, the smallest oocyte diameter measured was approximately 9 μm. Figure 9 shows rapid increases in both the means of the samples and in the maximum oocyte diameters measured for the period of February through April.

Instantaneous relative growth was defined by Brody (1945) on the basis of an increase in weight or volume. A measure of the increase in volume was used as the basis for calculating oocyte growth rates in this study. The natural logarithm of the volume at time one was subtracted from the natural logarithm of the volume at time two and the remainder was divided by the time difference between time two and time one. A day was chosen as a convenient unit of time to use in these calculations. The assumption was made that the physiological significance of a day did not change over the period of time that an oocyte grew.

Assuming that an oocyte grew from 9 μm to 340 μm in diameter between September 1, 1970 and March 1, 1971, the estimate of daily relative growth would be 6.1% per day (k=0.061). This would represent a maximum daily relative growth rate and would be necessary if

oocytes were to be produced and complete their growth between successive spawning seasons as suggested for Nucella lamellosa by Lambert and Dehnel (1974). More likely, oocytes grew from a mean diameter of 110 μm on September 1, 1970 to a diameter of 340 μm on March 1, 1971 at a daily relative growth rate of 1.9% per day (k=0.0188), based on the size-frequency distribution of oocytes presented in Figure 10. An increase in diameter from 340 μm to 510 μm from March 1, 1971 to May 1, 1971 gives an estimate of daily relative growth rate of 2% per day (k=0.020). On the basis of a smallest diameter of 9 μm on September 1, 1970 to a mean diameter of 80 μm on March 1, 1971, the growth rate was 3.6% per day (k=0.036). Growth based on the means increasing from 80 μm to 175 μm between March 1 and May 1 is 3.9% per day (k=0.039). The highest daily relative growth rate occurred between March and April when the mean diameter increased from 100 μm to 175 μm in 30 days, an increase of 5.6% per day (k=0.056). The question then may be not whether an oocyte could grow from a diameter of 9 μm to 340 μm in a period of 180 days at a daily relative growth rate of 2% per day, but how many oocytes can be grown at these rates per unit time. The fluctuations in means, ranges and maximum sizes measured during the period of a year seem to indicate that small oocytes are introduced during the entire year and may grow at different rates which are dependent both on the size of the oocyte and the amount of food

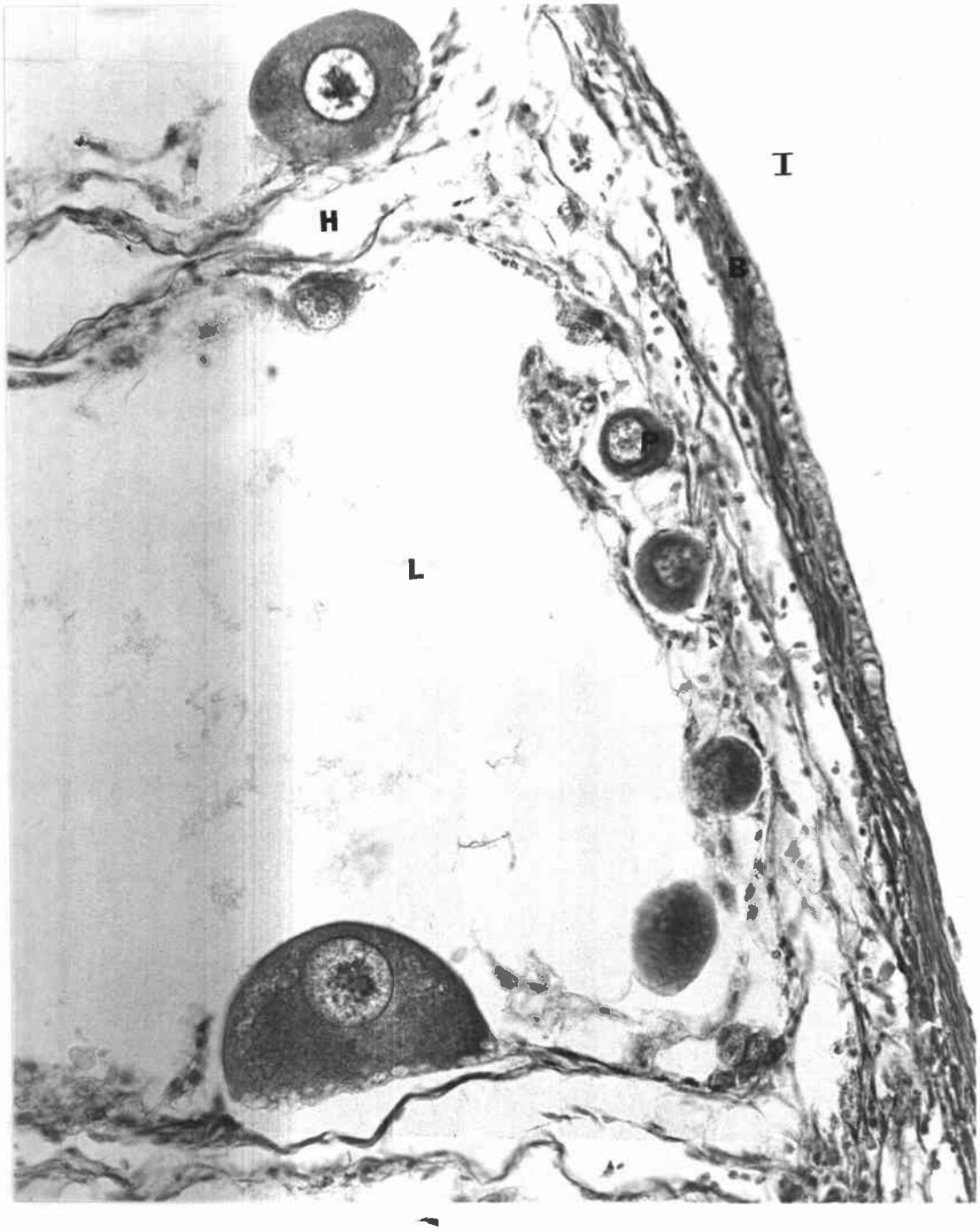

Figure 11. A portion of an ovarian tubule of a spawned Nucella lamellosa showing small primary oocytes attached to the tubule wall. The scale line represents ten microns. B = body wall; H = hemocoel; L = lumen at tubule; P = pre-vitellogenic oocyte.

available to the individual snail. Lambert and Dehnel (1974) have shown that Nucella lamellosa converts ingested food directly to gametes, and Gonor (1973a) has shown that Oregon populations of Strongylocentrotus purpuratus may be food limited. Pearse (1966) found that the Antarctic asteroid Odontaster validus produced more ova when food was more abundant.

Egg Capsules and Young

The egg capsule of Nucella lamellosa is vase-shaped, about 5.5 mm tall and 2 mm wide and is borne on a stalk about 2.7 mm long which holds the capsule away from the substrate. The apical end of the capsule is closed by a plug about 0.5 mm in diameter. The fertilized eggs are embedded in the capsule in a thick secretion. They are irregularly polygonal in shape and become rounded when dissected out of the capsule and exposed to sea water. The mean of 115 eggs measured from four capsules was 638 μm. Each capsule contained an average of approximately 29 eggs. Emlen (1966) counted 81 eggs per capsule at Port Townsend, Washington and Spight (1972), 20.8 at San Juan Island, Washington. Spight (1972) concluded that a linear relationship existed between capsule size and egg number and that larger females were capable of producing more eggs than smaller females. Most of the eggs laid by Nucella lamellosa are fertile (Ahmed and Sparks, 1970), and the embryos lack the expandable

mouth and esophagus used by other members of the genus for manipulating and ingesting food eggs within the capsule (Lyons and Spight, 1973).

Young snails emerged from egg capsules after 29 days of development during the summer of 1976. The capsules were deposited on the side of a holding tank at the upper edge of the water level in a tank in which sea water from Yaquina Bay was continuously circulated. During the 29 days, the water temperature fluctuated between 17° and 11.5° C. Emlen (1966) and Lambert and Dehnel (1974) estimated the developmental time for capsules in the field at approximately 140 days at Port Townsend, Washington and Brockton Point, Vancouver, British, Columbia, respectively. It seems likely that the developmental time for Nucella lamellosa at Coquille Point, Oregon is considerably less. The number of hatchlings found in five capsules ranged from 14 to 28, with a mean of 22.

Spermatogenesis

Spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperm were identified using the following criteria: 1) the relative size of the cell, 2) the relative size of the nucleus, 3) the amount and degree of basophilia of the cytoplasm, and 4) the abundance of cell types. Immediately after spawning, the testis of Nucella lamellosa appear to be empty of the later stages of

spermatogenesis (Figure 12). The tubules of the testis are constricted, and loose connective tissue and the hemocoelic space between the tubules is expanded. With the exception of spermatogonia, all the stages of spermatogenesis could be identified in the tubules of the testis during the entire year except immediately after spawning. The cell types are gathered in clusters, with cells within each cluster synchronized at the same stage of development, but different clusters within the tubule were not synchronized. Moreover, each tubule did not necessarily contain every type of cell. Proliferation seemed to take place from points along the tubule wall, with the descendants of those cells then forming a pyramid or clump extending into the lumen of the tubule.

Mature sperm were oriented with their flagellae extending in the lumen of the tubule (Figure 13). Sperm were observed by Feare (1970a) in Nucella lapillus and by Manzi, Calabrese and Rawlins (1972) in Urosalpinx cineria to have a similar orientation in the testis. When the quantity of sperm in an individual tubule was measured on a percent basis, a monthly average for three individuals clearly indicated a reduced amount during and immediately after the spawning period. However, variation among individuals from any one monthly sample was great (Figure 14). During the fall and winter, proliferation of gonial cells predominates. In the spring and summer the major activity in the testis is spermiogenesis and storage of sperm prior

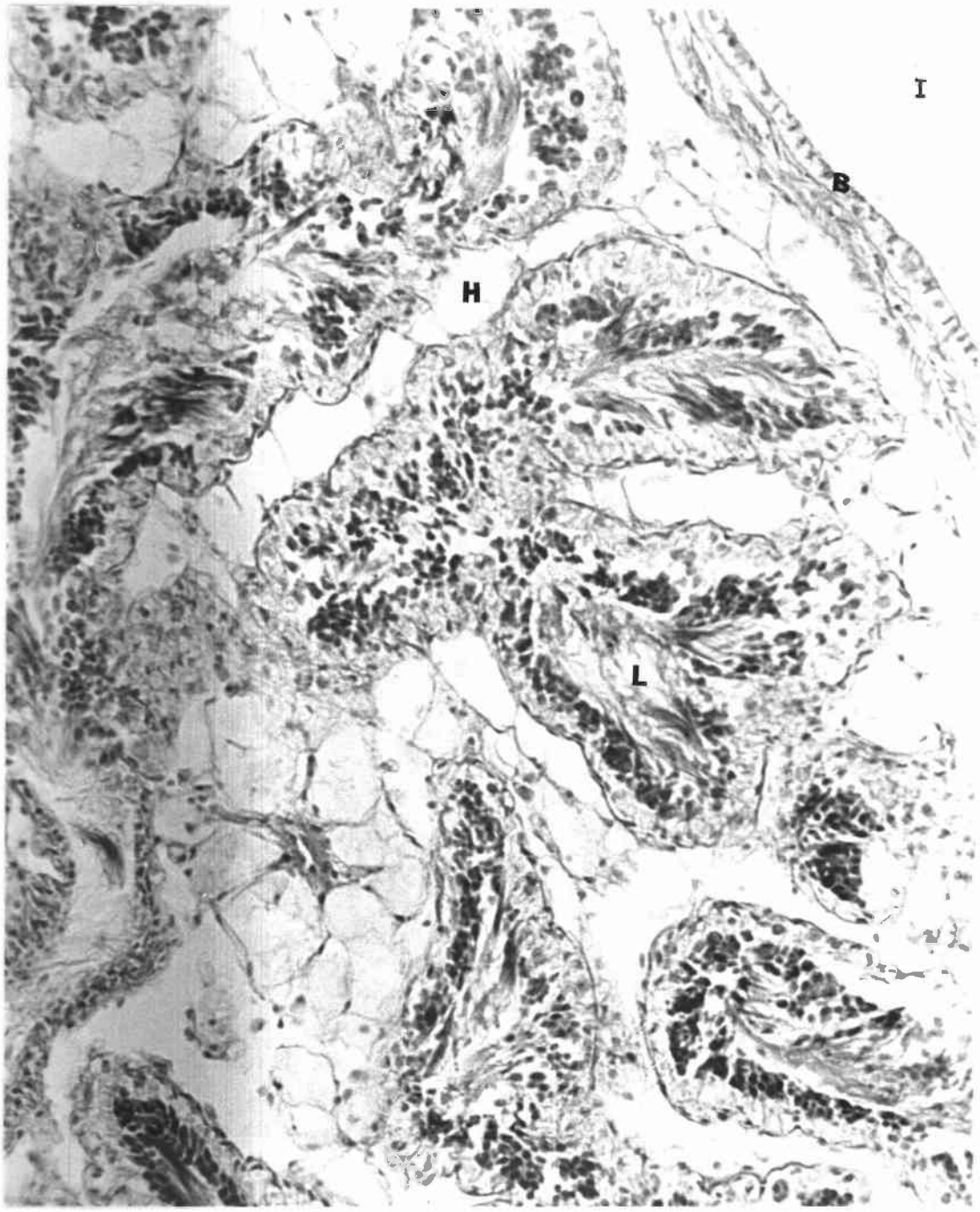

Figure 12. Section of the testis of a recently spawned Nucella lamellosa. The hemocoelic space has increased between the tubules. The scale line represents ten microns. B = body wall; H = hemocoel; L = lumen of tubule.

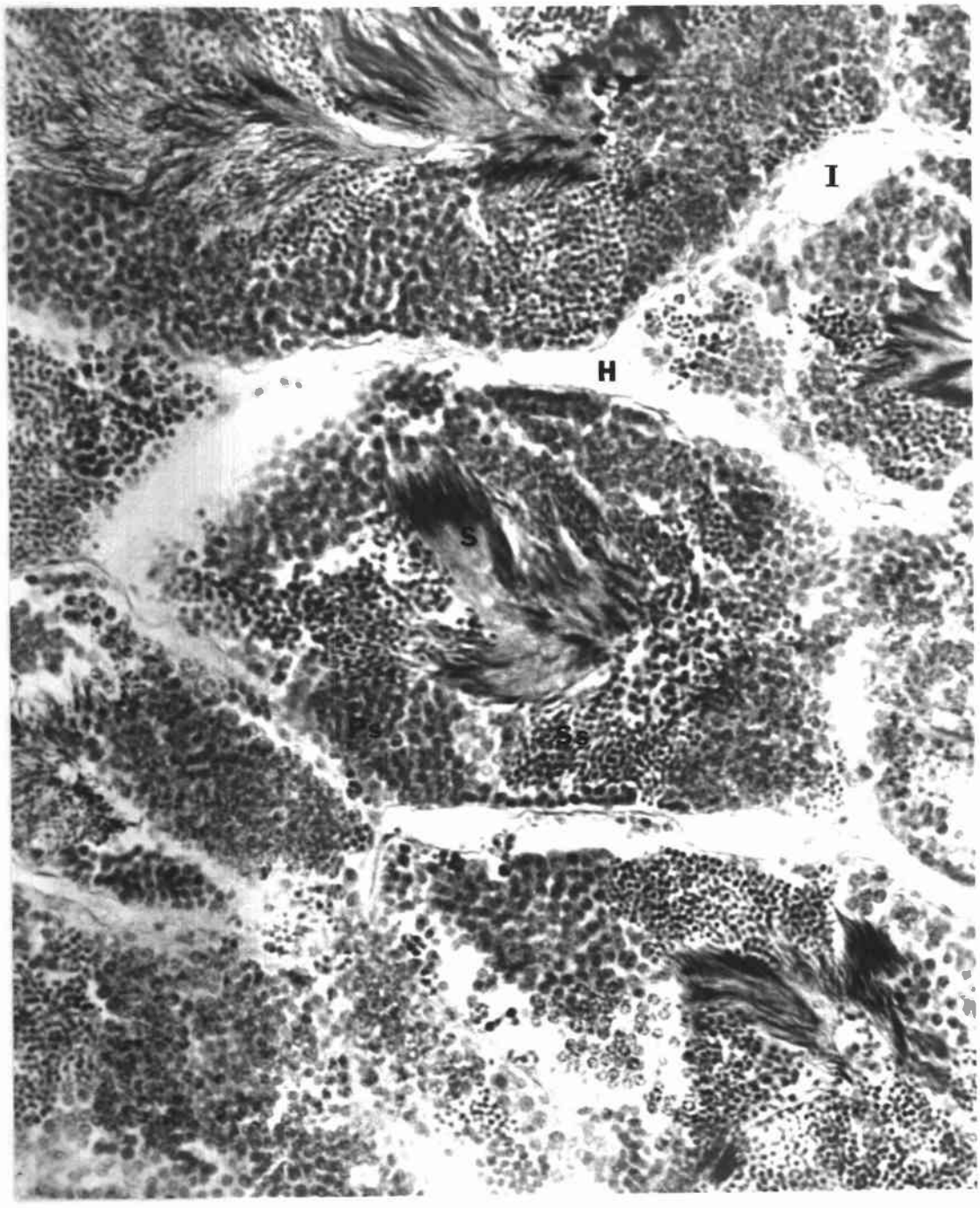

Figure 13. Cross section of a tubule from the testis of a ripe Nucella lamellosa. Hemocoelic space is reduced and sperm are oriented with their tails extending into the lumen of the tubule. The scale line represents ten microns. H = hemocoel; Ps = primary spermocyte; S = sperm; Ss = secondary spermatocyte.

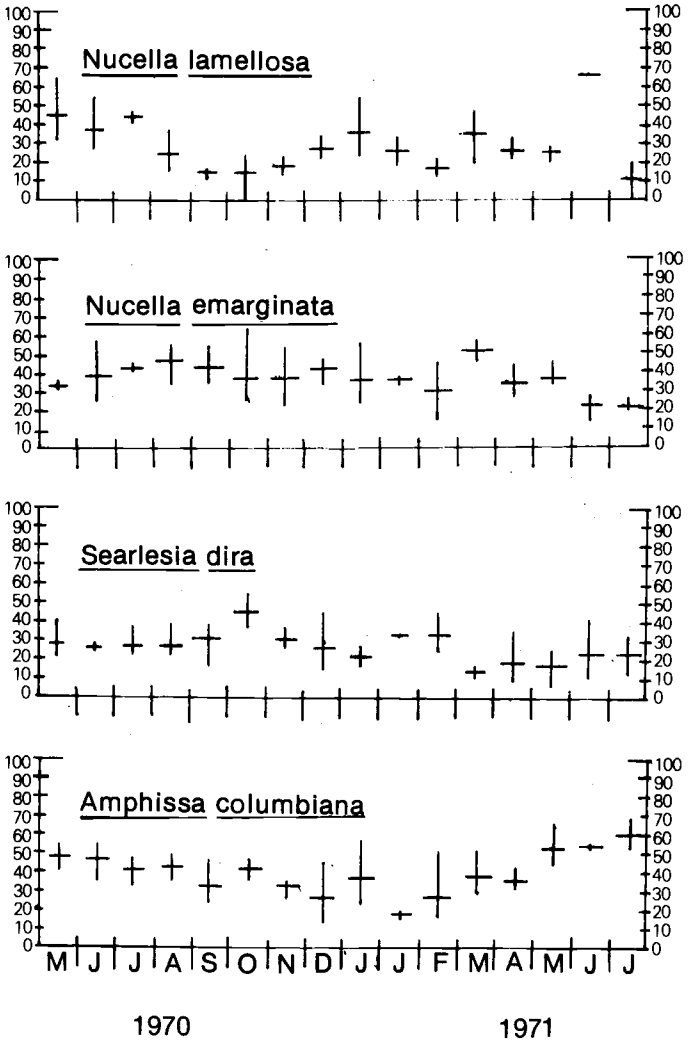

Figure 14. Range and mean of sperm in the tubules of the testis of Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana for the period from May, 1970 through July, 1971. Two samples were collected during January, 1971.

to copulation.

Nucella emarginata

Field Observations

Nucella emarginata has an intertidal vertical distribution from about +0. 3 to +1. 68 m (+1 to +5. 5 ft) above MLLW at Seal Rock. Percent exposure on a yearly basis at these levels would be approximately 2% and 60%, respectively (Figure 5). Large and small individuals are found throughout the vertical range. However, smaller individuals of Nucella emarginata have been found at the top of this species vertical range in Puget Sound (Bertness and Schneider, 1976). Sexually mature individuals aggregate to copulate and deposit egg capsules in crevices in the upper half of the range. The tough-walled capsules may be attached either to the sandstone substrate or to the shells of the barnacle Balanus glandula, with which the snails are associated and which serve as the main food source for this snail population. Emlen (1966) also found the range of Nucella emarginata to coincide exactly with the range of Balanus glandula, 0. 0 to +1. 52 m (0. 0 to +5. 0 ft), at Port Townsend, Washington. Both vertical and horizontal movements may be undertaken by these individuals in search of food. Vertical movement down into a mussel bed during the low tide period has been described by Glynn (1965) as a mechanism

for avoiding desiccation. Emlen (1966) found that Nucella emarginata at Port Townsend did not move vertically during low tide periods, but remained in the area of the large Balanus glandula and ceased feeding when exposed. Nucella emarginata were observed to have moved up approximately 0.3 m (1 ft) intertidally during the month of May, 1971 at Seal Rock.

There was no difficulty distinguishing sex on the basis of the presence of a penis in the male of this species; female Nucella emarginata do not possess a rudimentary penis as do female Nucella lamellosa. The sex ratio for animals collected during this study was 45% males to 55% females. This is not significantly different from a sex ratio of 1:1 based on a chi-square value of 2.95 and one degree of freedom. Since many of these animals were collected from aggregated breeding groups, it seems unlikely that only females congregate in the area where egg capsules are laid, as stated by Houston (1971). A more likely explanation for the observed preponderance of females in the sample is that males leave the aggregations earlier than do females, as observed by Spight (1974) for Nucella lamellosa at Shady Cove, San Juan Island, Washington.

Seventeen percent of the Nucella emarginata collected in this study were found to be infected with a trematode parasite. The infestation begins in the digestive gland and spreads to the gonad (Figure 15). Parasitic castration of Nucella lapillus by a trematode results

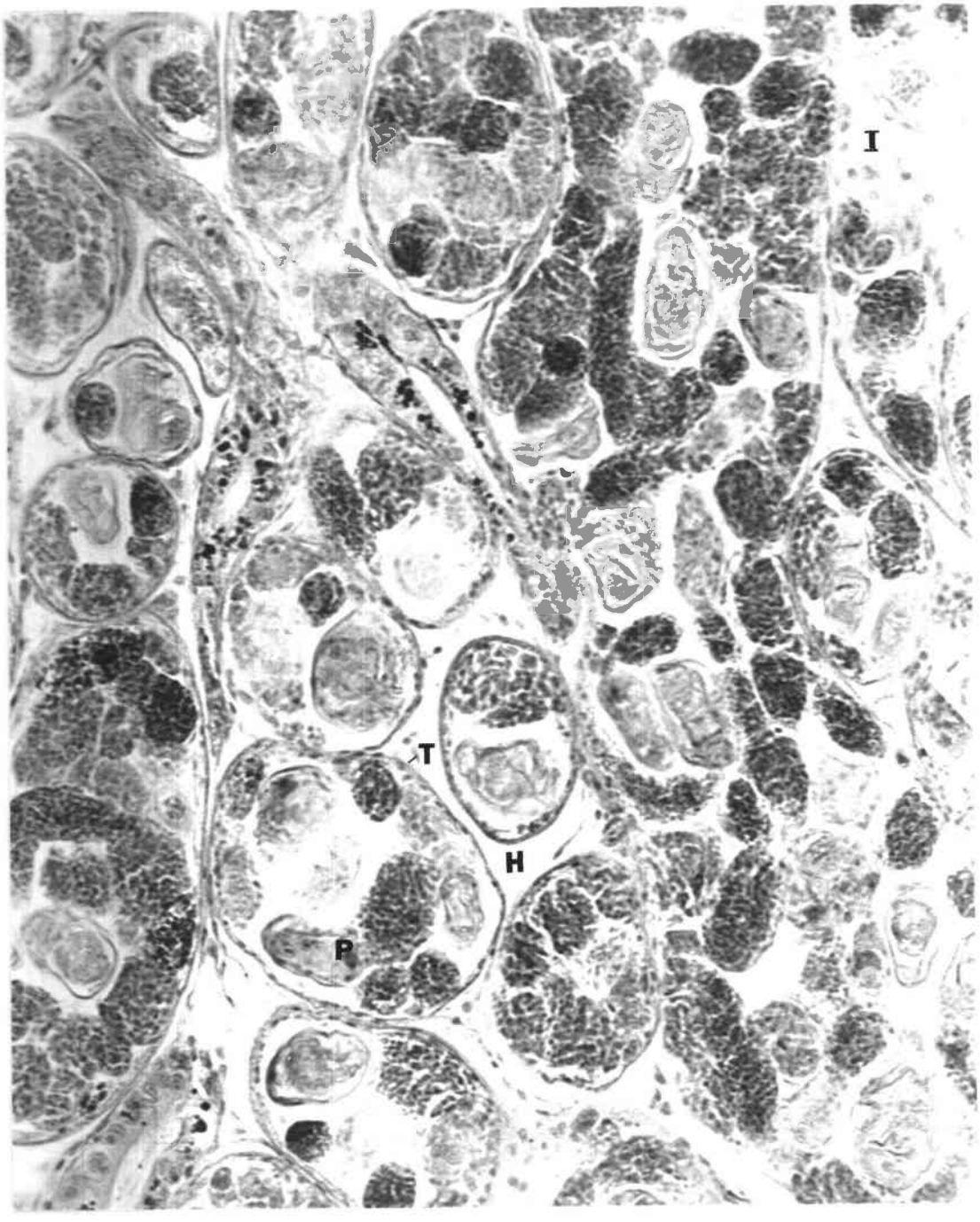

Figure 15. Cross section through the gonad of a female Nucella emarginata infected with a trematode parasite. No oocytes are present in this portion of the gonad. The scale line represents ten microns. H = hemocoel; Pa = parasite; T = tubule wall.

from deficiency of the nervous endocrine functions and is analogous to what happens during starvation (Feral, LeBreton and Streiff, 1972). No attempt was made to identify the parasite, but Cooley (1957) found the digenetic trematode Parorchis acanthus in the related East coast species Thais haemastoma.

The time of aggregation and egg laying for Nucella emarginata is not restricted to a brief period during the year. Rather, fresh egg capsules were found on February 6, October 16 and November 12 in 1970, and on January 26, April 24, May 25, June 23 and August 19 in 1971 at Seal Rock. More egg capsules were present in the winter and early spring than in the summer and fall.

Oogenesis

The same sequence of oogenic events described for Nucella lamellosa applies to Nucella emarginata. The sizes at which pre-vitellogenic oocytes become vitellogenic oocytes and these in turn become mature ova are smaller than for Nucella lamellosa since the average size of mature eggs and food eggs in the capsules is smaller, 180 versus 638 microns. Figure 16 illustrates the stages of development of primary ooctyes in the gonad of a female Nucella emarginata. The thickness of the ovary tubule wall decreases as the primary oocytes become larger. This may be due partly to crowding and partly to a reduced need for nutrients on the part of

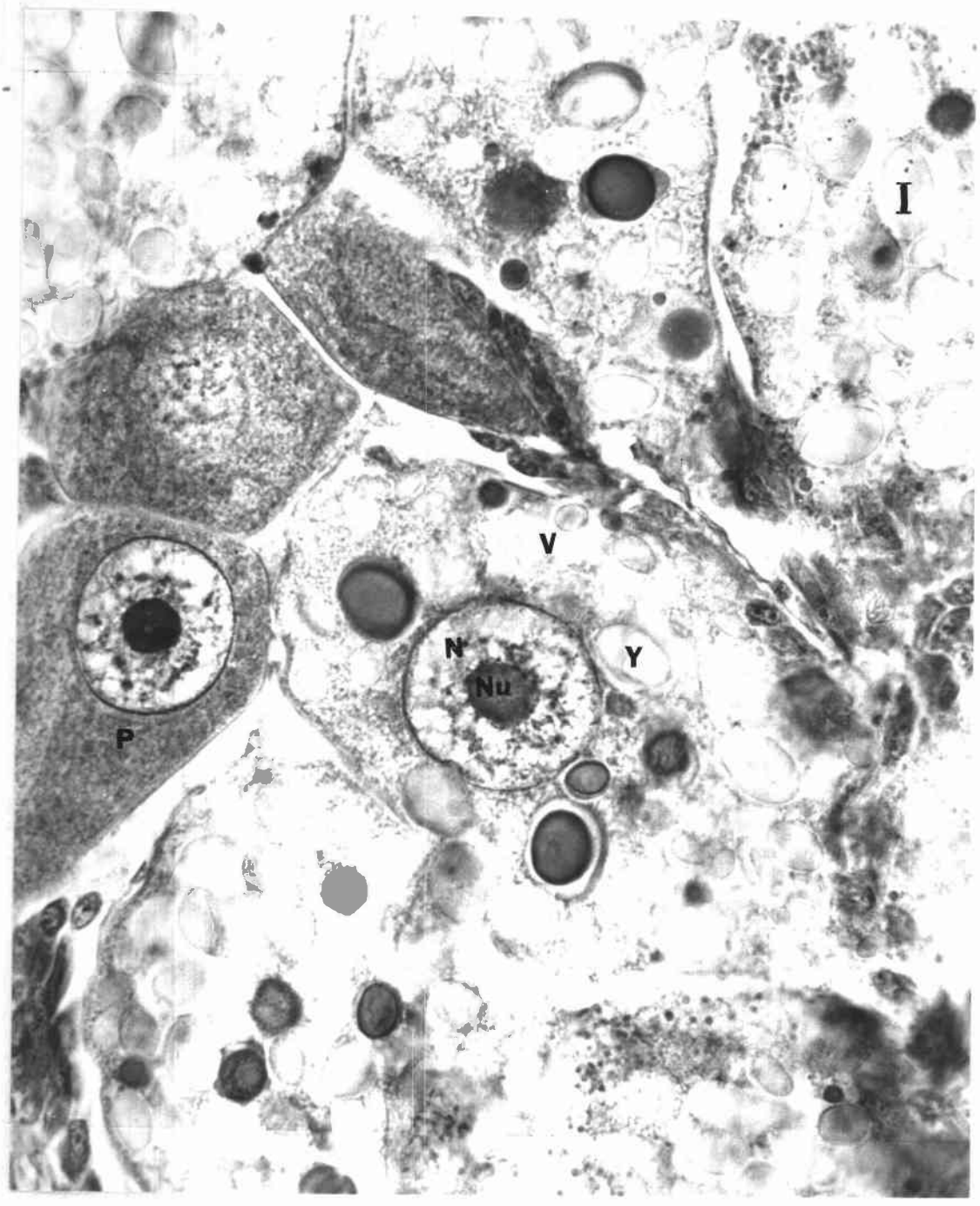

Figure 16. Cross section through the gonad of a female Nucella emarginata showing both a pre-vitellogenic oocyte and a vitellogenic oocyte sectioned through the nucleolus. The scale line represents ten microns. P = pre-vitellogenic oocyte; N = nucleus; Nu = nucleolus; V= vitellogenic oocyte; Y = yolk platelet.

primary oocyte. Again, it should be noted that all stages of oocyte development may be present at a single time in a single individual, a situation similar to that found by Houston (1971) in Nucella emarginata at Dillon Beach, California, and by Feare (1970a) for Nucella lapillus at Yorkshire, England. However, unlike spawned Nucella lamellosa, which have large, empty spaces in the tubule of the gonad, no individuals of Nucella emarginata were found to be in this condition. Possibly the time when spaces occurred in the gonad was short and therefore was not "captured" in monthly samples. Alternatively, individuals may not empty themselves completely at a single spawning and no large space may ever be created in the gonad. Multiple spawnings for individual females were observed by Emlen (1966), who suggested that this may be due to the lack of sufficient spawning sites for all the females to spawn at once. At Seal Rock no lack of suitable spawning sites was observed.

Determination of the Reproductive Cycle from Egg Diameter Data

Figure 17 depicts the range, mean and 95% confidence interval of oocyte diameters for the period of May, 1970 through July, 1971. The decreases of the means of the oocytes in samples from January through February and March through May, 1971 correspond with the major egg capsule deposition period observed in the field. The range

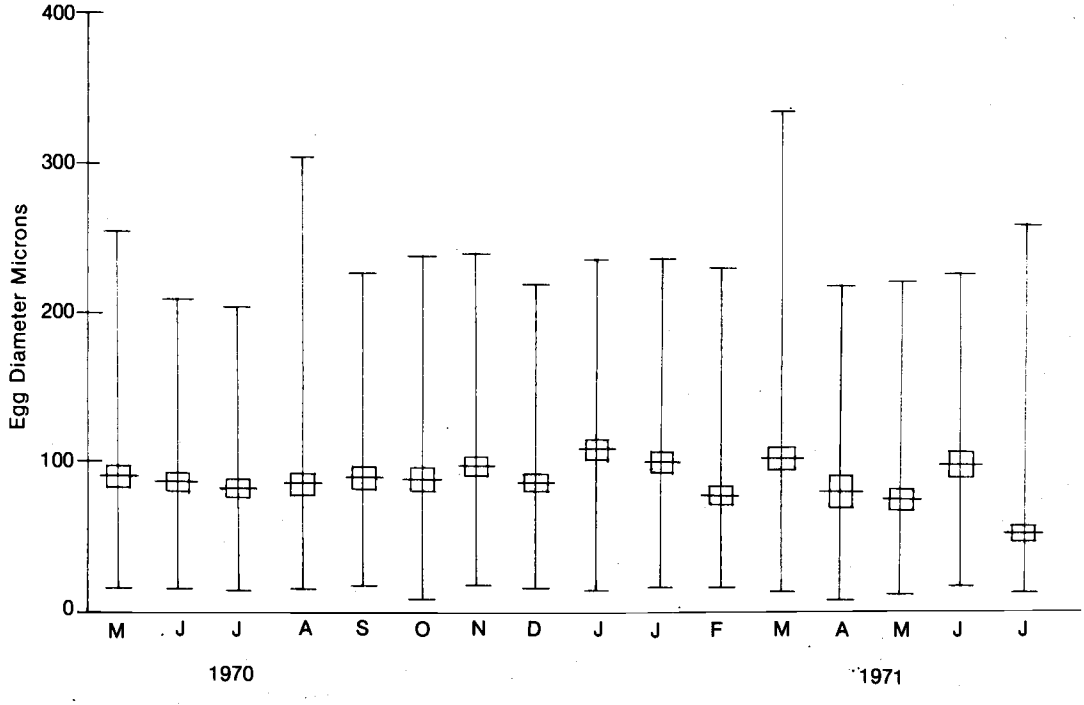

Figure 17. Range, mean and 95% confidence interval for the oocytes measured in Nucella emarginata from May, 1970 through July, 1971. The total number of oocytes measured per month is 250. Two samples were collected during January, 1971.

of oocyte size remained fairly constant throughout the year, indicating that the population is not closely synchronized, with a single breeding season.

The oocyte diameter frequency histograms presented in Figure 18 show a preponderant bimodal distribution for the smaller two-thirds of the size range measured. A break in the distribution appears between the 108 μm interval and the 130 μm interval in most months. From May through September, 1970, the ratio between the smaller and larger cohorts was 4:1. During October and November this ratio changed to 3:2, and in December it returned to 4:1, indicating that some of the large eggs were spawned. During January, 1971 the ratio was again 3:2. Both of the January sample ratios were 3:2, indicating no appreciable shift from the smaller cohort into the larger one. During February, the ratio changed to 4:1, indicating that either proliferation occurred or some of the large oocytes were spawned with no change in the number of small oocytes. In March the ratio shifted back to 3:2. During April and May the ratio was 4:1, again indicating probable spawning, followed by oocyte growth resulting in a 3:2 ratio during June. In July the cohort above 130 μm was completely gone.

The bimodality observed could only develop if the smaller oocytes, approximately 50 μm diameter, grew to establish the larger size classes while proliferation maintained the smaller size classes.

Since the average size of the eggs measured in a capsule was approximately 180 µm, one may assume that the reduction of these larger size classes represents spawning. The period when many large oocytes would have been available for spawning was from October, 1970 through March of 1971, with a second distinct peak in June, 1971. The times egg capsules were observed in the field were October 16 and November 12 in 1970 and January 26, April 24, May 25 and June 23 in 1971, approximately coinciding with the period of a distinct larger size cohort in the gonad.

The histograms in Figure 18 indicate a period of rapid oocyte growth during September and October, 1970. Using a mean diameter of 64 µm for the smaller cohort and 196 µm for the larger cohort, the daily relative growth rate would have been 11.1% per day (k=0.1119) for that period. Similar growth from the small cohort into the larger cohort seems to have taken place each month from October through June since the histograms did not change greatly even though spawning must have depleted the larger cohort. This would indicate that some oocytes complete their growth after spawning has started, a situation described by Gonor (1973b) for the echinoid Strongylocentrotus purpuratus. Since both spawnable-sized oocytes and the smallest size oocyte measurable were present in relatively large numbers (60%:40%) during October, it seems likely that recruitment from the population of oogonia took place just prior to the October sampling.

Figure 18. Oocyte diameter frequency histograms for Nucella emarginata for the period from May, 1970 through July, 1971. The total number of oocytes measured per month is 250. Two samples were collected during January, 1971.

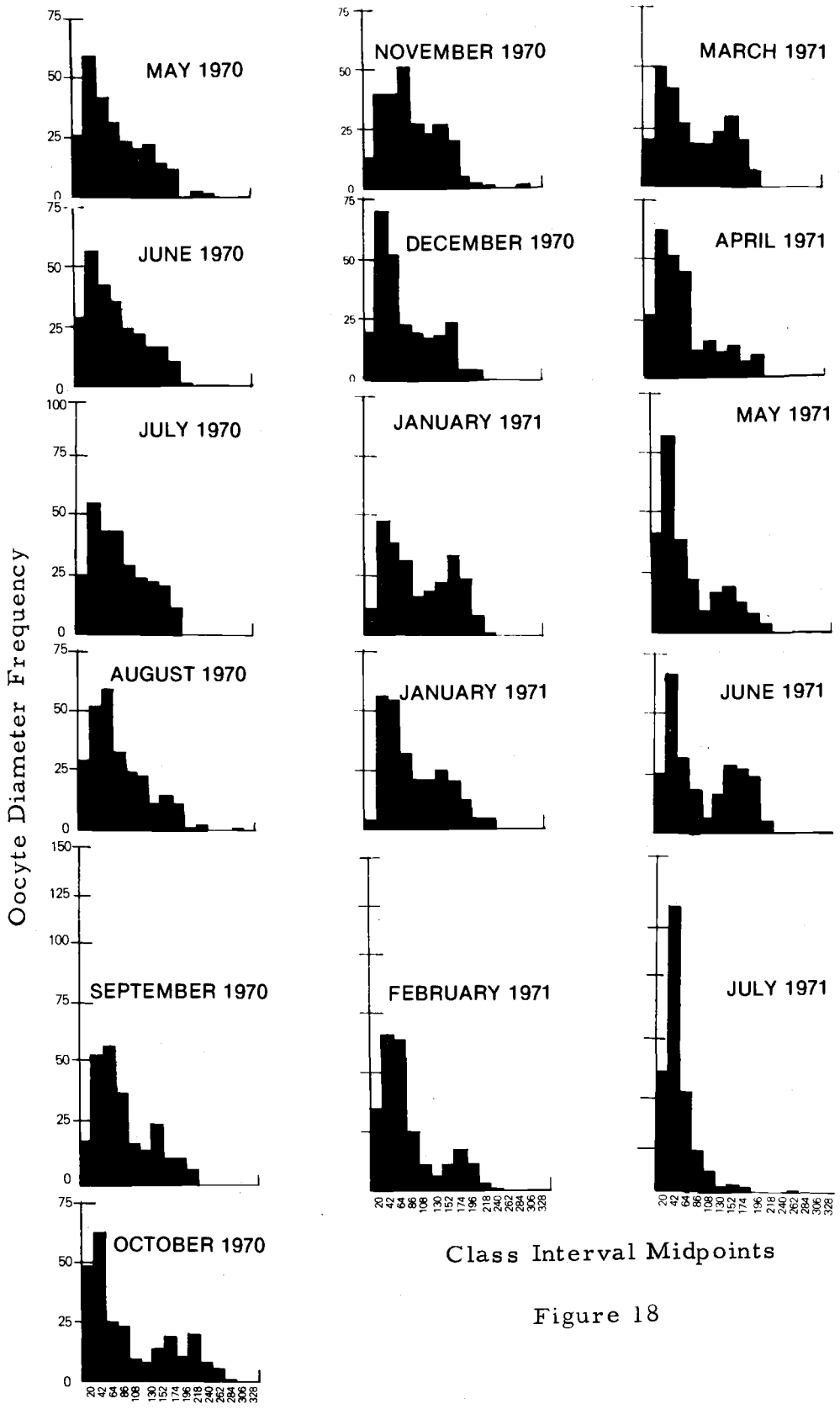
MAY 1970
NOVEMBER 1970
MARCH 1971
JUNE 1970
DECEMBER 1970
APRIL 1971
JULY 1970
JANUARY 1971
MAY 1971
AUGUST 1970
JANUARY 1971
JUNE 1971
SEPTEMBER 1970
FEBRUARY 1971
JULY 1971
OCTOBER 1970
Oocyte Diameter Frequency
Class Interval Midpoints

Figure 18

By late January, 1971 very few oocytes in the smallest size class (9 μm) were present, constituting 1% of the sample, but their number increased to 14% of the total sample during February. Two possible types of reproductive cycles are suggested by this data. One possibility is that proliferation takes place several times during the year, e. g., October, February and July, with growth to a spawnable size in the following three to four months. If oocytes grew at a constant rate from a size of 9 μm to 196 μm in a period of 90 days, their daily relative growth rate would be 10. 3% per day (k=0. 1027). If the assumption is made that proliferation began in October and that these oocytes grew from a diameter of 9 μm to a diameter of 64 μm between January and September, a daily relative growth rate of 11. 2% per day (k=0. 1119) would be required, and multiple spawnings for an individual female would be possible as observed by Emlen (1966).

It seems most likely that proliferation of oocytes in the Seal Rock population of Nucella emarginata begins in September and October and continues throughout the spawning season, which declines during June or July. If most of the large eggs have been removed from the gonads by this time, this would yield the large proportion of oocytes in the small cohort observed in July, 1971. Oocyte growth would then be at a very low level during the summer with only a few oocytes moving into the larger cohort. This is followed by a period of rapid growth just prior to or during the spawning season to bring

the oocytes to a spawnable size, resulting in an oocyte "lifetime" of 12 to 18 months. Feare (1970a) described an oocyte "lifetime" of 14 to 15 months in Nucella lapillus based on the number of pre-vitellogenic oocytes per unit area (microscope field) increasing prior to spawning of large vitellogenic oocytes. Nucella emarginata is clearly an organism which at this latitude exhibits multiple spawning periods throughout the course of a year.

Egg Capsules and Young

Egg capsules for Nucella emarginata were figured by Houston (1971) and in more detail by LeBoeuf (1971). The details of egg capsule morphology for the Seal Rock, Oregon population of Nucella emarginata are in agreement with those of LeBoeuf (1971) for a Dillon Beach, California population.

The larvae of Nucella emarginata are known to pass through the veliger stage inside the capsule while feeding on unfertilized food eggs. The number of eggs in a capsule is quite variable. From 300 to 1,000 eggs per capsule were counted by Emlen (1966) in capsules collected from Port Townsend, Washington. From 64 to 750 eggs per capsule were counted by LeBoeuf (1971) in capsules collected from Northern California; of these only three to thirty-nine developed to the juvenile stage. The mean diameter of 250 eggs measured out of a total of 361 from a capsule collected at Seal Rock was 180.84

(SD=12.92). LeBoeuf (1971) calculated an average diameter of 180 μm for eggs from capsules of Nucella emarginata at Dillon Beach, California.

The method of feeding on food eggs in the capsule has been described for Nucella emarginata by Lyons and Spight (1973), who indicate that individuals are only capable of feeding on their fellow veligers which are already dead and partly decomposed. The young snails crawl out of the egg capsule. Development time was 72 days for freshly collected egg capsules that were held at a constant temperature of 10° ±1° C. A developmental time of 80 days at a temperature of 8° - 10° C was observed by Emlen (1966) for Nucella emarginata.

Spermatogenesis

The same four criteria that were used in the identification of the various stages of spermatogenesis in Nucella lamellosa were applied to sections of the male gonad of Nucella emarginata.

The general appearance of the testis of Nucella emarginata is similar to that of Nucella lamellosa. All of the stages of spermatogenesis with the exception of spermatogonia could be identified in the tubules of the testis throughout the year in some individuals. Individual cell types were clustered, with the clusters all in the same stage of division. Each tubule did not contain all stages of

spermatogenesis, but all the stages could be found by observing several tubules within an individual. Mature sperm were oriented with their flagellae extending into the lumen of the tubule.

Spermiogenesis and storage of sperm prior to copulation are the major activities in an individual testis prior to the time that the snail breeds. Since Nucella emarginata breeds throughout the year, there is no period of time when the entire male population is synchronized, and individual males were not found with completely empty tubules in their testis (Figure 19), as were individuals of Nucella lamellosa. A quantitative estimate of the percentage of an individual tubule occupied by sperm in the testis of three animals per month is given in Figure 14. Variation among the individuals measured is as much as 100% (e. g., June and November, 1970; January and February, 1971). The range for the 15 month period of this study varied from 21% to 51%.

Searlesia dira

Field Observations

Searlesia dira occupies an intertidal vertical range from about -0. 3 to +1. 22 m (-1 to +4 ft) above MLLW in partially protected areas at Boiler Bay. Individuals would be exposed on a yearly basis approximately 3% of the time at the lower edge of the range and

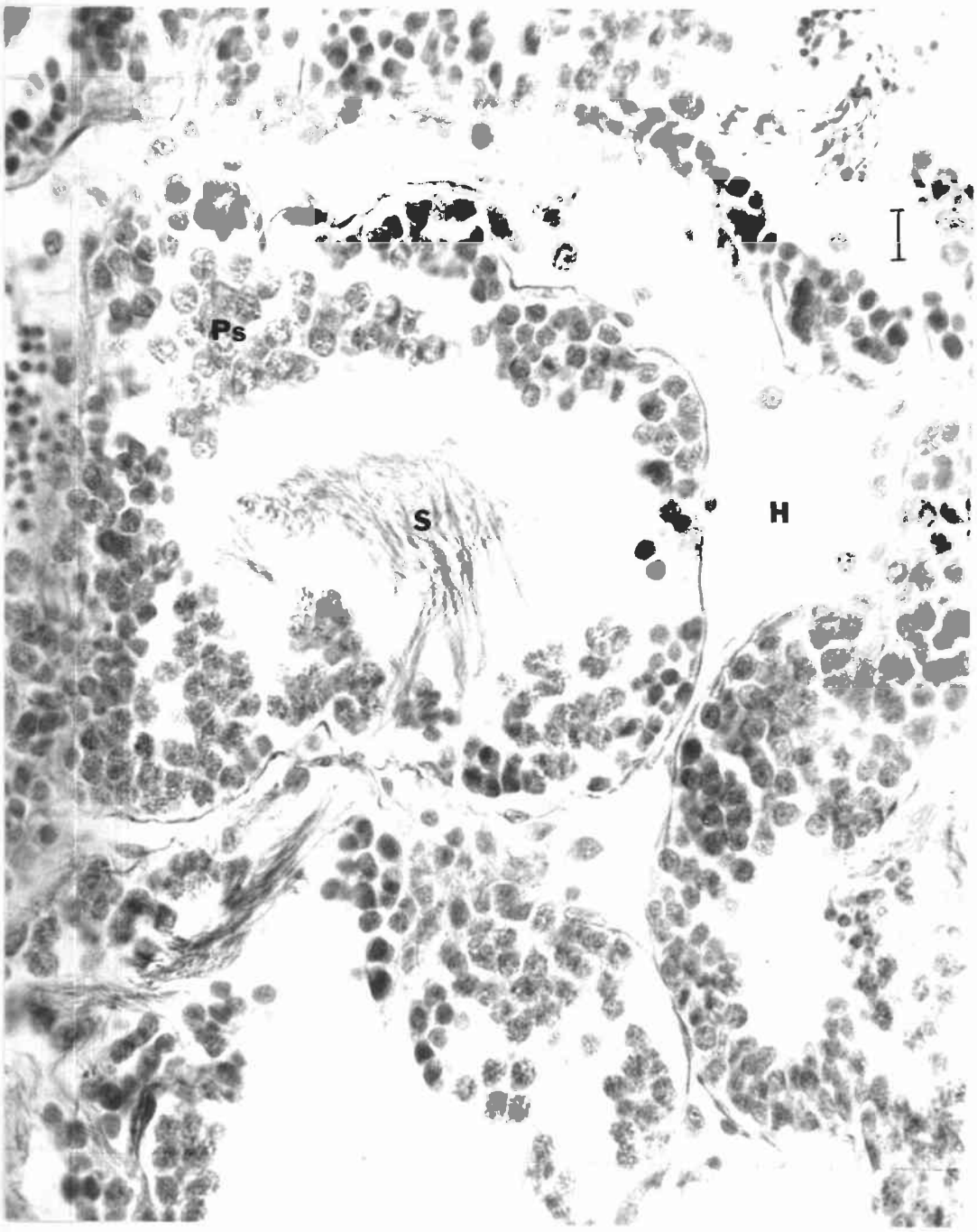

Figure 19. Cross section through a tubule in the testis of a partially spawned Nucella emarginata male. The scale line represents ten microns. H = hemocoel; Ps = primary spermatocyte; S = sperm.

approximately 43% of the time at the upper edge of the range (Figure 5). Commonly, Searlesia dira is associated with the red algae Rhodomela larix, which may provide physical protection from desiccation and some degree of protection from wave action. Smaller individuals occupy the upper part of the range, while sexually mature individuals occupy the middle to lower portion of the range, a distribution similar to that described for Nucella lamellosa. Individuals less than five mm long are relatively abundant during the late summer and are probably hatched beginning in February. Lloyd (1971) found average sized adults to be 25-30 mm long on intertidal rocky shores and 40-50 mm long on mudflats on San Juan Island, Washington.

Spight (1972) found that Searlesia dira was abundant on San Juan Island, occupying the mid-tidal range where it fed on barnacles. Lloyd (1971) observed that Searlesia dira spent low tide periods in gravel and tidepools and moved onto the rocks during high tide, in search of living or wounded prey. Observations of feeding by Searlesia dira on the Washington coast at Shi Shi, Mukkaw Bay and Portage Head indicate that it is a generalized higher order carnivore which eats barnacles and scavenges (Dayton, 1970). At Boiler Bay Searlesia dira was observed feeding on barnacles, other snails and pieces of smashed sea urchins. In the laboratory Searlesia dira readily fed on Mytilus that were opened for them. Feeding activity is seasonal on San Juan Island, Washington, decreasing during winter

and increasing in the spring to reach a maximum in the fall (Lloyd, 1971).

Adults of this species do not aggregate for breeding. Egg capsules were first observed at Boiler Bay on November 12, 1970 at the +0. 3 m (+1 ft) level. Subsequent observations of egg capsules were made on January 26 and April 24, 1971. The clear egg capsules are round if laid singly or irregularly elliptical if laid in clumps. Clumps contain from two to six egg capsules which are probably all from one snail since the snails were not observed to aggregate at any time during the year. The presence of a large penis in the male easily distinguishes the sexes after the snails have been partially removed from their shells. Males as small as 13. 2 mm long possessed a penis but it was less than one-fifth the length of the penis of a mature male. Both males and females appeared on an histological basis to be sexually mature at a shell length greater than 27 mm.

Oogenesis

The oocytes of Searlesia dira pass through the same staging of events as those described for Nucella lamellosa. The cytoplasm of pre-vitellogenic primary loctyes is darkly stained with hematoxylin. As these cells become vitellogenic, the accumulated yolk platelets stain lightly with eosin. All stages of oocyte development are present in a gonad at one time (Figure 20) except immediately

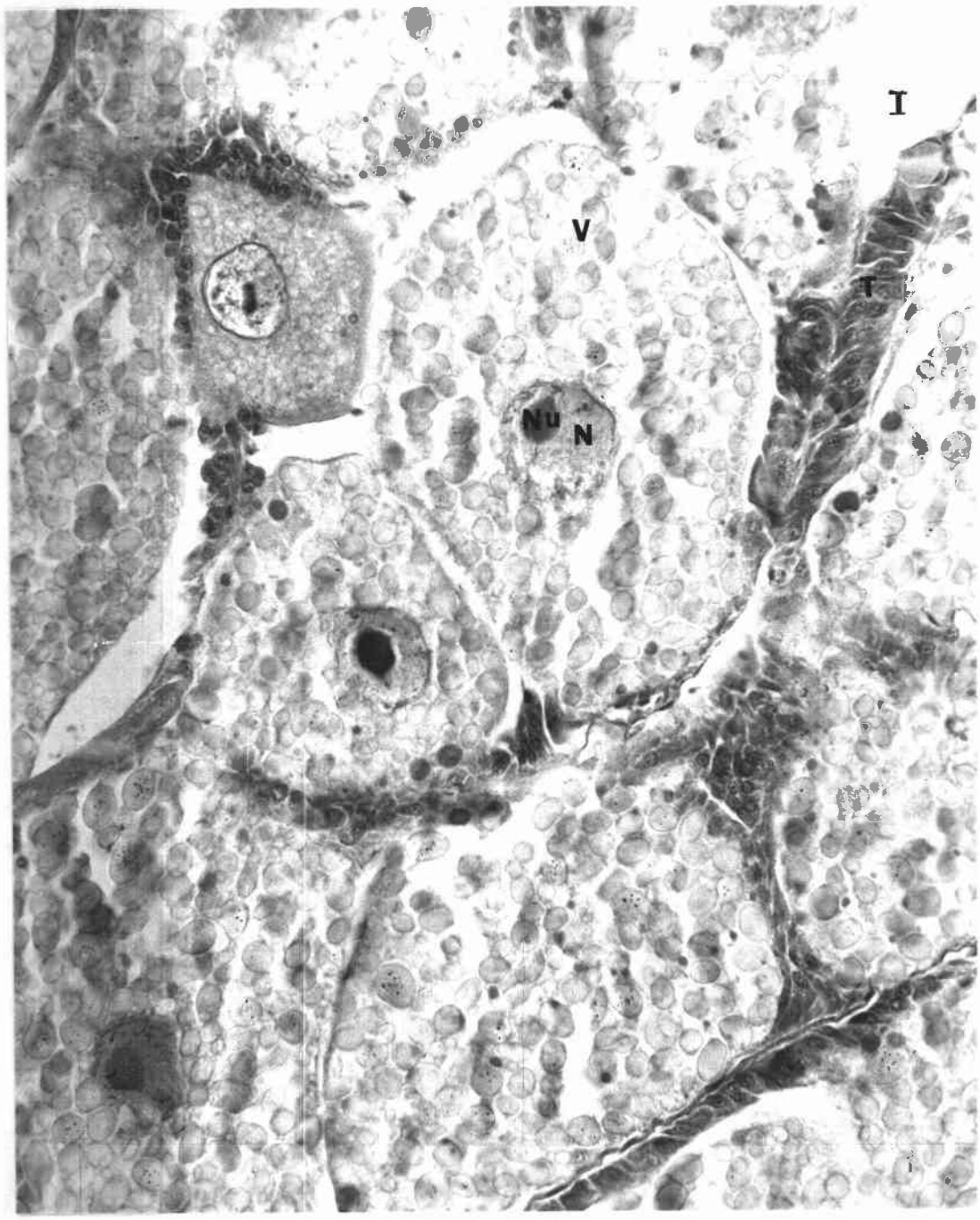

Figure 20. Cross section through the gonad of a female Searlesia dira showing several stages of vitellogenesis. The scale line represents ten microns. N = nucleus; Nu = nucleolus; T = tubule wall; V = vitellogenic oocyte.

after the animal has spawned (Figure 21). At this time vitellogenic oocytes are absent from the gonad and the pre-vitellogenic oocytes remaining in the tubules are oriented in a manner which indicates that they have been affected by the spawning of the post-vitellogenic oocytes. One end remains firmly attached to the tubule wall (Figure 21). Amoebocytes move into the partially emptied tubule and remove any traces of yolk platelets that may have been left behind when the post-vitellogenic oocytes were spawned. During the following month the diameter of the ovarian tubules is further reduced and the amoebocyte population in the lumen of the tubules decreases. Two functions could be served by the attachment of pre-vitellogenic and vitellogenic oocytes to the tubule wall. One might be to keep these immature oocytes from being removed from the tubule when the mature oocytes are spawned. A second might be the transport of yolk platelet material into the developing oocyte through the attachment point.

Determination of the Reproductive Cycle from Egg Diameter Data

Figure 22 depicts the range, mean and 95% confidence interval for the oocytes measured in Searlesia dira for the period of May, 1970 through July, 1971. The mean size measured decreased significantly between October and November, 1970 when spawning began and egg capsules were first observed in the field. The maximum size

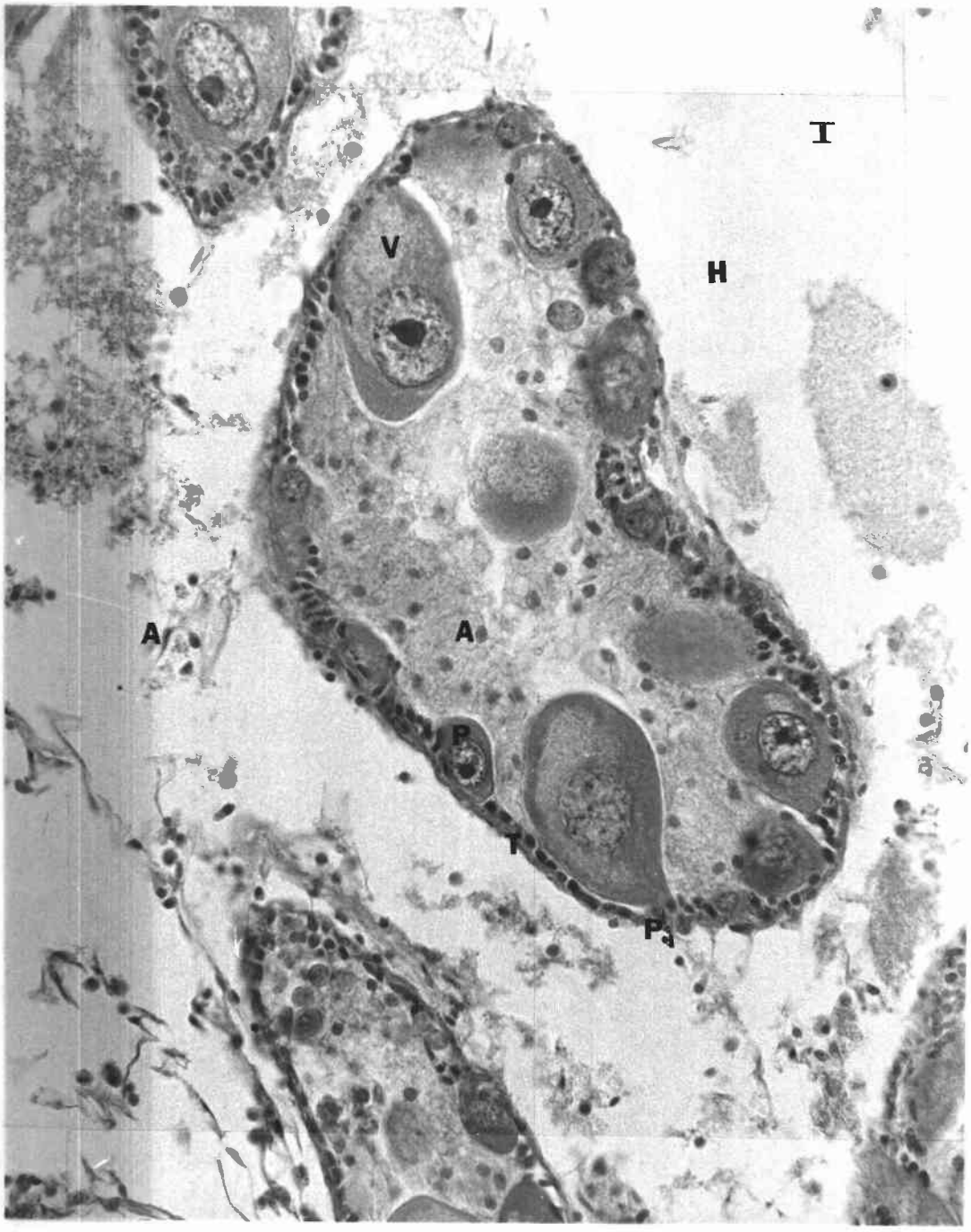

Figure 21. Cross section through the gonad of a spawned female Searlesia dira. Pre-vitellogenic and vitellogenic oocytes remain attached to the tubule wall. Amoebocytes are present in the lumen of the tubule, and the hemocoelic space between the tubules has increased. The scale line represents ten microns. A = amoebocyte; H = hemocoel; P = pre-vitellogenic oocyte; Pa = point of attachment; T = tubule wall; V = vitellogenic oocyte.

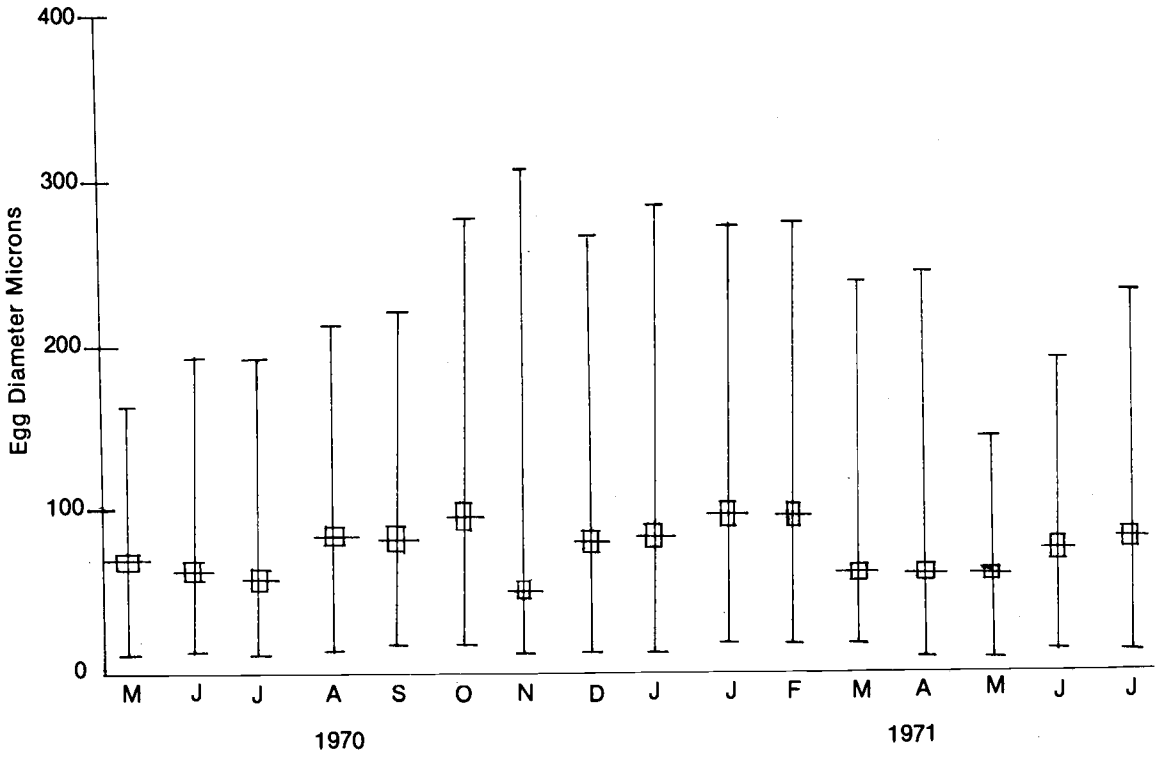

Figure 22. Range, mean and 95% confidence interval for the oocytes measured in Searlesia dira from May, 1970 through July, 1971.

measured decreased significantly from February to March, 1971 at the end of the spawning season, and large unspawned oocytes were removed by May, 1971.

Ooctye diameter frequencies are presented as histograms in Figure 23. During May, June and July, 1970 only small oocytes were present, with a range of 10 to 150 μm and a mean of approximately 55 μm. In August, 1970 growth within this small cohort shifted the mean to approximately 70 μm. This would require a daily relative growth rate of 2.4% per day (k=0.0241). Growth of some of the oocytes to a larger diameter established a bimodal curve with a second mean of approximately 175 μm by September. The curve remained bimodal through the entire winter, a period of seven months, until the end of the spawning period in April, 1971. Growth from the smaller cohort (mean=70 μm) to the larger cohort (mean=175 μm) between August and September required a daily relative growth rate of 9.2% per day (k=0.0916). In October the mean diameter of the larger cohort increased further to 240 μm, requiring a daily relative growth rate of 3.2% per day (k=0.0315). In November this large cohort was spawned. During the next five months a conort of oocytes began growth to larger sizes while the proliferation of oogonia and/or the transformation of oogonia to oocytes was at a rate sufficient to maintain the same proportion of small to large oocytes in the gonad. However, from January to March, 1971 the growth of middle-sized

Figure 23. Oocyte diameter frequency histograms for Searlesia dira for the period from May, 1970 through July, 1971. The total number of oocytes measured per month is 250. Two samples were collected during January, 1971.

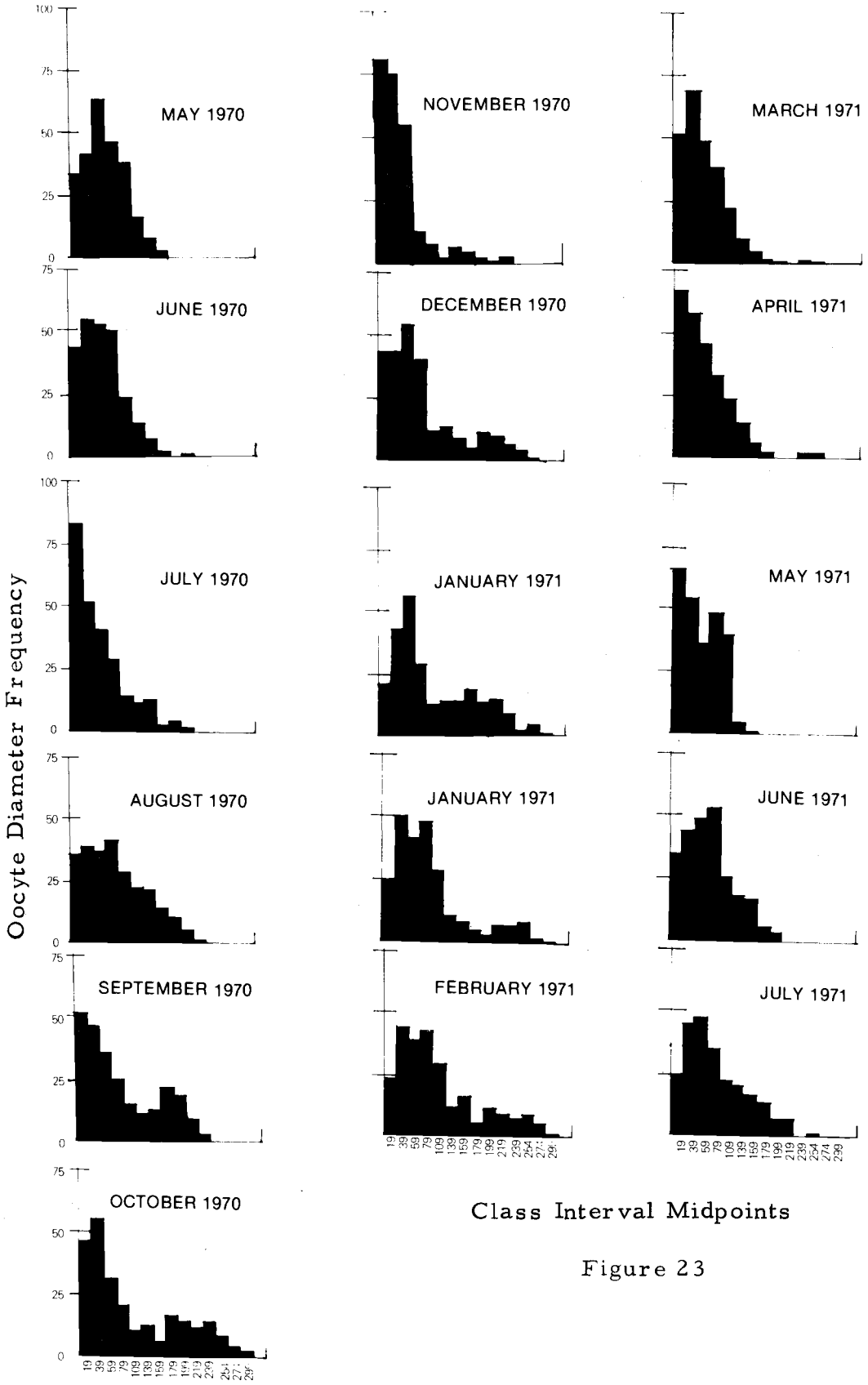
MAY 1970
NOVEMBER 1970
MARCH 1971
JUNE 1970
DECEMBER 1970
APRIL 1971
JULY 1970
JANUARY 1971
MAY 1971
AUGUST 1970
JANUARY 1971
JUNE 1971
SEPTEMBER 1970
FEBRUARY 1971
JULY 1971
OCTOBER 1970
Oocyte Diameter Frequency
Class Interval Midpoints

Figure 23

oocytes surpassed the rate of proliferation of oogonia and/or the transformation of oogonia to oocytes, resulting in a decrease in the number of oocytes in the smallest size class.

The period of March through July, 1971 was much like the period of May through July, 1970. Very little oocyte growth took place during May or June of either year. The July histograms for both years show the same skewness to the right indicating that the largest oocytes present have just begun to grow faster. This makes the July, 1971 histogram broader, with greater frequencies of the larger size classes, but never so great as the size classes below approximately 55 μm.

Since the smaller sizes are never depleted, proliferation must go on for sufficient time to replenish those that grow during the oocyte growth season (August of one year through March of the next). This would yield an annual cycle of oocyte development. Alternatively, only the sizes above approximately 50 μm may grow in any one year, and oocytes would then take two years to grow to a spawnable size, with a growth spurt in the second year. Since fairly large previtellogenic primary ooctyes are present in the gonad of recently spawned females, a cycle lasting more than one year seems most likely.

Egg Capsules and the Young

The egg capsules of Searlesia dira average four mm in diameter with a height of 1.8 mm. Each egg capsule is cemented to the substrate by a rim that is an additional 0.5 to 0.8 mm wide. This rim is composed of the same clear material as the egg capsule proper. The eggs are bright yellow and easily seen through the capsule. During November egg capsules were found to contain an average of 94 eggs. Earlier than November and later than April, egg capsules were found which contained as few as four eggs. The mean size of eggs measured from November capsules was 230 microns (N=917, S. D. =11.7). Lloyd (1971) found the number of eggs ranged from 50 to 175 in capsules collected on San Juan Island, Washington and that the mean diameter was 240 μm. All of these eggs appeared to be fertile, judging from the presence of cleavage furrows in each of them. As they are laid, the eggs are embedded in a jelly-like material so that they are not free to move. Egg capsules collected on November 12, 1970 and kept in the laboratory at approximately 10° C hatched in 70 days. Recently laid egg capsules observed at Boiler Bay on April 24, 1971 were gone by June 23, 1971. The juvenile snails are approximately two mm long when they crawl out of the egg capsule. Lloyd (1971) found the hatching time at San Juan Island, Washington to be four to five months but gave no indication of temperature.

Spermatogenesis

Application of the same criteria used to determine the stage of the testis of Nucella lamellosa and Nucella emarginata yielded similar results for Searlesia dira. Individual tubules may contain all stages of spermatogenesis but all of the tubules are not synchronized. Cell types appear in clumps, with the cells in a given clump all in the same stage of spermatogenesis.

The timing of the events of the reproductive cycle through measurement of the percent of a tubule occupied by sperm showed an increase from September to October, 1970 just prior to the spawning season (Figure 14), which is very similar to the oocyte growth spurt observed in female Searlesia dira (Figure 23). From October, 1970 through the first sampling period in January, 1971, the percent of sperm in the tubules decreased, corresponding with the presence of egg capsules in the field. An increase in the percentage of sperm in the tubules during late January and February, 1971 was followed by a sharp reduction in March, toward the end of the breeding season (Figure 14).

Immediately after spawning, the percent of sperm in the tubules of the testis is reduced; hence, the overall diameter of the tubule is reduced and the hemocoelic space between the tubules is increased.

Histological examination of both the ovaries and the testis of Searlesia dira over a 15 month period substantiate the presence of a

breeding season which begins in November and ends the following March or April.

Amphissa columbiana

Field Observations

The vertical range of Amphissa columbiana at Boiler Bay is from about -0.3 to +1.52 m (-1 to +5 ft) above MLLW. The species is restricted to relatively hard or smooth surfaces that are somewhat protected from direct surf. Typically this snail is found under boulders, in crevices, or under smooth basalt boulders resting on sediment if the boulders are not susceptible to being moved by the incoming tide or waves. On a yearly basis individuals are exposed about three percent of the time at the -0.3 m (-1 ft) level and about 70% of the time at the +1.52 m (+5 ft) level (Figure 5).

Amphissa columbiana was observed eating dead snails and urchins in the field and was often associated with large populations of the polychaete Spirorbis sp. In the San Juan Islands Amphissa columbiana has been identified as a low intertidal to subtidal organism which makes a living by scavenging (Spight, 1972). Small individuals were often encountered in the field but were not collected because of the possibility of confusing them with the smaller Amphissa versicolor. Amphissa versicolor reaches a shell length of approximately 12.5 mm and has 15 axial ribs on the next to the last whorl,

while Amphissa columbiana reaches a shell length of 25 mm and has 20 to 24 axial ribs on the next to the last whorl (Abbott, 1974). In an attempt to differentiate between the two species on the basis of shell length, the dry collection at the California Academy of Sciences, San Francisco, California was measured. A total of 56 Amphissa columbiana were measured (mean = 19.14, S. D. = 5.4) and a total of 38 Amphissa versicolor were measured (mean = 10.82, S. D. = 1.42). On the basis of these statistics, individuals less than 12 mm long were not used in this study.

No discernible differences in shell morphology exist between the males and females of Amphissa columbiana. The presence of a large penis distinguishes males from females of this species. Adults do not aggregate for breeding in the sense that the term is used for Nucella species. Males and females may be found together in a suitable habitat at all times during the year. However, they may be slightly separated in their vertical distribution since females were observed to move to the top of a sea water filled bucket when both males and females have been placed in the bucket together. Three hundred and four of the 400 individuals collected over a period of 15 months were sexed. One hundred fifty of these were males and 154 of these were females, yielding a sex ratio of 50:50. Newly laid Amphissa columbiana egg capsules were first observed in the field on November 12, 1970. Subsequent observations of fresh egg capsules

were made on January 26 and February 17, 1971. Egg capsules were found only at the 0.0 to +0.3 m (+1 ft) level on the underside of smooth basalt boulders.

Oogenesis

In the females of Nucella lamellosa, Nucella emarginata and Searlesia dira the ovary spreads over the surface of the digestive gland in the visceral mass. The ovary-digestive gland complex is twisted to the right which results in the ovary lying on the outside. In female Amphissa columbiana the ovary consists of many acini which have extensions of the digestive gland between them. The ovary-digestive gland complex is twisted to the right in this species also, which results in the ovary lying on the outside with a core of digestive gland extending into the middle of the ovary.

Oogonia appear to be located at one end of each acinus in Amphissa columbiana (Figure 24), rather than arising at any point along the entire length of an ovary as in Nucella lamellosa, Nucella emarginata and Searlesia dira, and occasionally mitotic figures may be seen in these cells. Developing primary oocytes remain attached to the lining of the acinus and are associated with nurse cells. Houston (1976) described a similar relationship within the ovary of Columbella fuscata. Primary oocytes undergoing vitellogenesis fill the larger part of the acinus. Identification of the stages of

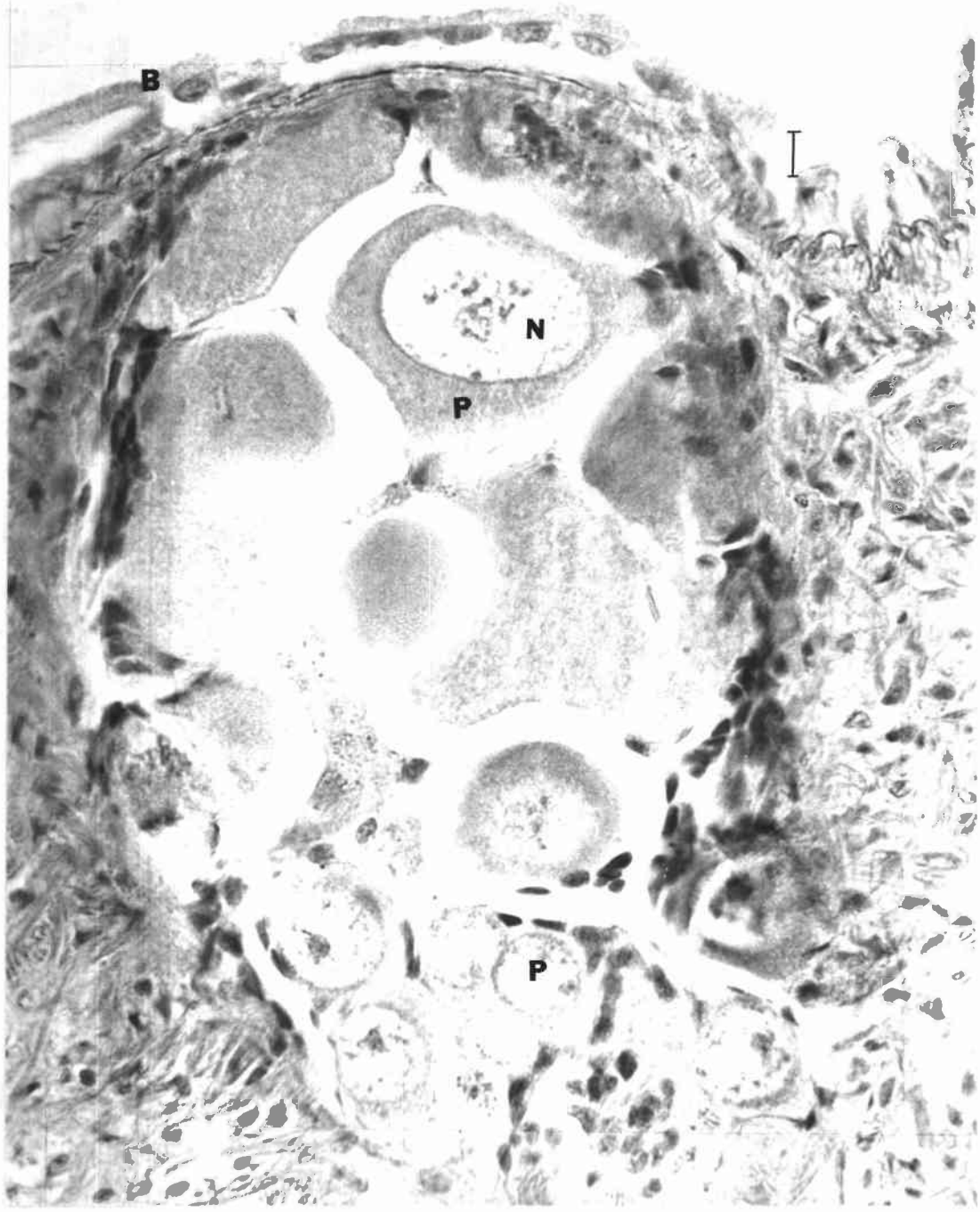

Figure 24. Cross section of one acinus in the ovary of a female Amphissa columbiana. Early primary oocytes are located at the bottom of the picture and pre-vitellogenic oocytes which have undergone some growth are displaced to the other end of the acinus. The scale line represents ten microns. B = body wall; N = nucleus; P = pre-vitellogenic oocyte.

oogenesis was based on the same criteria used for Nucella lamellosa. Both pre-vitellogenic and vitellogenic oocytes may be found in the ovary of Amphissa columbiana during the entire year.

Determination of the Reproductive Cycle from Egg Diameter Data

The range, mean and 95% confidence interval for the diameters of the primary oocytes measured from May, 1970 through July, 1971 for Amphissa columbiana are depicted in Figure 25. The mean diameter of oocytes in the samples increased from 46 μm in July, 1971 to 70 μm in August, 1971 prior to the first observation of egg capsules in the field. This rapid increase in diameter would have required a daily relative growth rate of 4.2% per day (k=0.042). Over the same period the maximum diameter measured increased from 180 μm to 240 μm. The mean and maximum size measured remained relatively constant from September, 1970 through February, 1971. Between February and March, 1971 both the mean and the maximum size measured decreased. February 17, 1971 was the last date during the cycle when newly deposited egg capsules were observed in the field.

The histograms presented in Figure 26 show that the oocytes below 25 μm diameter (16 μm class interval midpoint) constitute a high percentage of all oocytes measured in all months of the year. The

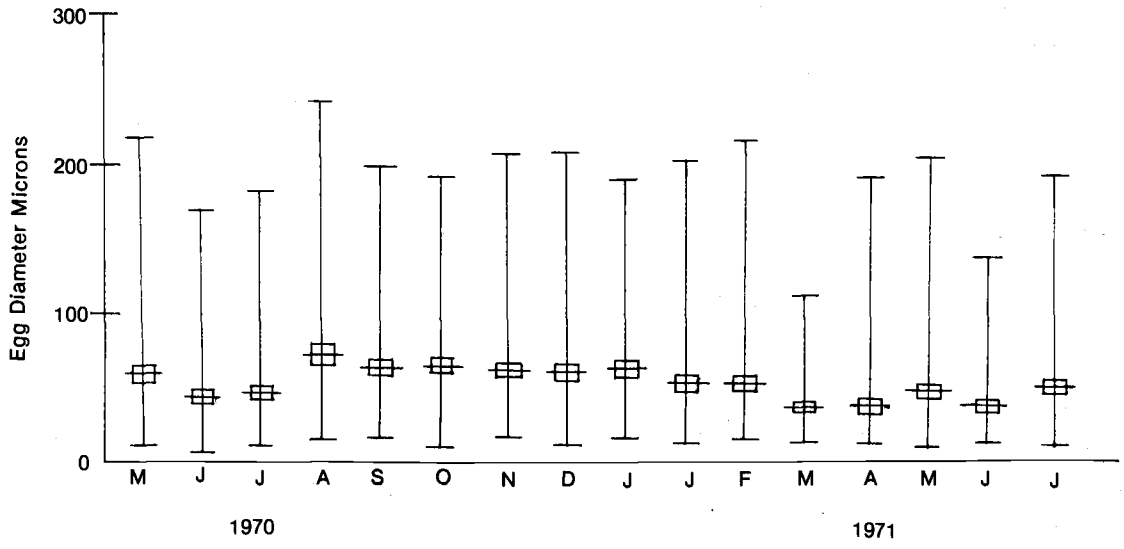

Figure 25. Range, mean and 95% confidence interval for the oocytes measured in Amphissa columbiana from May, 1970 through July, 1971. The total number of oocytes measured per month is 250. Two samples were collected in January, 1971.

Figure 26. Oocyte diameter frequency histograms for Amphissa columbiana for the period from May, 1970 through July, 1971. The total number of oocytes measured per month is 250. Two samples were collected during January, 1971.

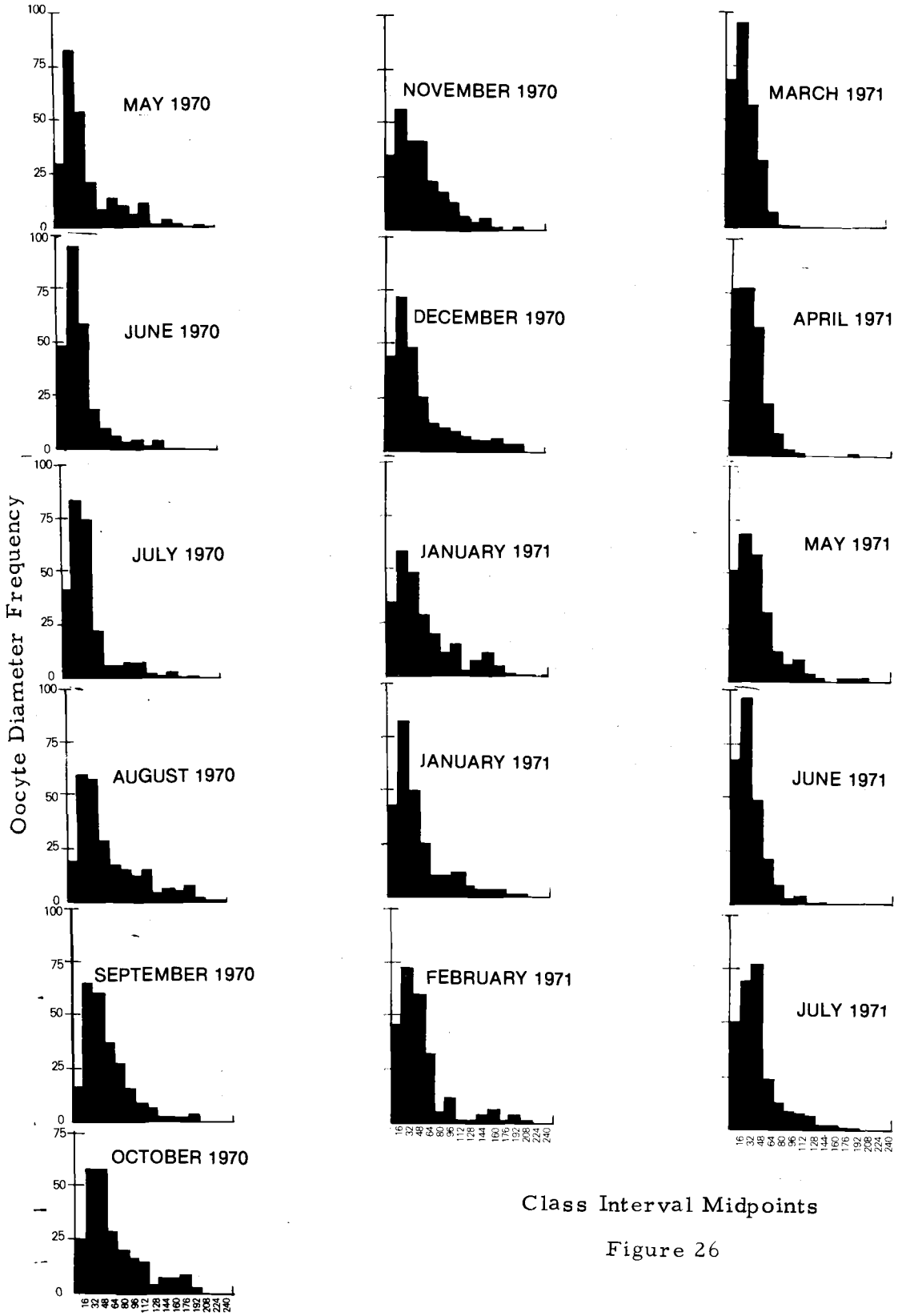
MAY 1970
JUNE 1970
JULY 1970
AUGUST 1970
SEPTEMBER 1970
OCTOBER 1970
NOVEMBER 1970
DECEMBER 1970
JANUARY 1971
JANUARY 1971
FEBRUARY 1971
MARCH 1971
APRIL 1971
MAY 1971
JUNE 1971
JULY 1971
Oocyte Diameter Frequency
Class Interval Midpoints

Figure 26

smallest size class measured dropped only from August to October when the histograms indicate rapid growth to the larger size classes by middle-sized oocytes. The relationship between the smaller cohort (range 8 to 70 μm, mean approximately 40 μm) and the larger cohort (range 70 to 250 μm, mean approximately 160 μm) was 4:1 during May, June and July of 1970. In August of 1970 this shifted to 3:2 as a result of the rapid growth of some of the middle-sized oocytes. This 3:2 relationship was maintained through November, when the first egg capsules were observed in the field, and returned to 4:1 in December, 1970. During the first sampling period in January, 1971, the ratio was again 3:2. By the second sampling period in late January, 1971, the ratio had returned to 4:1, indicating spawning of some of the larger oocytes. In February it was 4:1 and in March 4. 5:0. 5. Fresh egg capsules were seen on February 14 and by March, 1971 the animals had completed spawning. In April, 1971 the ratio was still 4. 5:0. 5, indicating very little growth of middle-sized oocytes into the large size classes. By May, 1971 the ratio had changed to 4:1, indicating some growth of middle-sized oocytes.

A daily relative growth rate of 2. 5% per day, based on the increase of the mean from 38 to 49 μm between April and May of 1971 and 38 to 50 μm between June and July of 1971, could maintain the distribution within the smaller cohort observed during the months of May, June and July of 1970 and 1971. Between July and August,

1970 a daily relative growth rate of 13. 9% per day (k=0. 1386), based on movement from the mean size of the small cohort, 40 μm, to the mean size of the larger cohort, 160 μm, would be required to establish the bimodal nature of the curve. Since the supply of smaller oocytes within the gonad is not depleted by this seasonal growth to larger sizes, some proliferation of gonia and their growth to larger sizes within the small cohort must take place throughout the year.

Egg Capsules and Young

The egg capsules of Amphissa columbiana average about 3 mm in diameter by 1. 5 mm high. They may be round if laid singly or slightly irregular if they are laid in clumps. The capsules lack supporting external ribs as described by Marcus and Marcus (1962) but have tiny papillae scattered over the outer surface. The top of the capsule has two concentric ridges which are circular. The juvenile snails emerge through the middle of the center ridge at hatching. The capsules themselves are clear and contain white eggs. Egg capsules collected on November 12, 1970 contained approximately 60 eggs each. The mean size of eggs measured from capsules was 194. 5 μm (N=126, S. D. = 14. 7). All of the eggs in each capsule appeared to be fertile based on the presence of cleavage furrows in the eggs. The eggs are embedded in a jelly-like matrix when they are laid. The hatching time for capsules laid in the laboratory on

February 17, 1971 was 72 days at 10° C. At the end of the 72 days the juvenile snails emerged from the capsule.

Spermatogenesis

Using the same criteria, the testis of Amphissa columbiana were found to be similar to those of Nucella lamellosa, Nucella emarginata and Searlesia dira. Individual tubules may contain all stages of spermatogenesis, but all of the tubules were not synchronized. Cell types appeared in clumps with the cells in a given clump all in the same stage of spermatogenesis (Figure 27). No individuals were found in which the overall diameter of the tubules of the testis was reduced and the intervening hemocoelic space increased, but the percentage of sperm in the tubules of the testis was found to be reduced during the mid-winter spawning season, December through February (Figure 14).

Statistical Summary

A summary of the statistical data on a monthly basis for oocyte measurements for Nucella lamellosa and Nucella emarginata is included in Table 1 and for Searlesia dira and Amphissa columbiana in Table 2. Table 3 compares the maximum oocyte size measured from sections with the mean of the monthly maximum oocyte size measured from sections and the mean egg size measured from

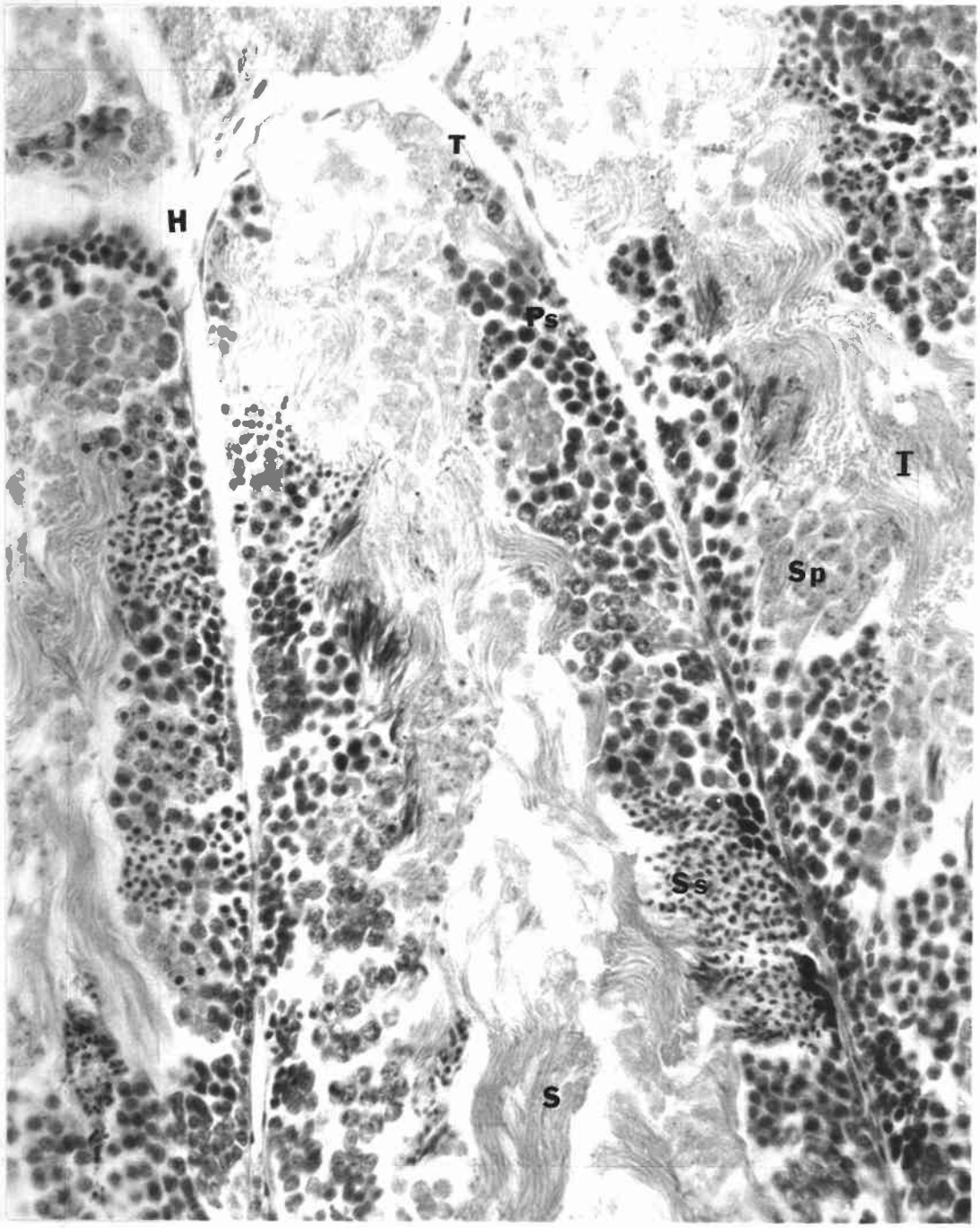

Figure 27. Longitudinal section through a tubule in the testis of a male Amphissa columbiana. In this ripe male all stages of spermatogenesis are represented. The scale line represents ten microns. H = hemocoel; Ps = primary spermatocyte; S = sperm; Sp = spermatid; Ss = secondary spermatocyte.

Table 1. Statistical analysis of monthly oocyte diameter measurements for Nucella emarginata and Nucella lamellosa. Diameters in microns.

N = number in sample; S. E. = standard error of the mean; S. D. = standard deviation; Var. = variance; C. V. = coefficient of variability

Month/year	N	Mean	S. E.	S. D.	Var.	C. V.
			Nucella emarginata			
May, 1970	250	89. 6	3. 4	52. 9	2797. 0	59. 0
June, 1970	250	85. 8	3. 2	50. 1	2509. 6	58. 4
July, 1970	250	83. 1	2. 8	44. 7	2002. 4	53. 8
Aug, 1970	250	84. 6	3. 1	49. 2	2419. 9	58. 2
Sept, 1970	250	89. 9	3. 3	52. 2	2720. 1	58. 0
Oct, 1970	250	88. 3	3. 4	53. 6	2867. 7	60. 7
Nov, 1970	250	96. 8	3. 0	47. 9	2295. 8	49. 5
Dec, 1970	250	86. 4	3. 2	51. 2	2626. 2	59. 3
Jan, 1971	250	108. 4	3. 7	58. 8	3454. 8	54. 2
Jan, 1971	250	99. 7	3. 4	53. 8	2891. 2	53. 9
Feb, 1971	250	77. 2	3. 3	51. 7	2669. 1	66. 9
March, 1971	250	102. 3	3. 8	60. 3	3637. 6	58. 9
April, 1971	250	81. 7	5. 2	51. 4	2645. 8	63. 0
May, 1971	250	75. 7	3. 3	51. 7	2672. 2	68. 3
June, 1971	250	98. 5	3. 9	61. 4	3775. 5	62. 4
July, 1971	250	50. 9	1. 9	29. 6	874. 3	58. 1
			Nucella lamellosa			
May, 1970	125	86. 0	4. 1	45. 8	2097. 5	53. 2
June, 1970	125	77. 4	3. 9	43. 9	1928. 5	56. 7
July, 1970	125	72. 3	5. 5	61. 2	3753. 7	84. 2
Aug, 1970	125	78. 1	6. 8	76. 5	5854. 7	98. 0
Sept, 1970	125	110. 1	8. 2	92. 1	8484. 6	83. 6
Oct, 1970	125	75. 1	5. 8	65. 2	4251. 7	86. 8
Nov, 1970	125	115. 4	7. 3	81. 2	6599. 6	70. 4
Dec, 1970	125	82. 1	4. 7	82. 1	2729. 8	63. 7
Jan, 1971	125	109. 1	8. 6	95. 6	9148. 5	87. 7
Jan, 1971	125	110. 9	6. 8	76. 1	5797. 9	68. 7
Feb, 1971	125	80. 4	4. 8	53. 3	2842. 8	66. 3
March, 1971	125	107. 2	7. 3	81. 2	6590. 9	75. 7
April, 1971	125	164. 4	12. 1	135. 2	18280. 4	82. 3
May, 1971	125	159. 7	11. 4	126. 9	16112. 4	79. 5
June, 1971	125	146. 2	11. 0	122. 9	15105. 4	84. 1
July, 1971	125	126. 1	8. 1	90. 7	8232. 6	72. 0

Table 2. Statistical analysis of monthly oocyte diameter measurements for Searlesia dira and Amphissa columbiana. Diameters in microns.

N = number in sample; S. E. = standard error of the mean; S. D. = standard deviation; Var. = variance; C. V. = coefficient of variability

Month/year	N	Mean	S. E.	S. D.	Var.	C. V.
			Searlesia dira			
May, 1970	250	67.8	2.1	32.7	1071.6	48.3
June, 1970	250	62.3	2.1	32.8	1072.9	52.5
July, 1970	250	56.7	2.5	40.0	1596.4	70.4
Aug, 1970	250	84.4	3.1	48.5	2351.9	57.4
Sept, 1970	250	82.3	3.7	58.3	3400.3	70.8
Oct, 1970	250	95.4	4.7	73.7	5429.7	77.3
Nov, 1970	250	50.4	2.6	40.9	1675.7	81.2
Dec, 1970	250	79.5	3.7	57.8	3345.0	72.8
Jan, 1971	250	83.6	3.7	58.6	3431.5	70.0
Jan, 1971	250	96.2	4.0	63.4	4022.4	65.9
Feb, 1971	250	94.1	4.0	63.6	4049.1	67.7
March, 1971	250	59.1	2.3	37.0	1367.4	62.6
April, 1971	250	58.2	2.5	39.2	1534.9	67.3
May, 1971	250	56.0	1.9	29.3	856.9	52.3
June, 1971	250	71.6	2.4	38.0	1446.3	53.1
July, 1971	250	79.4	3.1	49.1	2407.0	61.8
			Amphissa columbiana			
May, 1970	250	57.5	2.5	39.9	1592.1	69.4
June, 1970	250	44.3	1.8	29.1	847.8	65.7
July, 1970	250	46.2	1.9	29.6	873.8	63.9
Aug, 1970	250	70.1	3.0	47.6	2262.1	67.9
Sept, 1970	250	61.7	2.3	36.8	1354.6	59.7
Oct, 1970	250	65.3	2.7	42.2	1784.6	64.7
Nov, 1970	250	61.6	2.4	38.0	1444.8	61.7
Dec, 1970	250	59.4	2.8	44.4	1968.5	74.7
Jan, 1971	250	61.3	2.6	41.4	1711.7	67.5
Jan, 1971	250	52.8	2.4	38.2	1462.3	72.4
Feb, 1971	250	53.6	2.5	40.0	1601.9	74.7
March, 1971	250	38.0	1.1	17.0	289.5	44.7
April, 1971	250	37.9	1.3	20.9	437.4	55.2
May, 1971	250	49.5	2.0	30.9	957.9	62.5
June, 1971	250	38.5	1.3	21.1	443.6	54.7
July, 1971	250	50.3	2.0	31.8	1012.7	63.2

freshly deposited capsules for each of the four species. Both the maximum oocyte size measured from sections and the mean of the monthly maximum oocyte size measured from sections underestimate the mean egg size measured from capsules for Nucella lamellosa. This may be due both to the extremely large size of the eggs of this species and to the relatively small number of oocytes measured per month (25 per female for each of five females). For Nucella emarginata the maximum oocyte size measured from sections and the mean of the monthly maximum oocyte size measured from sections overestimate the mean egg size measured from capsules. For Searlesia dira and Amphissa columbiana the maximum oocyte size measured from sections overestimate the mean egg size measured from capsules, while the mean of the monthly maximum oocyte size measured from sections gave a very good estimate of the mean egg size measured from capsules.

Table 3. Comparison of the maximum oocyte size measured from sections with the mean of the monthly maximum oocyte size measured from sections and the mean egg size measured from freshly deposited capsules. Diameters in microns.

Species	Maximum from sections	Mean of monthly maximum from sections	Mean from capsules
Nucella lamellosa	533	416	638
Nucella emarginata	335	241	181
Searlesia dira	307	230	233
Amphissa columbiana	240	191	195

A two way analysis of variance using species and month as fixed factors was performed on the complete set of 14,000 oocyte diameters to test the null hypothesis that the means of the oocyte diameters of the four species are equal. The calculated F value of 23.54 (Table 4) indicates that the interaction of species with month is very significantly different from what one would have expected had the samples all been taken from the same universe ($F_{.001, 45^{\infty}} = 1.82$). To test whether the source of this variance was the species alone, an analysis of variance was computed using species as the numerator and residual or error as the denominator. The computed F value of 522.21 (Table 4) indicates that a very large source of the variance is due to species ($F_{.001, 3,^{\infty}} = 5.42$). The maximum egg size varies greatly and the various species egg deposition times do not coincide. To test the hypothesis that time was a significant contributor to the variance, an F value was computed using time (month) as the numerator and residual, or error, as the denominator. Here, again, the observed variance due to time was highly significant ($F_{.001, 15,^{\infty}} = 2.51$).

Even though all four species of snails deposit their eggs in capsules, the timing of their reproductive cycles and the various sizes of their eggs dictate that they be treated as separate entities. A single description of a reproductive cycle is not adequate to cover all four cases.

Table 4. Results of the analysis of variance on all 14,000 oocyte diameters.

df = degrees of freedom; F = F value; $F_{.05}$ = critical value; MS = mean square; p = probability; SS = sum of squares.

Tests	df	SS	MS	F	p	$F_{.05}$
numerator:						
species*month	45	3041114.4	67580.32	23.54	.05	1.39
denominator:						
residual	13936	39997288.1	2870.07			
numerator:						
species	3	4496411.8	1498803.92	522.21	.05	2.60
denominator:						
residual	13936	39997288.1	2870.07			
numerator:						
month	15	602046.7	40136.45	13.98	.05	1.67
denominator:						
residual	13936	39997288.1	2870.07			

Since the maximum egg size differs widely for the four species studied, the coefficient of variability was used to compare them. The coefficient of variability is an expression of the standard deviation as a measure of dispersion divided by the arithmetic mean as an average and is expressed as a percentage. If, in any of the four species the coefficient of variability were small, then the eggs measured in that species could be considered to be all of approximately the same size. This was not the case. All of the coefficients of variability were above 44. 7%, with the lowest number reached by Amphissa columbiana during March of 1971. This supports the idea that small oocytes are continually being introduced into these systems, rather than a group of oocytes arising at the same time and growing synchronously to maturity.

The values calculated for the standard error of the means are smaller for the larger sample sizes (N=250), indicating that these statistics are a more reliable measure of the actual population parameters then are the smaller sample sizes (N=1 25). However, a chi-square test on the size frequencies established for a sample size of 25 oocytes measured per female versus 50 for females of Nucella lamellosa indicated no significant differences.

The coefficient of variability of monthly means over the sampling period differed for each species, i. e. Nucella emarginata, 15. 3; Nucella lamellosa, 28. 5; Searlesia dira, 20. 8; Amphissa columbiana,

19.0. This appeared to indicate that the annual cycle of oocyte change from month to month was somewhat different for the four species. An analysis of variance was run on the four sets of coefficients of variability to test the hypothesis that there was no statistically significant difference between the four species in the amount of monthly variation in oocyte diameters during the 15 months (Table 5a).

The F value or variance ratio of 10.04 obtained by dividing the between species variance by the within species variance is greater than the five percent point (p=2.76) for 3:60 degrees of freedom thereby rejecting the null hypothesis. In fact, there is a highly significant variation among the monthly coefficients of variability for the four species since the F value of 10.04 exceeds the one percent point (p=4.13) for 3:60 degrees of freedom. Differences between the species in the monthly variation in oocyte mean size must be caused by species differences in oocyte growth and proliferation rates.

Comparisons of the homogeneity of variance of the sets of variances in pairs indicate that the variance of Nucella emarginata is significantly different from the variance of each of the other species (Table 5b). Nucella lamellosa, Searlesia dira and Amphissa columbiana show about the same annual variation in monthly variance, indicating that they are more seasonal in their oocyte size-frequency distributions. Nucella emarginata has significantly less monthly variation in oocyte diameter; that is, it has a more uniform

Table 5a. Analysis of variance of the coefficient of variability of monthly mean oocyte diameter for Nucella emarginata, Nucella lamellosa, Searlesia dira and Amphissa columbiana. Data from Tables 1 and 2.

df = degrees of freedom; F = F value (variance ratio); $F_{.05}$ = critical value; p = probability; SS = sum of squares; Var. = variance.

Source	SS	df	Var.	F	p	$F_{.05}$
Total	7318.42	63	116.17	1.43	.05	1.53
Between species	2446.58	3	815.53	10.04	.05	2.76
Within species	4871.84	60	81.20	--	--	--

Table 5b. Homogeneity of variance for Nucella emarginata, Nucella lamellosa, Searlesia dira and Amphissa columbiana. Data from Tables 1 and 2.

df = degrees of freedom; F = F value (variance ratio); p = probability; Var. = variance; $F_{.05}$ = critical value.

Species	Nucella emarginata (1)	Nucella lamellosa (2)	Searlesia dira (3)	Amphissa columbiana (4)
Var.	23.2318	147.8619	92.1958	61.5011

Source	df	F	p	$F_{.05}$
2/1	15/15	6.364	.05	2.40
3/1	15/15	3.968	.05	2.40
4/1	15/15	2.647	.05	2.40
2/4	15/15	2.404	.05	2.40
3/4	15/15	1.499	.05	2.40
2/3	15/15	1.603	.05	2.40

distribution of oocyte size-frequencies through the year than the other species. Despite the general similarities of these species in their reproductive mode, the seasonal aspects of the cycle differs from Nucella emarginata. The other three species are similar in this respect despite the differences in egg size between Nucella lamellosa and the other two species.

Comparison of the Reproductive Cycles in Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana

The oogenic cycle in female Nucella lamellosa probably begins with proliferation of oogonia in September, since very few small primary oocytes are present in the ovaries during the previous April to July when rapid growth to the larger sizes by middle-sized oocytes takes place (Figure 28). Proliferation appears to continue at a low level during the fall, winter and spring. By the following September, oocytes which are probably no more than one year old have grown to an average diameter of approximately 110 μm. Over the following winter period from September of one year to March of the following year, oocyte diameter increases from 110 μm to 340 μm. Rapid growth between March and May increases the oocyte diameter further to 510 μm just prior to the spawning season. An oocyte growth cycle, from the time of proliferation to spawning would then last about 20 months (Figure 28).

Figure 28. Relationship between oocyte diameter measured from sections and time. Oocyte cycles overlap, taking longer than one year to complete. The dashed line represents the spawning period.

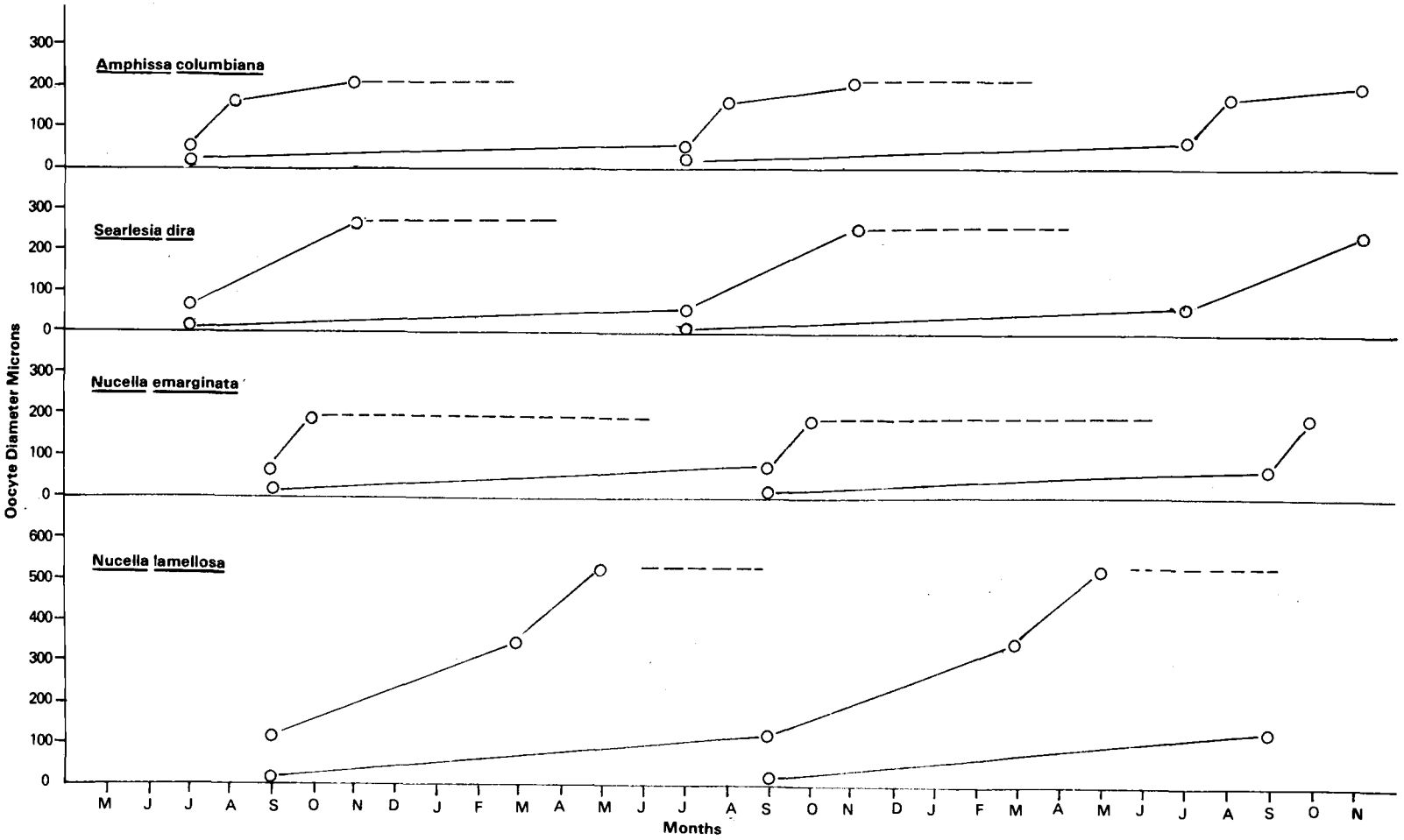
Amphissa columbiana
Searlesia dira
Nucella emarginata
Nucella lamellosa
Oocyte Diameter Microns
300
200
100
0
600
500
400
M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N
Months

Figure 28

Since the genus Nucella has been shown to cease activities either when exposed (Emlen, 1966, Feare, 1970b) or subjected to salinities below 8‰ (Arnold, 1972) Nucella lamellosa at Coquille Point probably ceases feeding during the very low salinities of December and January (Figure 4b). In order to maintain the measured increase in oocyte diameter, food stored in the digestive gland must be converted to gonad growth at this time. Active feeding during April, May and June prior to aggregating for spawning could provide nutrients for the increase in oocyte diameter from 340 μm to 510 μm observed during this period. Increased exposure, increased salinity or increased day length could stimulate animals to aggregate for breeding. While they are aggregated they cease feeding (Spight, 1972).

Stickle (1973) found that the oxygen consumption rate of male Nucella lamellosa on San Juan Island, Washington was highest during October and December during the times of copulation, and during March at the beginning of feeding after breeding. In females the oxygen consumption rate appeared to be more closely related to changes in water temperature.

Juvenile Nucella lamellosa emerge from the egg capsules any time from July through October. If these juveniles feed on small barnacles which in turn are feeding on diatoms in the bay, it would be an advantage for them to hatch when the diatom population was

greatest. Thum (1972) found that the amount of chlorophyll-a in the upper one half centimeter of sediments in Yaquina Bay increased at tidal levels of -0.6, 0.0 and +1.0 m (-2.0, 0.0 and + 3.0 ft) from October through November. No information is available on the time of Balanus settling in Yaquina Bay. However, barnacle nauplius and cypris stages are low during the winter and spring and high during the summer in the water column at the Coquille Point area (Zimmerman, 1972).

The oogenic cycle in Nucella emarginata probably begins in August or September following the end of the major part of the spawning season in June or July (Figure 28). Relatively slow growth from September of one year to September of the following year changes the diameter of the oocytes from 9 μm to 64 μm. This is followed by a period of rapid growth which increases the oocyte diameter to approximately 196 μm, a spawnable size. A single oocyte cycle from the time of proliferation to the time of spawning would then take about 14 months. Some oocytes grow from 64 um to 196 μm diameter during the entire spawning season from October of one year to June of the next year. During this time a low level of proliferation probably takes place to maintain the bimodality of oocyte diameter distribution observed in this species (Figure 18).

Reference to Figure 3b shows that Nucella emarginata began spawning when mean sea surface temperatures were beginning to

decrease in 1970 and continued through the period of mean sea surface temperature increase in 1971. Reference to Figure 7, which gives dai ly temperature ranges experienced by Nucella emarginata at the level from which egg capsules were collected as well as daily surface water temperatures, indicates that spawning began at the end of the period of upwelling in 1970. At that time temperatures experienced by individuals exposed by low tide at night at the +1. 4 m (+4. 5 ft) level were generally colder than sea surface temperatures. To provide spawnable-sized oocytes from October to June, either individuals had to feed at rather low temperatures when they were exposed to air or they had to transfer stored reserves from the digestive gland to the gonad. No correlation could be made between salinities and the definable events in the reproductive cycle of this species.

No information is available on the time of settling of Balanus which might serve as food for juvenile Nucella emarginata at Seal Rock, but Dayton (1970) has shown that Nucella emarginata individuals are important predators on newly metamorphosed Balanus on the Washington coast.

The oogenic cycle in Searlesia dira appears to take approximately 16 months to complete (Figure 28). Proliferation seems to take place in July, when some of the middle-sized oocytes are growing to larger sizes. These larger oocytes would complete their growth and be spawned by November. As oocytes continue to grow

to spawnable size from November of one year to March of the next year, the rate of proliferation decreases, but some proliferation probably takes place during this time since the supply of smaller oocytes in the gonad is never depleted by growth to larger sizes (Figure 23). During the first part of the cycle, lasting approximately 12 months, the diameter of an oocyte would grow from 10 μm to 55 μm. During the last three or four months of the cycle growth the oocyte diameter would increase to 240 μm, producing a spawnable sized oocyte.

No correlation with mean sea surface temperatures or salinities (Figure 3b) and the onset of reproductive activities in Searlesia dira could be made. Mean sea surface temperatures were decreasing on the open coast at the Columbia River lightship (Figure 3b) at the time egg capsules began to appear in the field. However, daily sea water temperatures at Whale Cove (Figure 6) were increasing steadily after upwelling ceased at the time Searlesia dira egg capsules first appeared in the field. Egg capsule deposition had ceased by the time upwelling began in May, 1971.

Proliferation in Amphissa columbiana seems to be at a low level during the entire year. Oocytes grow rapidly from July to August (Figure 28), increasing their diameter from 40 μm to 160 μm on the basis of the means of the two cohorts present at that time. Further growth over the next two months increases their diameter

to approximately 200 μm. These large oocytes are spawned between November of one year and March of the next. The largest number of small oocytes was present from March through June, 1971 (Figure 26). However, this may be misleading since no information on the number of oocytes per unit area of gonad was available. If proliferation occurred while large oocytes were being spawned and was completed by the end of the spawning season, this could account for the increase in the numbers of oocytes in the smallest size classes without an increase in their absolute numbers. A slow rate of growth beginning in June would decrease the number of oocytes in the smallest size classes if no proliferation took place at this time.

A 15 to 16 month cycle of oocyte production is likely for Amphissa columbiana (Figure 28), with proliferation taking place from July of one year through June of the following year and a slow rate of growth over a nine month period to a diameter of approximately 40 μm. Rapid growth during July could change the diameter to 140 μm by August and subsequent growth to a diameter of 200 μm by November spawning time. Amphissa columbiana shows the same egg capsule production period as Searlesia dira; it commences at about the time upwelling ceases and is finished by about the time upwelling begins the following year.

In all four species proliferation begins as soon as middle-sized oocytes begin to grow rapidly to a spawnable size. Rapid oocyte

growth begins in July for Searlesia dira and Amphissa columbiana and in September for Nucella emarginata and Nucella lamellosa.

Comparison of Oocyte Growth Curves for Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana

In Amphissa columbiana, Searlesia dira and Nucella emarginata oocyte growth curves show an increased rate of growth occurring two to three months before spawning begins (Figure 29). In Nucella lamellosa oocyte growth is approximately linear. In all four species proliferation begins as middle-sized oocytes grow to larger sizes and continues after spawning of the largest oocytes. Daily relative growth rates for oocytes are initially low, approximately 1.6% per day in all four species, increasing prior to spawning to 5.6% per day in Nucella emarginata, 4.9% per day in Searlesia dira and 4.0% per day in Amphissa columbiana but only 2.0% per day in Nucella lamellosa.

Constant Temperature Experiments

Individuals of all four species were kept at 5°, 10°, 15°, and 20° C for one year to determine the long term effect of constant temperature on feeding, growth, gonad maintenance and egg capsule deposition. Some individuals of each species did eat at each of the four temperatures. The animals kept at 15° C consumed the largest amount of food. Those kept at 10° and 20° C ate somewhat less, and

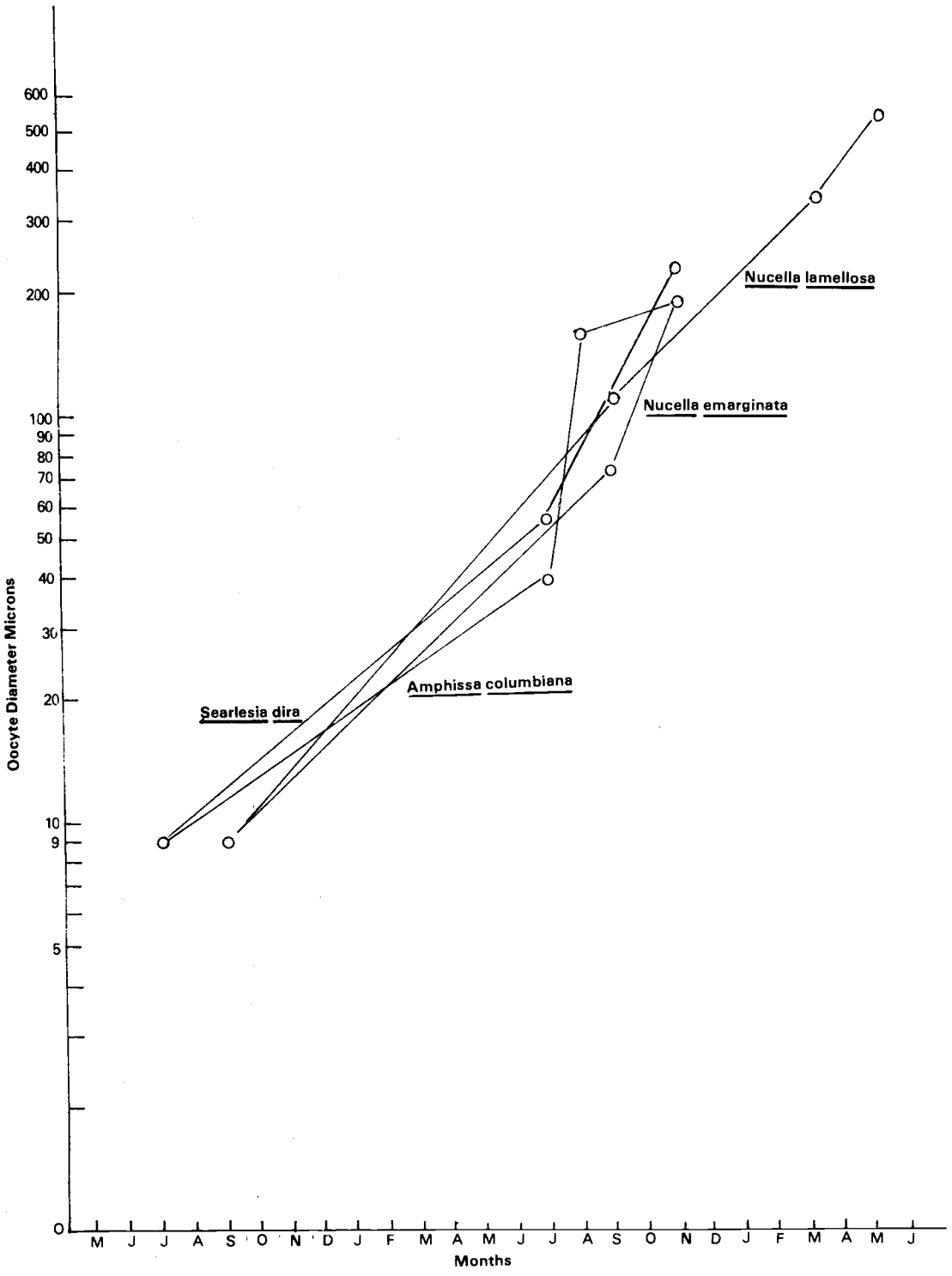

Figure 29. Comparison of oocyte growth rates calculated from oocyte volumes as a function of time.

those kept at 5° C at very little. Uneaten food in the form of opened mussels was removed at the end of one hour to avoid contamination of the aquaria. Barnacles were not removed from the aquaria.

Growth was measured on the lip of the aperture of the shell and was not an indication of length of the shell which is measured from the apex of the spire to the tip of the anterior (siphonal) canal. The results of the controlled temperature experiments are given in Table 6. Growth measurements are average values for a species at a given temperature. All four of the species grew at a temperature of 15° C, which was also the only temperature at which Nucella lamellosa grew. Nucella emarginata grew well at 15° C with lesser growth at 20° and 10° C. Both Searlesia dira and Amphissa columbiana grew at all four experimental temperatures, but Amphissa columbiana appeared to grow better at the higher temperatures.

Nucella lamellosa produced egg capsules only at the 20° C temperature. Nucella emarginata did not produce egg capsules at any of the experimental temperatures. Searlesia dira produced egg capsules at 5°, 10°, and 15° C. Amphissa columbiana produced egg capsules at the 10°, 15°, and 20° C temperatures. However, none of the four species produced a significant number of egg capsules at any of the experimental temperatures, including those at which feeding and growth took place. The few egg capsules that were produced contained embryos which developed normally, providing information on developmental time. Developmental time was similar for eggs

Table 6. The results of the controlled temperature experiments on feeding, growth and egg capsule production.

Temp °C	Species	No. at end of 12 mos.	Length (mm) size-range	Average growth (mm)	No. of egg capsules produced
5	Nucella lamellosa	7	49.1-37.0	--	--
	Nucella emarginata	11	19.9-17.1	--	--
	Searlesia dira	6	35.8-28.6	0.48	2
	Amphissa columbiana	6	20.2-12.6	0.16	--
10	Nucella lamellosa	8	51.0-29.4	--	--
	Nucella emarginata	10	19.8-16.3	1.60	--
	Searlesia dira	6	35.0-31.4	0.22	3
	Amphissa columbiana	10	19.5-14.0	0.30	12
15	Nucella lamellosa	7	48.7-31.5	3.95	--
	Nucella emarginata	6	19.9-17.8	3.93	--
	Searlesia dira	7	35.2-29.2	0.39	7
	Amphissa columbiana	7	16.4-10.2	0.36	3
20	Nucella lamellosa	9	51.8-28.9	--	14
	Nucella emarginata	9	22.4-16.8	2.02	--
	Searlesia dira	13	37.8-26.9	0.39	--
	Amphissa columbiana	14	23.6-10.9	0.53	6

deposited in the aquaria and eggs brought in from the field and held at the same temperature. Neither the timing nor the rhythm and rate of egg capsule production could be correlated with the naturally occurring spawning season.

Histology of the Gonad and Digestive Gland

Nucella lamellosa kept at 5° C for a period of one year exhibited small cells in the digestive gland, indicating that the animals had starved. Vitellogenic oocytes lacked membranes and appeared to be in the process of being resorbed. The testis of the males contained no sperm and the tubules were shrunken. Few amoebocytes were present in the testis, but the hemocoelic space between the tubules of the testis was quite large.

When held at a temperature of 10° C for a period of one year, only one-third of the individuals of Nucella lamellosa had reduced cells in the digestive gland. Starved males contained no sperm and starved females contained no large vitellogenic oocytes, although the starved females did contain pre-vitellogenic oocytes.

After one year at a temperature of 15° C, Nucella lamellosa males had no sperm in the tubules of the testis although the cells of the digestive glands did not give the appearance of those in starved animals. Females kept at this temperature contained pre-vitellogenic oocytes and either lacked large vitellogenic oocytes altogether or

contained vitellogenic oocytes which had lost their membranes and were in the process of being resorbed. The digestive glands of female Nucella lamellosa which were kept at 15° C did not give the appearance of those in starved animals.

At a temperature of 20° C Nucella lamellosa males lacked sperm in the tubules of their testis and showed a large amount of hemocoelic space between the tubules after a period of one year. Females contained pre-vitellogenic oocytes and yolk platelets which were associated with amoebocytes and being resorbed. The digestive glands of both males and females indicated that the animals had starved. Egg capsules were deposited within two weeks after the animals were brought in from the field to begin the experiment and were not produced as a direct result of this experiment.

Nucella emarginata held at a temperature of 5° C for a period of one year had sperm present in the tubules of the testis of the males and large vitellogenic oocytes present in the ovaries of the females. Two of the eleven individuals had small cells in their digestive glands and appeared to have starved. Two of the remaining individuals were heavily parasitized and their sex could not be determined. However, their digestive glands did not give the appearance of those in starved animals.

After a period one year at 10° C no Nucella emarginata individuals appeared to have starved. Two of the ten were heavily

parasitized and their sex could not be determined. Sperm were present in the tubules of the testis in the remaining three males. Large vitellogenic oocytes were present in two of the remaining five females; the other three females contained only pre-vitellogenic oocytes.

After one year at 15° C the digestive glands of all Nucella emarginata individuals contained small cells which gave the appearance of those in starved animals. Some sperm were present in the tubules of the testis of the males, and vitellogenic oocytes were present in the ovaries of the females.

Nucella emarginata kept at a temperature of 20° C for a period of one year had digestive glands which did not give the appearance of those in starved animals. Males lacked sperm in the tubules of the testis, but females contained large vitellogenic oocytes. One of the nine individuals remaining at the end of one year was so heavily parasitized that its sex could not be determined.

Searlesia dira held at 5° C for a period of one year had very small cells in the digestive glands, indicating that the animals had starved. The tubules of the testis in males were shrunken and possessed no sperm. No intact vitellogenic oocytes were present in the ovaries of the females.

When kept at 10° C for a period of one year, Searlesia dira did not appear to have starved; large cells were present in their

digestive glands. Sperm were present in the tubules of the testis of the males, and vitellogenic oocytes were present in the ovaries of the females, although some of the vitellogenic oocytes lacked distinct membranes.

No starvation was apparent in individuals of Searlesia dira after one year at a temperature of 15° C. Sperm were present in the tubules of the testis of the males. Two of the five females contained large vitellogenic oocytes and three of them contained only pre-vitellogenic oocytes.

At a temperature of 20° C the digestive glands of five of the 12 individuals remaining after one year contained small cells and appeared to have starved; the other eight Searlesia dira did not appear starved. However, males did not contain sperm in the tubules of their testis and females contained only pre-vitellogenic oocytes.

Amphissa columbiana held at a temperature of 5° C for a period of one year had normal digestive glands; no evidence of starvation was present. Sperm were present in the tubules of the testis of the males. Vitellogenic oocytes were present in the ovaries of the females. All of the vitellogenic oocytes appeared to have intact membranes. Large concentrations of amoebocytes were not present in any of the female gonads.

When held at a temperature of 10° C for a period of one year, Amphissa columbiana showed no signs of starvation in the digestive

glands. Sperm were present in the tubules of the testis in the males. Vitellogenic oocytes with intact membranes were present in the ovaries of the females.

Some of the individuals of Amphissa columbiana held at 15° C for a period of one year had small cells in their digestive glands and appeared starved. One male contained sperm in the tubules of its testis; the others did not. Two of the five females lacked vitellogenic oocytes in their ovaries. The remaining three females contained vitellogenic oocytes in their ovaries with intact membranes.

All individuals of Amphissa columbiana held at a temperature of 20° C for a period of one year appeared well fed. However, few sperm were present in the tubules of the testis of the males, and large vitellogenic oocytes were few in the ovaries of the females.

The relationship between the lack of gametes and small cells in the digestive glands which indicated starvation was consistent in all four species. Regardless of the temperature, well-fed individuals produced gametes and starved individuals did not produce gametes.

The quantity of food eaten by Nucella lamellosa appeared to have been inadequate to maintain the individuals and allow them to produce gametes at any of the experimental temperatures except 20° C and these were deposited soon after the experiment was begun. Lambert and Dehnel (1974) found that Nucella lamellosa tended to sacrifice the gonad during a period of starvation, while maintaining

reserves in the digestive gland. If Nucella lamellosa had eaten sufficient food they should have had mature gametes in their gonads when they were sectioned at the end of a year, but they did not. Searlesia dira appeared to have eaten sufficient food at all the experimental temperatures except 5° C. Some individuals of both Nucella emarginata and Amphissa columbiana appear to have eaten sufficient food at all the experimental temperatures and others did not.

Interpretation of Experimental Data

A summary of the condition of the digestive gland, growth, and the condition of the gonads for the controlled temperature experiment animals is found in Table 7. On the food source used, Nucella lamellosa fed and grew only at 15° C; below this temperature feeding was insufficient to support growth and gonad production. While Nucella lamellosa grew at 15° C, either the quantity of food consumed or its quality was insufficient to maintain the gonad. At 20° C, increased metabolic demand apparently was great enough to leave insufficient nutritional reserves for either growth or gonad maintenance.

Nucella emarginata maintained some gametes at each temperature used. It grew best at 15° C, and large oocytes were also produced at this temperature. However, the condition of the digestive

Table 7. Condition of the digestive gland, growth and the condition of the gonads of controlled temperature experiment animals.

Species	Temp. C	Condition	Growth	Sperm	Oocytes
Nucella lamellosa					
	5	all starved	none	gonads degenerate	gonads degenerate
	10	30% starved	none	none	pre-vitellogenic
	15	not starved	maximum	none	pre-vitellogenic
	20	all starved	none	none	pre-vitellogenic
Nucella emarginata					
	5	18% starved	none	present	pre-vitellogenic, vitellogenic
	10	not starved	41% of maximum	present	pre-vitellogenic, vitellogenic
	15	all starved	maximum	present	pre-vitellogenic, vitellogenic
	20	not starved	51% of maximum	none	pre-vitellogenic, vitellogenic
Searlesia dira					
	5	all starved	maximum	none	pre-vitellogenic
	10	not starved	46% of maximum	present	pre-vitellogenic, vitellogenic
	15	not starved	81% of maximum	present	pre-vitellogenic, vitellogenic
	20	38% starved	81% of maximum	present	pre-vitellogenic
Amphissa columbiana					
	5	not starved	30% of maximum	present	pre-vitellogenic, vitellogenic
	10	not starved	57% of maximum	present	pre-vitellogenic, vitellogenic
	15	starved	68% of maximum	none	pre-vitellogenic
	20	not starved	maximum	present	pre-vitellogenic, vitellogenic

gland indicated that the animals were under nutritional stress at this temperature. At 20° C, the animals grew 51% of the 15° C amount, the digestive gland indicated adequate nutrition and large oocytes were maintained in the gonad. While the growth at 10° C was only 41% of that at 15° C, the animals fed sufficiently to maintain good reserves in the digestive gland and some produced sperm or large oocytes. Feeding at 5° C was insufficient to produce growth and 18% of the animals were in a starved condition. However, the gonads did not degenerate in this species at 5° C, unlike those of Nucella lamellosa at this temperature.

Amphissa columbiana was the most eurythermal of the four species in growth, nutrition and gonad maintenance. A clear, positive correlation between temperature and growth was evident, with growth at 5° C reaching 30% of that at 20° C. This species maintained digestive gland nutritional reserves at all temperatures. The gonads also maintained at all temperatures, but at 20° C few gametes were present, indicating some long term thermal stress.

Searlesia dira also showed growth at all temperatures, but at 5° C feeding was insufficient to maintain either digestive gland reserves or gamete production in the gonad. While there was growth at 20° C, increased metabolic demands resulted in depleted digestive glands and no large oocytes in the ovaries.

Temperatures near the environmental means, in the 10° to

15° C range, permitted some growth, nutrient storage and gamete production in all four species. Consequently neither the direct nor indirect effects of temperature could be the cause of the lack of egg capsule deposition observed in these experiments. Since the quality of the food used in the experiments was adequate to support growth and maintain normal appearing gametes in the gonad, food quality was probably not the cause either. Breeding and egg capsule deposition in all four of these species is apparently induced by an external environmental feature absent from the experiments. Photoperiod was controlled at 12 hrs light: 12 hrs dark for the 5°, 10°, and 15° C temperatures, but was not controlled for the 20° temperature. The constant water levels used in the experiments are not found in the natural environment and may have not provided the natural sequence of events required to induce egg capsule deposition.

Spight (1972) reared Nucella lamellosa from hatching to maturity under laboratory conditions similar to those used in these experiments. He observed no breeding of these animals, indicating that some cue was absent from his experiments also. Nucella lamellosa collected during the height of the egg capsule deposition period and transported for 12 hours in an ice chest subsequently deposited no additional egg capsules. Possibly the animals were "turned off" by the period of abnormally low temperature.

DISCUSSION

Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana all occupy wide intertidal vertical ranges which vary dramatically in environmental characteristics at their extremes. Each of these species produce relatively few large eggs which are deposited in capsules. Nucella lamellosa, Searlesia dira and Amphissa columbiana migrate to the lower portion of their vertical ranges to deposit capsules. The environmental conditions necessary for rapid oocyte growth, e. g. food or feeding time, or required for successful development of the eggs and larva may be found only at the lower edge of their vertical distribution. In contrast, Nucella emarginata deposits egg capsules near the upper limit of its vertical range.

Field Observations

Field observations of Nucella lamellosa at Coquille Point indicated that smaller individuals were found in the upper portion of the range and larger, sexually mature individuals in the middle to lower portion of the vertical range. Bertness and Schneider (1976) described a similar distribution for this species in Puget Sound. They found by experimentation that smaller individuals had higher thermal limits. This size distribution may be due in part to the size of available food organisms. Barnacles of the genus Balanus constitute the

primary food item of this species. One would expect that barnacles that settle lower in the intertidal and are submerged more of the time and therefore able to feed longer would grow larger than those that settle in the higher intertidal. It would not be profitable for large snails to spend their time eating small barnacles and so the large snails would tend to remain in the area of suitably-sized prey organisms.

Sexually mature individuals aggregate at the lower edge of the population vertical range to deposit egg capsules during the breeding season. At Coquille Point egg capsules are deposited during June, July and August, considerably later than mid-winter egg capsule deposition periods described for the Seattle and Port Townsend, Washington and Vancouver, British Columbia populations (Emlen, 1966; Spight, 1972, 1974; Lambert and Dehnel, 1974). The probable cause for this mid-summer egg capsule deposition period at Coquille Point is the period of seasonally reduced salinity experienced at Yaquina Bay (Figure 4b). Arnold (1972) has observed Nucella lapillus to become totally inactive at salinities less than 8‰. Mid-winter and not mid-summer is the time that Nucella lamellosa egg capsules may be found at the Seal Rock and Boiler Bay collection sites. Seasonally reduced salinities do not occur at these sites as they do at the Coquille Point site.

The vertical distribution of Nucella emarginata at Seal Rock

coincides exactly with the distribution of its primary prey, Balanus glandula. A similar distribution was found for this species by Emlen (1966) at Port Townsend, Washington. Bertness and Schneider (1976) found smaller individuals of Nucella emarginata at the top of the vertical range of this species in Puget Sound. In contrast to Nucella lamellosa, Nucella emarginata deposits egg capsules in the upper half of its vertical range. Small aggregations are formed by this species at the time of breeding. The time of deposition of these egg capsules is not restricted but occurs from October to July at Seal Rock. Continuous spawning in a Nucella emarginata population in the Bodega Bay area in northern California, with somewhat increased activity from late November through February, has been observed by Houston (1971). Continuous spawning has also been observed for Nucella emarginata at Port Townsend and at San Juan Island, Washington (Emlen, 1966; Spight, 1972). Dehnel (1955) found egg capsules for Nucella emarginata from February through August in Santa Monica Bay, California and from June to September in Mount Edgecumbe, Alaska (the entire time that observations were made there).

Variability in breeding season, depending upon environmental conditions, is also known for another member of this genus. Restricted spring and early summer breeding seasons have been described for Nucella lapillus by Colton (1916) and Moore (1938), but

in some localities this species is known to breed throughout the year (Moore, 1938). The range of Nucella lapillus in Europe extends from 37° N to 71° N because of the Gulf Stream, while on the American shore its range is restricted to 41° 37' N in the south by warm water and 51° N in the north by the cold Labrador current (Hughes, 1972). A single restricted breeding season in October characterizes the Halifax, Nova Scotia populations of Nucella lapillus studied by Hughes (1972). These results and those reported here indicate that environmental salinity and temperature regimes greatly influence the time of the breeding season in intertidal Thaisinid snails.

The vertical distribution of Searlesia dira could not be correlated with any particular food item. Individuals were observed feeding on barnacles, other snails and pieces of smashed sea urchins indicating that it is a generalized higher order carnivore. Lloyd (1971) found feeding activity to be seasonal on San Juan Island, Washington, decreasing during winter and reaching a maximum in the fall.

As with Nucella lamellosa and Nucella emarginata smaller individuals occupy the upper part of the vertical distribution while larger, sexually mature individuals occupy the middle to lower portion. Downward vertical movement must take place with age in this species since older individuals are found predominately in the bottom half of the range. Egg capsules are deposited in the

bottom half of the range, although adults were not seen to aggregate for breeding. Egg capsules were present at Boiler Bay from November to April. Lloyd (1971) observed egg capsules of this species during April on San Juan Island, Washington.

Amphissa columbiana occupies the same vertical distribution as Searlesia dira at Boiler Bay. Slight habitat differences separate the two species, Amphissa columbiana preferring more protected microhabitats than Searlesia dira. Amphissa columbiana was classified as a scavenger by Spight (1972), but at Boiler Bay it was often associated with large populations of the polychaete Spirorbis sp. and it may be a carnivore as are other members of the family Columbellidae. Egg capsules were observed from November through February and were often associated with small aggregations of sexually mature individuals.

On the basis of field observations of egg capsules, Nucella lamellosa, Searlesia dira and Amphissa columbiana all have restricted breeding seasons, in contrast to the breeding season of Nucella emarginata, which is unrestricted.

The one physical parameter that Searlesia dira and Amphissa columbiana have in common with respect to their breeding times is that egg capsule deposition does not begin until after the period of summer upwelling has ceased. Consequently temperatures at the level where egg capsules are deposited are beginning to rise (Figure 6).

Seasonal Appearance of Ripe Sperm in Males

Spermatogenesis in Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana continues throughout the year. The percentage of sperm in tubules of the testis did decrease during the spawning season, but no correlation with seasonal environmental temperature or salinity changes could be made.

Houston (1971) indicated that the males of Nucella emarginata were ripe throughout the year at Dillon Beach, California, and that there were no histological changes in the course of the year in the prostate gland. Feare (1970a) concluded on the basis of the percent of a unit area occupied by the various stages of spermatogenesis on a yearly basis that the complete cycle of spermatogenesis in Nucella lapillus takes 14-15 months with a 2-3 month overlap between successive cycles. A spermatogenic cycle of this duration may occur in the species studied here. However, the process of spermatogenesis in the opisthobranch Phyllaplysia taylori has been shown to take about 20 days and the process of spermiogenesis as little as one day by using autoradiographic methods (Beeman, 1970). No autoradiographic studies have been conducted on long-lived neogastropods to determine the time required for spermatogenesis in these species.

Determination of Reproductive Cycle from Oocyte Diameter Measurements

Determining the histological condition of the gonad to stage the timing of the events of gametogenesis will yield a precise definition of the stages of the gametogenic cycle. The results may be quantified by measurement of oocyte diameters or the counting of the number of oocytes in a given stage of oogenesis per unit area of gonad tissue. If proliferation took place once during the year, one would expect a large number in the smallest size class at one point during the year. As these small oocytes grew during the course of the year, the relative number of small oocytes in the sample would be expected to decrease. Their rate of decrease would not be constant since the probability of finding the nucleolus of a small oocyte in section would be greater than the probability of finding the nucleolus of a larger oocyte in a section of the same thickness. The sections used in this study were thin with respect to the size of the large oocytes.

In none of the four species did this expected sequence happen. The histograms indicate that proliferation took place both during the time that middle-sized oocytes were rapidly growing to larger sizes and during the time that eggs were being deposited in capsules. In all four species, proliferation maintains a large number of small oocytes in the gonads at all times; the gonad is never inactive. This may be related to large egg size since egg growth, which is a long

process, is not further delayed by a quiescent period necessitating a start-up time. Whenever food, temperature and other environmental constraints or signal factors are adequate, there are oocytes available so that growth can proceed directly.

The results of this quantitative study indicate that the time required to produce an egg is approximately 20 months in Nucella lamellosa, 16 months in Searlesia dira and Amphissa columbiana and 14 months in Nucella emarginata. Mean egg sizes measured from freshly deposited capsules were 638, 233, 181 and 195 μm, respectively. The 20 month oogenic period of Nucella lamellosa may be related to its egg size which is three times that of the other species. However, the two month range of difference between the other species is obviously not related to mature egg sizes which are very similar.

It is clear from these results that the timing and duration of egg production in these neogastropods is not solely dependent upon the relatively large egg size that the group as a whole produces. Differences in the duration of egg production may reflect different adaptations to prey which themselves provide different qualities and quantities of food required for egg growth, or to differences between the snails in efficiency of food use.

By staging and counting oocytes per unit area of ovary tissue, Feare (1970a) determined that the oogenic cycle in Nucella lapillus, with an egg diameter of 187 μm, lasted 14 to 15 months. This

species has a two to three month overlap between successive annual breeding cycles. The same results were obtained in this study for Nucella emarginata, which has a similar egg size, by measuring a constant number of oocytes per month.

Lambert and Dehnel (1974) stated that oocytes of mature size occupy the ovary of Nucella lamellosa most of the year except for about three months after spawning. During this time they found that the smaller size classes begin to reappear in the ovarian tubules, until five months after spawning oocytes of mature size had regained the percentage frequency that existed before spawning. Their implication is that proliferation and growth of an oocyte to a spawnable size take approximately one year. However, their data do not show a time in the year when their smallest size class (0-100 μm) constitutes less than 50% of the monthly sample measured. It is possible that their choice of 100 μm intervals for the oocyte diameter range prevented the observation of the entrance of very small primary oocytes into the population. By measuring the same number of oocytes per month, but for a longer period of time (15 months instead of 12 months), and dividing the oocyte size range into 40 μm intervals, an oocyte "life cycle" of approximately 20 months was arrived at in this study.

Using similar histological techniques to those employed in this study, oogenesis was found to take approximately 13 months in the

asteroid Pisaster ochraceus, 18-24 months in the antarctic asteroid Odontaster validus and 24 months in the brooding asteroid Leptasterias hexactis. Oocytes of spawnable size in these species are 150, 150 and 800 μm in diameter, respectively (Mauzy, 1966; Pearse, 1965; Chia, 1968).

A comparison of the time it takes these species to make their various-sized eggs is found in Table 8. Larger eggs take longer to make, yet within a given egg size (those of Searlesia dira, Amphissa columbiana, Nucella lapillus and Nucella emarginata are all approximately 200 μm in diameter) a variable production time exists. This same phenomenon is true of the 150 μm diameter eggs of Odontaster validus and Pisaster ochraceus. For these two asteroids differences in the duration of egg production probably reflect adaptations to specific environments. Pisaster ochraceus is a temperate species while Odontaster validus is found in the antarctic. In Strongylocentrotus purpuratus the small eggs are produced in less than one year (Gonor, 1973b).

Statistical Tests

The statistical tests employed indicated that Nucella lamellosa, Searlesia dira and Amphissa columbiana show approximately the same seasonality in their oocyte size-frequency distributions, even though egg size differs greatly among the three species. All of these species

Table 8. Comparison of production time, egg diameter and egg volume.

Species	Time months	Egg diameter μm	Egg volume mm^3
Nucella lamellosa	20	638	0.136
Searlesia dira	16	233	0.007
Amphissa columbiana	16	195	0.004
Nucella lapillus	15	187	0.003
Nucella emarginata	14	181	0.003
Leptasterias hexactis	24	800	0.263
Odontaster validus	18	150	0.002
Pisaster ochraceus	13	150	0.002
Strongylocentrotus purpuratus	10	80	0.0003

deposit their egg capsules in the lower portion of their intertidal vertical ranges. The same statistical tests showed that Nucella emarginata, with an egg size approximately the same as those of Searlesia dira and Amphissa columbiana, has a more uniform distribution of oocyte size-frequencies throughout the year. This species deposits its egg capsules in the upper portion of its range. Seasonality of egg capsule deposition may depend upon the vertical position of the species in the intertidal region instead of egg size.

Comparison of Oocyte Growth Rates

Daily relative growth rates are initially low in all four species, approximately 1.6% per day, increasing two to three months prior to spawning to 5.6% per day in Nucella emarginata, 4.9% per day in Searlesia dira and 4.0% per day in Amphissa columbiana. The maximum daily relative growth rate for the curve presented for Nucella lamellosa is 2.1% per day. The asteroids Leptasterias hexactis, Pisaster ochraceus and Odontaster validus also take longer than one year to complete oocyte growth. During the first year the daily relative growth rate for Leptasterias hexactis oocytes is approximately 1.3% per day, increasing to 3.5% per day during the final five months of its growth (daily relative growth rates calculated by Gonor, 1973b). The oocyte then "rests" until it is spawned. An oocyte may reach terminal size by August but is not spawned until December (Chia,

1968). The oocytes of Pisaster ochraceus grow at a daily relative growth rate of 1% per day from September to April, the rate increasing further to 3.7% per day until it reaches a spawnable size in December. Spawning may take place any time from December to April (Mauzey, 1966).

In contrast, the daily relative growth rates for oocytes of the antarctic asteroid Odontaster validus are 5.3% per day from May to September, 1.9% per day from September to April and 0.2% per day from April to October (calculated from mean diameters of cohorts published by Pearse, 1965). An oocyte then "rests" until the spawning season of June through September.

Oocyte growth curves for the broadcast fertilizers Comanthus japonica and Haliotis crocheroidii are concave downward (Holland, Grimmer and Kubota, 1975; calculated from data in Webber and Giese, 1969). Oocyte growth in these species is initially rapid, 28.4% per day in Comanthus japonica and 6% per day in Haliotis cracheroidii and decreases toward the end of the cycle to 1.2% per day and 3.7% per day, respectively. Gonor (1973b) found the daily relative growth rate curve of Strongylocentrotus purpuratus to be only slightly concave upward. Each of these species completes oogenesis in less than one year.

Based on these few species for which quantitative data exist, oocyte growth appears to have different forms in species with an

oogenic cycle which spans more than one year and overlaps successive breeding seasons. Growth of the oocytes of these species is characterized as being initially slow and increasing toward the end of the cycle, producing an oocyte growth curve that is concave upward. Oocyte growth in species which produce eggs in less than one year is characteristically rapid immediately after proliferation and tapers off toward the end of the cycle, producing an oocyte growth curve that is concave downward.

The quantity of food available per unit time may limit the growth of small oocytes in species in which spawning cycles overlap. Priority is given to the large oocytes in the ovary that will be spawned in the present year. Oocytes of the next year's spawning season are proliferated at this time, but their early growth appears to be controlled so that all of the relatively limited food supply is used to complete growth in as many older oocytes as can be matured within one season.

Relationship between Oocyte Growth and Feeding

In all four species proliferation of oocytes seems to begin as soon as middle-sized oocytes start to grow rapidly to a spawnable size. This rapid oocyte growth begins in July for Searlesia dira and Amphissa columbiana, and in September for Nucella emarginata and Nucella lamellosa. Food abundance is increasing at these times due

to coastal upwelling (Zimmerman, 1972). Some lag would be expected prior to the increased primary production being incorporated in prey organisms available to higher level carnivores.

Feeding rates may be increased in July due to elevated temperatures, although assimilation may be relatively poor. Increased assimilation from a given amount of food ingested may come about either through the lack of food or through the effect of lowered temperature on the feeding rate. Lasker and Giese (1954) found that both of these increased the retention time of food in the gut, making digestion more complete in the echinoid Strongylocentrotus. Reduced temperatures from November of one year through February of the following year might increase assimilation during the spawning season for Amphissa columbiana, Searlesia dira and Nucella emarginata when rapid oocyte growth in all three species is taking place.

In Nucella lamellosa at Coquille Point the last growth phase is delayed until March through May apparently because of the reduced salinities in Yaquina Bay during December and January and the resultant inactivity of the species. Controlled temperature feeding experiments indicated that none of these species did well when held at a temperature of 5° C for a period of one year.

Sutherland (1970) found that growth and reproduction in a high intertidal population of the limpet Acmaea scabra were seasonal and directly related to food availability while growth and reproduction of

a lower intertidal population of the same animal, at the same location, were more constant, reflecting the constant availability of food. By starving the chiton Katharina tunicata beginning at the time when gametogenesis and oocyte growth is initiated, Nimitz and Giese (1964) found that the rate of development and differentiation of gametes was not affected, but the number of gametes produced in starved individuals was reduced.

Further evidence for nutritional control of reproductive cycles in prosobranchs comes from the work of Stickle (1973). He found that seasonal changes in the rate of shell deposition were not responsible for the reproductive cycle periodicity of body component indexes. There was no linear or curvilinear correlation between the shell length-weight relationship and the monthly sampling dates for Nucella lamellosa from San Juan Island, Washington. A tremendous increase in visceral mass index due to the availability of optimal size barnacles for predation by Nucella lamellosa just prior to the spawning season was described by Stickle (1973, 1975) for this same population. Spight and Emlen (1976) concluded that Nucella emarginata responded to an increasing food supply by increasing its spawning frequency, Nucella lamellosa responded to an increasing food supply by growing larger and in subsequent years producing more eggs at each restricted spawning season.

Wilson (1969) correlated gonad resorption in the estuarine

mussel Xenostrobus securis with lowered salinities, approximately 3.6‰, and consequent poor feeding and/or osmoregulatory stress associated with the lowered salinity. He also found that growth did not occur during periods of lowered salinity. Arnold (1972) has shown that Nucella lapillus becomes totally inactive at salinities less than 8‰. Feare (1970b) found winter aggregations of Nucella lapillus which lasted as long as six months in exposed habitats. Nucella emarginata tends to cease activity when exposed to air (Emlen, 1966), and Nucella lamellosa has been shown to sacrifice the gonad first when starved (Lambert and Dehnel, 1974). Short-term starvation may be brought about by either exposure and consequent inability to feed or by reduced salinities in an estuary and consequent osmotic stress. Presumably both exposure and lowered salinities would have less of an effect on a large organism than on a juvenile.

Synchrony of Gametogenesis and Breeding Cycles

Environmental stimuli may impinge upon the reproductive processes of animals when vitellogenesis and rapid oocyte growth begins, but it is unlikely that very exact coordination of spawning would result from this. Better synchrony may be achieved by having proliferation and oocyte growth induced by separate stimuli.

Loosanoff (1937) stated that in the clam, Venus mercenaria, "discharge of ripe eggs evidently removes the factors inhibiting

production and growth of a new crop." The presence of ripe coelomic oocytes in the polychaete Cirratulus cirratus inhibits previtellogenic development of immature oocytes (Olive, 1973). Elvin (1974) found circumstantial evidence that in Mytilus californianus the presence of mature oocytes inhibited mitotic activity leading to the production of oogonia from stem cells. He suggested that mature oocytes might secrete substances which inhibit mitosis.

Some proliferation must take place during the time egg capsules are being deposited to maintain the bimodality of the oocyte size-frequency distributions observed for all four of the species compared in this study. This type of control, with chemical feedback within the organism itself, is very attractive as an explanation for initiating proliferation within an individual. More work is needed to determine what specific chemicals might be given off by either rapidly growing oocytes or by fully grown oocytes that would inhibit mitosis.

The possibility that proliferation of gametes is under neurosecretory control should not be excluded. The synchrony of the short autumn breeding season of the polychaete Arenicola marina is under the control of a cerebral hormone which stimulates gametocyte maturation (Howie, 1966). The proliferative phase of spermatogenesis in Arenicola marina is under the control of a mitosis promoting hormone released from the prostomium and an inhibitory substance produced by spermatocytes which interferes with the activity of the

mitosis promoting hormone (Howie and McClanaghan, 1965). In the polychaete Cirratulus cirratus the ovary alternates between a proliferative phase when the terminal oocytes are present and a non-proliferative phase when they are absent (Olive, 1971). Clark (1965) found that removal of the supraesophageal ganglion appeared to have no influence upon the proliferation of oocytes from the germinal epithelium in Nereis diversicolor. However, once the oocytes reached 30 μm diameter removal of the ganglion resulted in accelerated growth. Rapid growth of oocytes is inhibited by a hormone from the supra-esophageal ganglia in this species (Clark, 1965).

Himmelman (1975) was able to induce spawning in Strongylocentrotus droebachiensis, Tonicalla lineata and Tonicella insignis by exposing them to phytoplankton collected with a 50 μm mesh net. The larvae of Strongylocentrotus dorebachiensis are planktotrophic and need phytoplankton as a food source for part of their larval life. The larvae of Tonicalla lineata do not feed on phytoplankton. However, stable water conditions brought about by increased insolation of the surface water and the associated phytoplankton bloom in the spring may provide favorable conditions for larval development in terms of temperature. If the zooplankton population increase lagged far enough behind the phytoplankton bloom, larval stages with no need to feed on the phytoplankton would be relatively free from predation by zooplankton.

Animals that pass through their larval stages inside an egg capsule would still need a readily available source of food when they hatched out. It would be to the advantage of the adults of such a species to be synchronized for breeding by some environmental cue which might precede favorable food conditions for newly emerged juveniles some months in advance. Nucella lamellosa, Nucella emarginata and Searlesia dira all feed on barnacles as adults. No morphological differences exist in the radula between the juveniles and adults of these species. Therefore, it seems logical that these juvenile snails may feed on newly metamorphosed barnacles.

In a study of Yaquina Bay, Zimmerman (1972) found barnacle nauplius and cypris numbers to be highest at upstream stations between Coquille Point and Toledo from March through October with peaks tending to occur during the summer months. He further stated that "almost no animals were present here during the winter." March through October coincides with the high salinity period in Yaquina Bay (Figure 4b). March is also the time when rapid growth of oocytes to a spawnable size began in Nucella lamellosa. The quantity of food available in March due to increased salinity and consequent increased feeding activity on the part of Nucella lamellosa is probably responsible for the increased oocyte growth rate in this species at this time. The quality of food available, e. g. substances produced by fertilized eggs inside the barnacles, may be the cue that sets in

motion the aggregation and spawning behavior of these snails. Egg capsules deposited in the summer when barnacle larval stages are highest would produce juvenile snails at the time when a suitable food supply in the form of newly metamorphosed barnacles was present. Strathmann (personal communication) has shown that Balanus sp. settling in Sinclair Inlet, Kitsap County, Washington is heaviest during March and April, continuing through September. Nucella lamellosa juveniles emerge from egg capsules there during March and April (personal observation).

Nucella emarginata has a relatively unrestricted breeding season at Seal Rock. Egg capsules were found there from October through July. Zimmerman (1972) found succession at the mouth of Yaquina Bay to be less dramatic than it was between Coquille Point and Toledo. Barnacle nauplius and cypris populations there were highest in the spring and fall, and numbers were low during the summer (June and July) and very low during the winter (November, December and January). Low numbers of barnacle larvae would indicate that the larvae had metamorphosed and settled out of the water column, and could provide at that time food for newly emerged Nucella emarginata juveniles. The extended breeding season of this species shows a positive correlation with the presence of newly metamorphosed barnacles in its open coast habitat as well as does Nucella lamellosa in its enclosed bay habitat.

Amphissa columbiana and Searlesia dira both began to deposit egg capsules after the period of upwelling had ceased. Rapid growth of oocytes to a spawnable size in these species occurred during the period of upwelling. During the summer, upwelling causes higher food availability for filter feeding organisms which should in turn be available to higher order carnivores or scavengers such as Amphissa columbiana and Searlesia dira.

The developmental times for the embryos of Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana are all highly temperature dependent. A variable larval developmental time dependent on temperature would be an adaptive advantage to an organism if it would delay emerging of the juveniles until conditions were optimal for success of the young.

Seasonal temperature fluctuations have been shown to be important for synchronizing the reproductive cycles of many temperate marine species (Thorson, 1946; Gunter, 1957; Giese, 1959; Kinne, 1963). However, as the above examples demonstrate, temperature may not be the direct causative factor.

Strategy of Egg Numbers and Egg Size

Both large eggs and extra-embryonic material around gametes, such as egg capsules, are metabolically expensive to produce. This cost may be balanced by the production of fewer eggs than in

broadcast fertilizers (such as the abalone (Haliotis crocheroidii studied by Webber and Giese, 1969) and increased chance of survival of the young. The question then becomes one of how a species can optimize its fecundity in a given environment.

The model presented by Smith and Fretwell (1974) provides a way of representing the relationship between energy expended on individual offspring and the fitness of parents. Their model is based on the assumption that "at any point in an organism's life history there is an optimum percentage of available energy that should be diverted to reproduction to maximize the parents' total contribution to future generations." Fitness of offspring may be a function of size at hatching. Spight (1976a) stated that a large hatchling will survive environmental stress better than a small one of the same type because it: 1) tolerates physical stress more readily; 2) may be eaten by fewer predators; 3) can withstand starvation longer; 4) may travel further to find food or shelter; and 5) has a larger food supply since it may eat larger organisms than a smaller hatchling can.

Nucella emarginata hatch larger, 1.2 mm, than Nucella lamellosa, 1.0 mm, and because of their larger size should face a lesser risk of death by dehydration and/or overheating (Spight, 1976a). Stress experienced by an embryo may be environmental (temperature, salinity, pH, dehydration) or biological (predation, competition for food).

Neogastropod embryos may be placed in capsules either with or without food eggs. Nucella emarginata deposits egg capsules which contain a relatively constant number of food eggs but a variable number of fertile eggs (Spight, 1976b). Embryos reach the same size in crowded capsules but variable sizes in uncrowded capsules since an individual has a greater chance of obtaining and consuming more food eggs in an uncrowded capsule. The unrestricted breeding season exhibited by Nucella emarginata would require variable embryo size at hatching since environmental conditions may vary drastically over a long hatching period. Of the four species studied, Nucella emarginata has the longest breeding season, from October to July, on the Oregon coast. Nucella emarginata embryos of more northerly populations, San Juan Island, Washington, hatch at a larger size than more southerly ones, Dillon Beach, California (Spight, 1976b). Having a constant number of food eggs and a variable number of embryos per capsule is an optimum reproductive strategy for this species.

In contrast, Nucella lamellosa has a very restricted breeding season in Yaquina Bay, June through August, and does not deposit food eggs in the capsules. Since the environmental conditions under which the embryos emerge from the capsules would be more constant because the capsules are deposited at the lower edge of the species vertical range where exposure is less, the optimum strategy would be to have all embryos emerge at the same size.

Searlesia dira and Amphissa columbiana have the same type of restricted reproductive season as Nucella lamellosa. Both species deposit egg capsules in the lower portion of their vertical ranges and it would seem these species could optimize reproduction by having all embryos emerge at the same size.

In species from seasonally varying intertidal environments, selection for the developmental advantages of large eggs impose other adjustments. Eggs sufficiently large to be advantageous apparently cannot be produced in the same season they are spawned. This limit is apparently one set on oocyte growth rate by temperature sensitive limitations of metabolic efficiency or seasonal variations in the supply of sufficient food to meet all demands of metabolism and growth.

In temperate zone shore neograstropods and other carnivores, the total time required for egg growth appears to be dependent upon final size. The large eggs of brooding and capsule using forms cannot be produced within one season. Many species have been shown to have a relatively short period of accelerated oocyte growth during which at least half the growth is accomplished and final egg size is reached at the appropriate season. This clearly shows that the oocyte production system does not operate near its potential efficiency much of the time. Environmental constraints on reproduction and synchrony with seasonal cues seem to operate at this time.

SUMMARY AND CONCLUSIONS

1. Samples of 25 individuals for Nucella lamellosa, Nucella emarginata, Searlesia dira and Amphissa columbiana were collected monthly from May, 1970 through July, 1971. The shell length of each snail was measured prior to fixation of the animal for histological examination and quantitative determination of oogenesis.
2. Nucella lamellosa, in Yaquina Bay, has a restricted breeding season during June, July and August. Nucella emarginata, at Seal Rock, has an unrestricted breeding season from October to July. Searlesia dira and Amphissa columbiana, at Boiler Bay, have a restricted breeding season from November through March. All four species deposit their eggs in capsules in the intertidal region. However, those of Nucella emarginata are deposited at the upper edge of the species vertical distribution and those of the other three species are deposited at the lower edge of their vertical distributions.
3. Mature egg size was found to vary among the four species: Nucella lamellosa, 638 μm; Nucella emarginata, 181 μm; Searlesia dira, 233 μm; Amphissa columbiana, 195 μm.
4. Oocyte diameter measurements, plotted as size-frequencies against time, indicated a bimodal frequency distribution in

each of the four species prior to the time of spawning. On this basis, oogenesis in Nucella lamellosa requires a minimum of 20 months; in Searlesia dira and Amphissa columbiana, 16 months; in Nucella emarginata, 14 months. In none of the four species is the gonad completely empty of gametes and "resting" at any time during the cycle.

5. An analysis of covariance on the coefficient of variability for oocyte diameters for each species on a monthly basis indicated that Nucella lamellosa, Searlesia dira and Amphissa columbiana all have similar seasonal reproductive cycles. In Nucella emarginata there is no distinct seasonality to the reproductive cycle. Rather, individuals may spawn repeatedly, or synchrony at the total population level may be unnecessary.
6. Daily relative growth rates calculated for each species on the basis of oocyte volumes are initially low and increase during the last third of the oogenic cycle in Amphissa columbiana, Searlesia dira and Nucella emarginata. In Nucella lamellosa, daily relative growth rates are fairly constant throughout the oocyte growth cycle.
7. A decrease in the percent of testis tubules occupied by sperm coincided roughly with the breeding season in all four species.
8. Minimum embryo developmental times were found to be 29 days for Nucella lamellosa at a temperature that fluctauted between

17° and 11.5° C, 72 days for Nucella emarginata, 70 days for Searlesia dira and 72 days for Amphissa columbiana at a temperature of 10° C.

9. All four species kept at constant temperatures near the environmental means, in the 10° to 15° C range, for a period of one year, showed some growth, stored some nutrients and produced some gametes. However, breeding and egg capsule deposition did not occur to any great extent and presumably the necessary environmental cue was absent from these experiments.

10. Egg capsule deposition times for Nucella lamellosa and Nucella emarginata show positive correlations with the availability of suitable food in the form of newly metamorphosed barnacles for emerging juveniles.

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APPENDIX

Table 9. Shell length, penis length, state of gonad, color of gonad and sex of 162 Nucella lamellosa collected from a breeding aggregation at Coquille Point, Yaquina Bay, Oregon June 24, 1976.

CGF = capsule gland full; R = ripe, oozed gametes when cut; S = spawned, no gametes oozed when cut; M = male; F = female; P-S = present, small 3 mm long; P-L = present, large 5 mm long; PS = partially spawned, CGE = capsule gland empty.

Length (mm)	Penis present/ absent	State of gonad spawned ripe	Color of gonad	Sex
46.20	P-S	R/CGF	yellow	F
40.90	P-S	S/CGE	yellow	F
47.85	P-L	S	brown	M
46.50	P-L	S	brown	M
43.80	P-L	S	brown	M
47.30	P-L	S/CGE	yellow	F
48.85	P-L		brown	M
46.50	P-S	R/CGF	yellow	F
43.70	P-S	PS	yellow	F
41.85	P-S	PS	yellow	F
45.00	P-S	R/CGF	yellow	F
40.25	P-L	S	brown	M
34.05	P-L	immature?	gold?	?
42.90	P-S	PS/CGF	yellow	F
48.00	P-S	R/CGF	yellow	F
45.20	P-S	R/CGF	yellow	F
46.60	P-L	S	gold	M
45.50	P-S	PS/CGE	yellow	F
48.20	P-S	PS/CGE	yellow	F
51.50	Absent	R/CGF	yellow	F
46.70	P-S	R/CGF	yellow	F
46.20	P-S	R/CGF	yellow	F
50.40	P-S	R/CGF	yellow	F
40.70	P-L	S	gold	M
44.00	P-S	R/CGF	yellow	F
46.70	P-S	R/CGF	yellow	F
42.25	P-L	S	brown	M
46.30	P-L	S	gold	M
43.10	P-S	PS	yellow	F
45.40	P-S	R/CGF	yellow	F
44.10	P-S	R/CGF	yellow	F
41.60	P-L	PS	gold	M
37.65	P-S	PS	yellow	F
46.80	P-S	R/CGF	yellow	F
42.35	P-L	R	gold	M
49.10	P-S	R/CGF	yellow	F
43.50	P-S	R/CGF	yellow	F
47.45	P-S	PS	yellow	F
44.70	P-S	R/CGF	yellow	F

Table 9. (Continued)

Length (mm)	Penis present/ absent	State of gonad spawned ripe	Color of gonad	Sex
39.50	P-L	PS	gold	M
42.55	P-L	R	gold	M
46.00	P-S	PS/CGE	yellow	F
40.55	P-S	R/CGF	yellow	F
46.45	P-S	R/CGF	yellow	F
43.00	P-L	R	gold	M
45.20	P-S	R/CGF	yellow	F
48.00	P-S	R/CGF	yellow	F
49.20	P-S	R/CGF	yellow	F
49.55	P-L	R	gold	M
44.80	P-L	R	gold	M
47 35	P-S	R/CGF	yellow	F
50.00	P-S	R/CGF	yellow	F
41.40	P-L	PS	brown	M
39.90	P-L	R	gold	M
45.75	P-L	R	gold	M
42.60	P-S	R/CGF	yellow	F
47.30	P-S	R/CGF	yellow	F
44.90	P-S	R/CGF	yellow	F
41.00	P-S	R	gold	M
40.70	P-L	R	gold	M
39.40	P-L	S	brown	M
43.45	P-S	PS/CGE	yellow	F
44.55	P-S	R/CGF	yellow	F
45.25	P-S	R/CGF	yellow	F
45.25	P-S	R/CGF	yellow	F
42.90	P-S	R/CGF	yellow	F
47.00	P-S	R/CGF	yellow	F
39.40	P-L	PS	gold	M
42.75	P-S	PS/CGF	yellow	F
49.20	P-L	R	gold	M
47.40	P-S	R/CGF	yellow	F
46.45	P-S	R/CGF	yellow	F
46.20	P-L	PS	gold	M
42.10	P-S	R/CGF	yellow	F
42.60	P-S	R/CGF	yellow	F
41.20	P-S	R/CGF	yellow	F
49.50	P-L	R	gold	M
47.65	P-S	R/CGF	yellow	F
48.00	P-S	R/CGF	yellow	F
45.20	P-L	R/PS	gold	M
51.45	P-S	R/CGF	yellow	F
41.80	P-S	R/CGF	yellow	F
48.85	P-S	R/CGF	yellow	F
47.50	Absent	$/CGF	yellow	F

Table 9. (Continued)

Length (mm)	Penis present/ absent	State of gonad spawned ripe	Color of gonad	Sex
48.90	P-L	S	brown	M
46.30	P-L	PS	brown	M
41.00	P-L	R	gold	M
47.50	P-L	S	brown	M
50.10	P-S	R/PS	yellow	F
38.65	P-L	R	gold	M
44.60	P-L	R	gold	M
49.30	P-L	R	gold	M
43.45	P-L	PS	gold-brown	M
45.90	P-S	R/CGF	yellow	F
41.20	P-S	R/CGF	yellow	F
36.20	P-L	S	brown	M
41.80	P-S	R/CGF	yellow	F
45.65	P-L	R	gold	M
43.50	P-S	R/CGF	yellow	F
46.00	P-L	PS	gold-brown	M
41.10	P-S	R/CGF	yellow	F
42.45	P-L	R	gold	M
44.50	P-S	R/CGF	yellow	F
41.40	P-S	PS/CGF	yellow	F
46.30	P-S	R/CGF	yellow	F
43.00	P-S	R/CGF	yellow	F
39.80	P-L	R	gold	M
42.80	P-L	PS	gold-brown	M
45.40	P-S	R/CGF	yellow	F
44.20	P-L	R	gold	M
45.40	P-S	R/CGF	yellow	F
47.90	P-L	R	gold	M
39.90	P-L	R	gold	M
44.30	P-S	R/CGF	yellow	F
45.40	P-S	R/CGF	yellow	F
44.10	P-S	R/CGF	yellow	F
41.40	P-L	R	gold	M
44.00	Absent	PS	yellow/splotchy	F
40.25	P-S	R/CGF	yellow	F
39.55	P-L	R	gold	M
42.70	P-L	PS	gold-brown	M
44.30	P-S	R/CGF	yellow	F
36.35	P-L	R	gold	M
40.60	P-L	PS	gold-brown	M
47.15	P-S	R/CGF	yellow	F
43.35	P-L	R	gold	M
37.60	P-S	R/CGF	yellow	F
43.85	P-L	PS	gold-brown	M
46.80	P-L	PS	gold-brown	M

Table 9. (Continued)

Length (mm)	Penis present/ absent	State of gonad spawned ripe	Color of gonad	Sex
40. 50	P-S	R/CGF	yellow	F
39. 20	P-L	PS	gold-brown	M
42. 00	P-S	R/CGF	yellow	F
46. 65	P-S	R/CGF	yellow	F
41. 00	P-L	S	brown	M
46. 65	P-L	PS	gold-brown	M
47. 15	P-S	R/CGF	yellow	F
41. 95	P-L	S	brown	M
45. 00	P-L	R	gold	M
43. 20	P-S	R/CGF	yellow	F
42. 25	P-S	R/CGF	yellow	F
40. 60	P-L	PS	gold-brown	M
44. 95	P-S	R/CGF	yellow	F
40. 10	P-S	R/CGF	yellow	F
39. 10	P-L	R	gold	M
47. 75	P-S	R/CGF	yellow	F
49. 30	P-S	R/CGF	yellow	F
42. 55	P-S	R/CGF	yellow	F
46. 80	P-S	R/CGF	yellow	F
46. 65	P-S	R/CGF	yellow	F
43. 00	P-S	R/CGF	yellow	F
49. 00	P-S	R/CGF	yellow	F
39. 50	P-S	PS/CGF	yellow	F
39. 20	P-L	S	brown	M
39. 50	P-S	R/CGF	yellow	F
43. 50	P-L	S	brown	M
44. 00	P-L	R	gold	M
47. 65	P-S	R/CGF	yellow	F
40. 90	P-L	R	gold	M
39. 30	P-S	R/CGF	yellow	F
47. 10	P-S	R/CGF	yellow	F
39. 70	P-L	R	gold	M
43. 35	P-S	R/CGF	yellow	F