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Along the central and southern Oregon coast and on San Juan Island, Washington, the lined chiton *Tonicella lineata* is very abundant in the lower intertidal levels on rocky shores. It is usually found on encrusting coralline algae which, with epiphytic diatoms, make up the major portion of its diet. On the central and southern Oregon coast, *T. lineata* are closely associated with the purple urchin *Strongylocentrotus purpuratus* and are often found in the burrow of the urchin. This association, together with algal cover, protects *T. lineata* from desiccation during periods of tidal exposure, whereas on San Juan Island the lack of an intertidal urchin reduces the diversity of habitats available to *Tonicella*. The asteroids *Pisaster ochraceous* and *Leptasterias hexactis* are the most common predators of *T. lineata*. The average growth in length of twelve *T. lineata* studied for ten and one-half months was 0.40 cm (range 0.88 to 0.06 cm) with great variation among animals 2.0 to 3.0 cm in body length. Some *T. lineata* exhibit a homing behavior.

The reproductive cycle was studied by determination of a monthly gonad index and examination of gonad histology. In 1968 and 1969 Tonicella lineata showed a distinct annual reproductive cycle along the central Oregon coast, spawning between 1 April and 15 April in 1968 and during the middle of April in 1969. Southern Oregon coast data is inconclusive, but spawning in 1968 and 1969 probably occurred in February or March. In 1968 and 1969 spawning took place during May and June on San Juan Island. Tonicella lineata thus shows a latitudinal difference in the timing of its annual reproductive cycle. Histological examination of the gonads revealed a close correlation of gamete buildup and release with gonad index changes. Gamete production starts immediately after spawning. The gonads of T. lineata do not go through a resting period as has been observed for those of the chitons Katharina tunicata, Mopalia hindsii and Cryptochiton stelleri. Comparison of gonad index and histological data with sea surface temperature data shows that gametogenic activity increases while the water temperatures are dropping.

Spawning behavior is described and data are presented which suggest that there may be a correlation between the time of spawning and the lunar phase.

The development of Tonicella lineata is described from the time of fertilization through metamorphosis. The features described

follow closely those which have been described for other chitons except for the description of previously unnoted structures on the pretrochal region of the trochophore larva. In laboratory cultures the development of the trochophore stops between 110 and 160 hours post-fertilization, depending upon the temperature. Further development will take place only when the larvae are presented with the proper settling substrate of encrusting coralline algae. In substrate choice experiments, T. lineata larvae settle only on encrusting coralline algae and on pieces of tile previously soaked in an encrusting coralline algae extract.

After settling, the trochophore larvae go through a drastic metamorphosis within twelve hours, losing the apical tuft and prototroch, taking on the shape of a small chiton, and developing seven shell plates. When 30 days old the young chitons have a fully developed radula and feed on encrusting coralline algae.

Ecology and Reproductive Biology of *Tonicella lineata* (Wood, 1815) (Mollusca-Polyplacophora)

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ECOLOGY AND REPRODUCTIVE BIOLOGY OF *Tonicella lineata* (WOOD, 1815) (Mollusca-Polyplacophora)

INTRODUCTION

The lined chiton *Tonicella lineata* (Wood, 1815) (Figure 1) is not only one of the most beautiful chitons to be found along the Oregon coast and in the San Juan Island, Washington region, but is also one of the most abundant.

Yakovleva (1952) described *T. lineata* as a typical North Pacific chiton form in that it is found in the Sea of Japan, on the Aleutian Islands, and along the western coast of North America. Johnson and Snook (1927) report *T. lineata* are found from Alaska to San Diego but that they are not common south of Monterey, California.

Little is known about the biology of *T. lineata*. Yakovleva (1952) lists it as inhabiting the sublittoral along the Eastern Pacific coast of the USSR. Ricketts and Calvin (1962) briefly discuss its position in the intertidal along the California coast. The taxonomic position of *T. lineata* is discussed by Leloup (1945) and Yakovleva (1952).

The purpose of this study was to examine the reproductive biology and some aspects of the ecology of this chiton. The aspects of the reproductive biology studied were: the annual reproductive cycle, gametogenesis and its timing in relation to the annual cycle, spawning behavior, developmental features, and larval substrate

selection. Since T. lineata was found to have a restricted diet consisting mainly of encrusting coralline algae which also is the substrate upon which most adults are found, the possibility that the larvae might show a specific settling response towards this substrate was also examined.

The central Oregon coast and the San Juan Island study areas were chosen because T. lineata are abundant at these localities and to determine whether latitudinal differences in annual reproductive cycle timing occur. Habitat differences between the two regions were also studied.

The importance of temperature in regulating the timing of annual reproductive cycles in marine invertebrates has been discussed by Giese (1959). Hedgpeth and Gonor (1969) have pointed out that temperature appears to be the most important single factor in synchronizing reproductive periodicities while light and other factors may play secondary roles. The majority of reproductive cycle studies on Pacific coast chitons have been done by Giese and his associates. The species involved, Katharina tunicata, Mopalia hindsii, and Cryptochiton stelleri, were studied in the Monterey Bay, California area. Annual inshore sea surface temperatures for the central Oregon coast differ significantly from those of the surface waters of the Monterey Bay, California area. The mean monthly temperature at Monterey Bay, California (Bolin and Abbott, 1963)

is always several degrees above the typical mean for central Oregon coastal stations.

All of the chitons used in the central California coast studies have higher intertidal ranges than does T. lineata and therefore are exposed to air temperatures longer than is Tonicella. T. lineata are at ambient sea surface temperature more of the time than the other species. Possibly a more meaningful correlation between reproductive cycle activities and sea surface temperatures can be made for Tonicella lineata than for the previously studied chitons.

This paper describes the ecology and reproductive biology of Tonicella lineata from the Oregon coast and San Juan Island and compares these results with what is known from similar studies on other chitons, most of which have different intertidal ranges than T. lineata and were studied at areas with different temperature regimes from that of the study areas.

DESCRIPTION OF STUDY AREAS

Central and Southern Oregon Coast (Figure 2)

The principal study area on the central Oregon coast was at Yaquina Head, five miles north of Newport, Oregon (lat. 44° 37'' N, long. 124° 02'' W). Other study areas were at Boiler Bay, 14 miles north of Newport, and Strawberry Hill, 25 miles south of Newport.

Hedgpeth and Gonor (1969) review the sea surface temperature regime of the Oregon coast and the effects that the California and Davidson currents have on this regime. The annual mean sea surface temperature for selected shore stations along the Oregon coast is between 10° C and 12.5° C with a range of 5 to 7 degrees. In December and January the yearly low of 8° C is reached.

Yaquina Head

Yaquina Head is a large basaltic outcropping 5 miles north of Newport, Oregon. The study area was located on a flat basaltic bench in the lower intertidal. This bench had an abundant population of the purple sea urchin, Strongylocentrotus purpuratus, and a dense algal cover consisting mainly of the brown algae Hedophyllum sessile and the red algae Odonthalia lyalii.

Boiler Bay

Boiler Bay is located 14 miles north of Newport, Oregon. Tonicella were collected from a sandstone bench on the south side of the bay. The bench had a rich cover of Hedophyllum and an abundant purple urchin population on it.

Strawberry Hill

Only one collection was made from this location where Tonicella lineata are very sparse. The collection was made from tide pools located in the mid-intertidal region. Strawberry Hill is located 25 miles south of Newport.

Cape Arago

Cape Arago (lat. 43° 20.3'' N, long. 124° 22.5'' W) is located on the southern Oregon coast near Coos Bay and Charleston, Oregon. The study site was inside a small bay located on the south side of Cape Arago. The bay was protected from the outer ocean by large sandstone outcroppings and received little wave action. Tonicella were collected from a gently sloping sandstone area with an algal cover and urchin population very similar to the Boiler Bay collection site.

San Juan Island (Figure 3)

The principal study areas were five locations on San Juan Island, Washington approximately lat. 48° 30" N, long. 123° 05" W. The most intensively studied locations were at San Juan Park (Smallpox Bay) and Deadmans Bay. Both of these study sites were located on the western side of San Juan Island and faced Haro Strait. Other observations were made at Cattle Point on the extreme southern tip of the island, MarVista on the western side of the island near the southern tip, and Turn Island on the eastern side of San Juan Island. Except in stormy weather, there was little wave action at any of the San Juan study sites. Only during winter storms did the wave action on the western side of the island approach the force that is normally present along the central Oregon coast study sites throughout the year. The monthly means of sea surface temperatures near the Friday Harbor Laboratories for the period 1935 to 1952 ranged from 7.1° C to 11.1° C (Coast and Geodetic Survey, 1962). The highest temperatures were usually reached in July and August and the lowest in January and February. Connell (1970) reports that the maximum daily air temperature at Olga (13 km northwest of Friday Harbor) between 1958 and 1966 ranged from 26° C to 33° C while the minimum ranged from -16° C to -3° C. Connell (1970) also describes the tidal regime at Friday Harbor.

San Juan Park

Most of the San Juan Island animals examined for reproductive activity were collected from the lower intertidal at San Juan Park. The collection area faced northwest and had a steep slope. The major rock type was a metamorphosed sedimentary chert, black in color and very hard (McLellan, 1927). Tonicella were collected from the lower parts of the zone marked by the brown algae Hedophyllum sessile and from lower areas that were extensively covered with encrusting coralline algae.

Deadman's Bay

This study site was located about four miles south of San Juan Park. The rock type was similar to that found at San Juan Park. The lower intertidal where the collections were made had a very gentle slope and supported an extensive algal cover consisting mainly of Hedophyllum sessile. Underneath this algal cover were extensive areas encrusted with coralline algae. The exposure of this collecting site was directly west.

Cattle Point

Cattle Point is located on the extreme southern point of San Juan Island. This site and the MarVista site sustained the greatest

wave action during winter storms of any San Juan locales included in this study. The collection site was on the western side of the point at the base of a steep sandy bluff. There was a very gentle slope to the lower intertidal area and the main rock type was a metamorphosed sedimentary rock. Tonicella were collected from the lower intertidal where the marine grass Phyllospadix scouleri provides most of the cover.

Turn Island

Turn Island is located on the east side of San Juan Island. The collection site was a steep slope where T. lineata were gathered from the undersides of rocks one to three feet in diameter. The rock here resembled that of the San Juan Park site.

MarVista

The collection site was directly down the cliff from the MarVista Resort. The rock type was mainly igneous with some tuffaceous graywackie. Exposure of the collecting site was southwesterly with a very steep slope. Tonicella were collected from the middle of the exposed Hedophyllum area.

ECOLOGY

Habitat Description

Central and Southern Oregon Coast

The usual microhabitat of T. lineata during tidal exposure along the Oregon coast is in the lower intertidal associated with encrusting coralline algae and in burrows under the purple sea urchin Strongylocentrotus purpuratus (Figure 4). They can also be found in empty sea urchin burrows, near the base of the green anemone Anthopleura xanthogrammica, or between the anemones and rocks. A few individuals are found in crevices or, more rarely, in the open. The most critical factor in determining the occurrence of T. lineata seems to be their obligatory association with encrusting coralline algae.

T. lineata are very abundant from approximately the +1 foot tidal level above MLLW down to those levels exposed by the lowest tides. In the San Juan Island area, they were found to extend subtidally for some distance. The subtidal limit has not been determined for the Oregon coast. The upper limit of their range corresponds to the upper limit of Hedophyllum sessilis, just below the lower limit of Mytilus californianus, although at this point Tonicella are not very abundant. At their upper range, they are found around the holdfasts of Hedophyllum where encrusting coralline algae are also usually present.

T. lineata are also found in high tide pools in or above Mytilus californianus, but only in those pools whose bottoms are covered with encrusting coralline algae.

The low tide microhabitat (under urchins, under algae, etc.) permits the animals to remain in dark, damp areas free from desiccation during periods of exposure. This would be important especially when the low tides occur during the mid-day in the spring and summer. Tonicella placed out on rocks in the sun immediately show photonegative behavior and move back into the darker microhabitat. In May, 1968, at Yaquina Head, the positions of 300 T. lineata during a low tide were noted with the following results: 68 percent were under urchins in burrows, 20 percent next to anemones or slightly under their bases, 8 percent in crevices, and 4 percent in the open. In some cases three and four animals were found under a single urchin. In the winter when the low tides are at night, more T. lineata can be found in the open than during the spring and summer, although large numbers are still found under urchins and other cover.

At Yaquina Head collections for this study were taken from the lower part of the range of Hedophyllum and just below this in the area characterized by Odonthalia and S. purpuratus.

In these areas the coralline algae encrust the substrate in an almost continuous sheet. Other conspicuous members of the biota in these area are the red algae Odonthalia flocossa, S. purpuratus,

and A. xanthogramica.

Collections at Cape Arago (Southern Oregon) and Boiler Bay (Central Oregon) were taken in the lower part of the vertical range of Hedophyllum. These two collection sites are characterized by soft sandstone, allowing S. purpuratus to form rather deep burrows which become lined with encrusting coralline algae; it is in these burrows the T. lineata are found. Below the Hedophyllum area encrusting coralline algae form a continuous cover on the rock and T. lineata are abundant.

San Juan Island

In this area, as along the Oregon coast, T. lineata are found in association with encrusting coralline algae, but the microhabitat differs significantly from that on the Oregon coast. In the San Juan Island region, S. purpuratus are seldom found intertidally; a common subtidal urchin species, Strongylocentrotus drobachiensis, occasion- occurs intertidally. As a result, at a low tide the intertidal in this region offers few urchins or urchin burrows to shelter the chitons. Here during periods of exposure the position of T. lineata is usually beneath foliose algae at the level of Hedophyllum and Phyllospadix, in crevices, or in the open in the lower intertidal where the algal cover is sparse. When a low tide occurs during the late morning or at mid-day, the lower Tonicella not covered with algae become very

dry in appearance and lack moisture on the shells and girdle. This condition was rarely observed on the Oregon coast because the chiton-urchin association protects the chiton from desiccation.

Collections were made at three main areas on San Juan Island; Deadman's Bay, San Juan Park, and Cattle Point (Figure 3). At Deadman's Bay and San Juan Park, T. lineata are abundant in the lower range of Hedophyllum where encrusting coralline algae are also abundant. At Cattle Point Tonicella are abundant in the lower intertidal where they were associated with encrusting coralline algae under the Phyllospadix and Hedophyllum. At all collection sites and in particular at Deadman's Bay, the chitons were also found in high tide pools with bottoms encrusted with coralline algae. With the exception of those found in high tide pools and a few small individuals less than 2 cm long found higher among the barnacle Balanus cariosus, the upper limit of the majority of Tonicella in the San Juan Island area was found to be the top of the vertical ranges of Hedophyllum and Phyllospadix. The lower limit of Tonicella at these sites was not established, but at Shady Cove they were found at 50 feet. All subtidal T. lineata collected in this study were associated with encrusting coralline algae.

In summary, the absence of intertidal urchins in this region greatly reduces the diversity of microhabitats available to Tonicella during low tide. In the higher intertidal range, T. lineata use algal

cover or crevices as protection against desiccation at low tide. In the lower intertidal the only protection available is crevices or an occasional anemone, and many individuals are found in the open during low tides.

Other Habitat Notes and Observations--San Juan Island and Yaquina Head

On San Juan Island many intertidal T. lineata were found occupying a bare spot on the rock, the same size as the chiton and devoid of the encrusting coralline algae which covered the surrounding substrate. At Yaquina Head a few T. lineata located along the sides of coralline-lined channels and on lower intertidal benches exposed only a few times a year were found to have bare spots underneath them. At both sites the location of these chitons was the lower intertidal, below the range of Hedophyllum.

Whether or not this bare area represents a permanent "home" is not known, although it would seem that the chitons would have had to occupy the site for some time to wear the coralline algae away to the bare rock.

During one collection at San Juan Park, eight of twenty T. lineata on a lower bench were found to be associated with bare areas. No other counts were taken to determine the frequency of chitons associated with bare areas.

At San Juan Park five T. lineata on such bare areas were marked with a diamond scriber by scratching sets of identifying lines into the valves and the bare area of rock beneath each chiton. Over a two month period the locations of these animals were noted during fourteen low tide observations. After one month all five animals were still associated with their original bare area but movements had taken place between observations. The anterior ends of the animals were found at different ends of the bare area on different observations, indicating a turn of at least 180 degrees since the last observation. At the end of two months one animal had been lost, but four were still located on the bare area on which they were originally marked. The data on these five animals indicates that at least part of the T. lineata population probably exhibits homing behavior.

The movement of twelve additional marked T. lineata was followed. These animals were located in five coralline-lined tide pools which ranged in length from one to eight feet. The water depth ranged from two to eight inches. All of the pools were located in the lower vertical range of Fucus gardneri at San Juan Park. None of the animals were associated with bare coralline areas. Fourteen observations were made over a two month period. At the end of one month two animals had been lost, but the remaining were followed for another month. The marked animals were always found in the tide pools where they were originally marked, but were usually

located at a site in the tide pool different from that of the previous observation.

Movement of animals associated with urchins and anemones has not been monitored, so it is not known whether they return to the same anemone, urchin, or urchin burrow.

Feeding

Microscopic examination of T. lineata stomach contents and fecal pellets showed that encrusting coralline algae makes up a major part of their diet. Scrapings of the coralline algae from which the animals were collected resemble the stomach contents. A dilute hydrochloric acid solution will cause the stomach contents and fecal pellets to bubble, followed by disappearance of the coralline. The other major dietary element found in the stomach contents were epiphytic diatoms from the encrusting coralline. The diatoms varied seasonally in abundance. Other epiphytes are also present, but in a substantially lower proportion than diatoms.

The fecal pellets were mainly composed of small pieces of calcium carbonate and digested and undigested diatoms. Fecal pellet color can be used as an indicator of diatom intake. White to pink fecal pellet color signifies low intake of diatoms while a greenish hue results from high intake.

No attempts were made to quantify food intake, although

animals examined at all times of the year had full stomachs and intestines. Since no movement was observed during exposure, it is felt that all feeding takes place when the animals are covered by the tides. Continuous feeding was shown by animals kept in the laboratory on coralline algae in running sea water.

Exactly what portion of the coralline algae is eaten by T. lineata is not known, but they do not scrape the algae down to the bare rock as I have observed Acmaea mitra, a limpet from the same habitat, to do. Rasmussen (1968) feels that Tonicella scrape off only the layers above the algae meristem.

Predation and Mortality

The asteroids Pisaster ochraceus and Leptasterias hexactis are probably the most prevalent predators on T. lineata. During the period of this study at Yaquina Head, I have observed P. ocracheus eating T. lineata sixteen times and L. hexactis eating T. lineata ten times. While removing urchins to collect T. lineata, on six occasions I have found a partially eaten chiton held next to the mouth of the urchin. Menge (1970) has shown that in the San Juan Island region T. lineata are the third most important food for Leptasterias hexactis in terms of calories available and that this asteroid feeds on Tonicella five to twelve percent of the time.

During the first half of June, 1968, twelve dead T. lineata

were observed at Boiler Bay being held on the aboral surface of the purple urchin S. purpuratus by its tube feet. In all cases the chiton was being held with its foot exposed to the sun. During this period a series of late morning low tides coincided with temperatures that were unusually warm for the Oregon coast at that time of year. A heat kill of considerable numbers of sea urchins was simultaneously in evidence (Hedgpeth and Gonor, 1969). It is felt that this chiton mortality was due to the combination of high temperatures and the T. lineata-purple urchin association.

On June 15, 1968, when six of the twelve chiton mortality observations were made, the mean internal temperature (for methods see Gonor, 1968) for thirty T. lineata under urchins or at their sides was 23.28° C (range 18.5° C to 25.6° C) at 10:45 a.m. PDT. Most T. lineata with an internal temperature above 20° C had large areas of the girdle margin lifted from the substrate. The girdle margin is normally held against the substrate. During laboratory thermal death point experiments on T. lineata, I observed that considerable girdle lift occurs when the water temperature is between 20° and 23° C. This girdle lift, exposing more gill area to the air or water, takes place as the oxygen demand of the animals increases with increased temperature. In the field and laboratory a chiton showing extensive girdle lift can easily be removed from the substrate, whereas normally it must be pried off. Mortality in the field due

to a high temperature probably takes place in the following way: The sea urchins, which usually cover themselves with stones, pieces of wood and algae, use their tube feet to dislodge the adjacent chitons once girdle lift has taken place. The chitons are then moved to the urchin's aboral side where they come to rest with the foot and gills exposed to the sun and wind. Death is probably due to high temperatures and/or desiccation.

Population Samples

Although this was not a demographic study, data were collected to gain some understanding of the populations being sampled for reproductive cycle studies. Total lengths were measured by allowing the chitons to attach to a plastic sheet, marking the positions of the ends after the animals assumed normal length, and measuring the distance between the marks with calipers.

Southern Oregon Coast--Cape Arago

On May 15 and 16, 1968, the total length of 363 T. lineata was measured (Figure 5). Every T. lineata that could be found within a 20 foot wide transect between the approximate tide levels of -1.0 and +2.0 in reference to MLLW (0.0) was measured. The area sampled was a sloping bench covered at the higher levels with Hedophyllum and on the lower levels with encrusting coralline algae and purple

sea urchins. This sample was taken in the same general area in which the reproductive cycle samples were taken. The mean total length of the sample (n=363) was 2.58 cm (Figure 5) and the range was 0.6 cm to 4.2 cm. The majority of the larger chitons (above 3.5 cm in total length) were found below the 0.0 level (MLLW) while the small ones (below 2.0 cm) were found throughout the sampled area. As can be seen from the histogram in Figure 5, the majority of the animals are between 2.0 and 3.3 cm in total length. The mean length for all the gonad index samples from this site was in the size range that included the majority of the population present at the Cape Arago sample site (Table 6).

Central Oregon Coast--Yaquina Head

At Yaquina Head a population sample was made by measuring every T. lineata in a tide pool (11 feet long by 2 to 3 feet wide). This pool was located in the mussel bed area, which is higher in the intertidal than the bench site where monthly reproductive cycle samples were taken. The water depth in the pool at exposure varied from two inches to eighteen inches, and the pool was lined with a rich growth of encrusting coralline algae. Also present in the pool at the time of measurement were numerous purple urchins and green anemones. Four Pisaster and seven Leptasterias were also present. The sample size was 216 and the total lengths ranged from 0.7 cm

to 3.9 cm. The mean total length for the sample was 2.33 cm (Figure 6). The low numbers in the three to four centimeter length range are possibly due to predation by the Pisaster and Leptasterias present in the tide pool. Although the mean length and ranges for the reproductive samples from Yaquina Head (Table 5) indicate that there are larger animals on the bench area, the high tide pool measurements indicate that at Yaquina Head, as at Cape Arago, the samples used in the reproductive cycle study do not include individuals from the far extremes of sizes present in the population.

At Yaquina Head as at Cape Arago, the largest animals are found in the lower intertidal (Figure 7). while smaller ones are found equally abundant throughout the intertidal range. On July 10, 1968, on a low tide of -2.1 feet below MLLW, a sample of the largest animals that could be found was made for gonad index determination. The animals obtained were the largest found at Yaquina Head in two years of sampling. Fourteen animals were collected, ranging in size from 4.8 cm to 3.4 cm, with a mean length of 3.96 cm. On July 25, 1968, a sample was made of the largest animals available in the regular collecting area at a level of MLLW to +1.0 feet above MLLW. This yielded 15 animals ranging in total length from 2.6 cm to 3.4 cm and with a mean length of 2.89, almost 1 cm less than the mean of the lower sample. The largest animal in two years of collection from the higher bench area was 4.0 cm, but animals this

size or near this size are quite rare; at the lower levels T. lineata above 3.5 cm in length are very common.

The larger size of the lower intertidal animals may be due to the short exposure period making a high proportion of time available for feeding. Data from the tide gauge on the dock at the Marine Science Center, Newport, Oregon, show that the -2 foot level for the years 1967, 1968, and 1969 was exposed only 0.43 percent of the time while the +1 foot level was exposed 11.7 percent of the time and the +2 foot level 19.6 percent of the time. This would mean that the lower, larger animals from the -2 foot level at Yaquina Head would have approximately 10 to 20 percent more feeding time than the higher bench animals from the +1 foot to +2 foot level. Additionally, the diatom cover on the encrusting coralline was much greater at the -2 foot level than at the higher intertidal levels at the time the lower sample was taken and areas showing extensive diatom grazing occurred around most of the T. lineata collected at the lower level. More feeding time and possibly more available nutrients due to a denser diatom growth are probably the main reasons for the observed size difference.

San Juan Island

Population samples were not made at the collecting sites on San Juan Island. The T. lineata sampled from this region for gonad

index samples are larger than those sampled from the Oregon coast (Table 7). Animals larger than 3.5 cm are very common at all sample sites on San Juan Island; at Cattle Point animals above 4.0 cm in total length are very common. No measurements were taken at the sample sites to determine if there is a size difference in relation to intertidal height.

Growth

Some growth data were obtained from Yaquina Head T. lineata. In the tide pool from which the population sample was made, 48 animals were marked and measured on April 30, 1968. Marking was by a combination of scratches on the various valves. On March 13, 1969, ten and one-half months after marking, 12 of the 48 marked animals were recovered and measured for growth (Table 1). The average increase in length for the 12 recovered animals was 0.40 cm. The largest amount of growth observed was 0.88 cm and the smallest 0.06 cm. Growth within the 2.0 to 3.0 cm size range showed great variation (Figure 8).

Growth of young settled T. lineata was followed on laboratory reared animals at the Friday Harbor Laboratories. Forty-five days after settlement, 10 young chitons had an average length of 0.75 mm, with a range of 0.65 mm to 0.83 mm.

The smallest animals ever observed in the field were 5 mm and

5.7 mm long. These were found at Yaquina Head on May 2, 1968. Judging from the spawning times of T. lineata at Yaquina Head (between April 1 and 15 for 1968 and 1969) and the growth data for the young settled chitons (0.75 mm in 45 days) and those in the tide pool (average 4.0 mm in 11 months), these 5 mm and 5.7 mm animals were about one year old.

If the average growth rate of 4.0 mm in one year for the tide pool animals is valid and remains somewhat constant for various size classes, then some of the larger animals collected may have been six, seven, and eight years old.

Subtidal Range of T. lineata and Associates

To determine how far T. lineata occurred subtidally, a transect from the 10 foot to 70 foot water depth was made at Shady Cove on the west side of San Juan Island one mile north of Friday Harbor Laboratories. At every ten foot interval in depth all chitons observable in front of the diver were collected.

T. lineata, abundant in the intertidal, were found down to 50 feet. At 20 feet another Tonicella form resembling T. lineata was present. This form ranged on down to the 70 foot limit of the transect. Because this form is not found in the intertidal, it will be referred to as the subtidal form. Tonicella insignis (Reeve, 1847) was present in the samples from 60 feet and 70 feet. All three

forms were found on encrusting coralline algae and fecal pellet examination indicated coralline makes up a major part of the diet of each form. Further work is being done on the taxonomic position of the subtidal form.

ANNUAL REPRODUCTIVE CYCLE

Materials and Methods

Except when the weather and tidal conditions would not permit, monthly samples of Tonicella lineata were taken for gonad analysis at Yaquina Head. Additional central Oregon coast samples were taken at Boiler Bay and Strawberry Hill. For comparison with the central Oregon coast samples, monthly samples were also collected during selected periods at Cape Arago and on the west side of San Juan Island. The number of animals collected at each sampling, usually between 20 and 35, was dependent upon weather and tidal conditions. The reproductive state of each sample was assessed by determining a gonad index value for at least ten animals and by histological examination from prepared slides of the gonad of at least ten others.

All collections, except those from Cape Arago, were transported to the laboratory in plastic buckets one-fourth full of sea water. The Cape Arago collections were placed in plastic bags half full of sea water and taken to the Marine Science Center in an iced styrofoam chest. Upon arrival, the animals were transferred to an enamel pan containing fresh sea water. After the chitons were attached, magnesium chloride anaesthetizing solution (Light et al., 1961) was added

and the tray was floated in a sea water table. Once the animals were relaxed, total lengths were measured and the animals processed for gonad index determination. Some gonads were also fixed for histological examination.

The gonad index of each animal was determined by removing the gonad by disecction and placing it and the remainder of the animal in separate, preweighed aluminum pans. These pans were then dried in an 80° C oven for three or four days or until the weights remained stable. The gonad index was calculated according to the following formula.

$$\text{Gonad Index (GI)} = \frac{\text{Dry weight of gonad}}{\text{Dry weight of total animal}} \times 100$$

Dry weights were used rather than wet weights or volumes because of the small size of the unripe gonads and because it was felt that dry weights would give greater accuracy and precision.

In specimens used for histological examination, the gonad was removed and placed in Bouin's fixative. None of the gonads remained in the fixative longer than six months. Following dehydration and paraffin infiltration, eight to ten micrometer thick sections were cut and stained with alum Hematoxylin and Eosin.

Temperature data cited in this study are from two sources: (1) Central Oregon Coast--Daily afternoon surf temperatures recorded

at Agate Beach, one-half mile south of the Yaquina Head collecting site (Figure 9). Gonor, Thum, and Elvin (1970) summarize this data from 1968-1970. (2) Southern Oregon Coast--Surface water temperatures at Charleston, Oregon (3 miles north of Cape Arago) were taken from the reports by Gilbert and Wyatt (1969 and 1970) (Figure 10). Concurrent temperature data were not available for the San Juan Island study area.

Results

Gonad Index Data

Central Oregon Coast

The gonad index data for the central Oregon coast have been summarized in Table 2 and Figure 9. Figure 9 also summarizes the daily Agate Beach surf temperatures for comparison with the gonad index data. The sizes of animals sampled for gonad index data from the central Oregon coast are given in Table 5.

In 1968 the highest mean gonad index (10.89) observed at Yaquina Head occurred on the first of April. At this time the gonad in both sexes filled a considerable part of the posterior body cavity area and oozed gametes when removed from the body cavity. Fifteen days later the gonad index had dropped to 1.74, indicating that within this time period a major spawning had taken place. As the range of

gonad indices within the April 15th sample was small (Table 2), a rather synchronous spawning is implied for the entire population at Yaquina Head at that time (Figure 9).

On April 19, 1968, a gonad index sample from Strawberry Hill had a mean value of 1.10, which is not significantly different from the April 15, 1968, Yaquina Head value of 1.74. At the end of April, 1968, the gonad index value for Yaquina Head reached its yearly low (0.96). A sample taken at Boiler Bay on May 16, 1968, had a mean value of 1.12. This data suggest that intertidal T. lineata along the central Oregon coast from Strawberry Hill on the south to Boiler Bay in the north, a distance of 40 miles, spawned between the period of April 1 and April 15. At Yaquina Head the high mean gonad index value for 1969 (10.60) was noted in the middle of March. One month later, in the middle of April, the mean value had dropped to 2.90, a drop of 7.70, which indicates that a major spawning occurred (Figure 9). The mean value continued to drop in May and June, possibly indicating that small amounts of spawning were taking place during this period. Observations were discontinued after the June, 1969, sample. In both 1968 and 1969 a small drop in the mean was recorded just prior to the occurrence of the highest gonad indices observed. The significance of this drop is not known. It may have been caused by either a small amount of spawning or sampling variation.

San Juan Island

Gonad index data for San Juan Island is summarized in Table 4 and Figure 11. The sizes of animals sampled for gonad index data from San Juan Island are given in Table 7. The first sample taken was in March, 1968, when the mean gonad index value was 6.89. This was also the highest gonad index observed in 1968.

During the next collection, May, 1969, taken at Cattle Point, San Juan Island, T. lineata were seen spawning in the field. The gonad index of this sample showed a mean drop of 1.67, but a wide range of values, from 11.8 to 1.3, was observed. The population was sampled during spawning, but this wide variation in gonad index values suggests that not all individuals had spawned by that time.

The observed low gonad index for 1968 was in September (3.44), but no samples were taken between May and September. A lower point was probably reached in July or August and the September values probably represent an increase over the actual low. In November, 1968, T. lineata around San Juan Island showed remarkable synchrony. The values obtained for three gonad index samples taken on two successive days at Turn Island (4.18), Cattle Point (3.34), and San Juan Park (3.90) were very close.

The high in 1969 was noted in the April (7.53) and May (7.59) samples. There was a drop of 3.52 from the May value to the June 1

value of 4.07. This drop would indicate that spawning had taken place and coincides with the observation of spawning in the laboratory on May 30. The value obtained for June, 1969, probably represents a population only partially spawned out and more spawning followed. On June 2, 1969, fifteen animals at San Juan Park were examined in the field to determine the size of the gonad. Some gonads still filled a good portion of the posterior body cavity and oozed gametes when cut, while others were much smaller, had a shrunken appearance, and did not ooze gametes; others were intermediate between these two conditions.

On June 18, 1970, fifteen T. lineata at San Juan Park and fifteen at Cattle Point were examined in the field, none of which had gonads that filled a large portion of the body cavity and many appeared to be spawned out. This would indicate that, as in 1968 and 1969, a major spawning probably took place in late May or early June.

Southern Oregon Coast

The gonad index data for the southern Oregon coast (Cape Arago) is summarized in Table 3 and Figure 10. The sizes of animals sampled for gonad index data from the southern Oregon coast are given in Table 6. The first sample from Cape Arago was taken in April, 1968, and was found to have a mean gonad index value of 3.83. The next two samples (in May and June) both showed

a decrease from the April value with the 1968 low occurring in June (1.48). The last sample taken at Cape Arago was obtained in January, 1969, and yielded the highest value (8.51) obtained from any sample at this location.

While the main spawning time in 1968 was probably in March and April, since no February or March samples were taken this remains undemonstrated. The slow decrease from April, 1968, to June, 1968 indicates that a number of spawnings took place through this time period.

Description of Gonads and Gametogenesis

The gonad of Tonicella lineata, like the gonads of other chitons (Hyman, 1967), is a sac-like structure located mid-dorsally just in front of the pericardium. The size of the gonad depends upon its ripeness and at the ripest condition may extend posteriorly over the pericardium and anteriorly to the area of the second valve. As the testes is red to orange in color and the ovary is green, the sex of T. lineata can be determined by the color of the gonad.

The floor and sides of both the testis and ovary are thrown into folds which project into the gonadal lumen. These folds are arranged in transverse rows and are called lamellae or tissue platelets. The platelets have a central support axis of connective tissue and muscle fibers. This same supportive tissue is continuous around the gonad

where, covered by a thin epithelium, it makes up the external covering. The germinal epithelium is located between and on the platelets. Nimitz (1964) and Selwood (1968) provide a more detailed description of chiton gonads.

The tissue platelets of the testes are like those described above except that an epithelium covers the germinal epithelium on the plate and interplate area. The spermatogonial layer is found next to the supportive tissue and next to it is the spermatocyte layer. Both of these layers form continuous bands, although their width and density changes depending upon the ripeness of the gonads. The spermatocytes give rise to the spermatids in a patchy distribution at first and then in a continuous band. Sperm formation also takes place first in isolated pockets and then becomes continuous along the lamellae. Increase in the thickness of the spermatogonial and spermatocyte layer and the production of spermatids and mature sperm cause the epithelial cells that cover the germinal epithelium to change their shape from cuboidal to flat. This epithelium then forms a barrier between the spermatozoa in the lumen and the spermatozoa still associated with the platelet and interplatelet germinal epithelium. After spawning has taken place and the layers of the germinal epithelium are reduced in size, the cells of the surrounding epithelium return to their cuboidal shape. Nimitz (1964) points out that because of this rapid return to their original shape,

the contact between the epithelial cells and the underlying connective tissue is probably not broken.

Small oocytes form within the germinal epithelium on the floor and sides of the ovary between and at the base of the tissue platelets. At the time of formation, the young oocyte becomes enclosed within a layer of follicle cells which are also derived from the germinal epithelium (Anderson, 1969). The flattened follicle cells covering the young oocyte have elongate nuclei. The oocytes bulge into the lumen of the ovary as they grow and their cytoplasm becomes very basophilic. Next, the oocyte becomes pear-shaped with a narrow stalk-like region attached to the platelet. Due to the growth of the platelet, the oocyte is carried dorsally into the lumen of the ovary.

Just prior to or at the time the oocyte becomes pear-shaped, the basophilic cytoplasm becomes filled with vacuoles called "areolae" by Gabe and Prenant (1949). Selwood (1968) and Anderson (1969) feel that this event just precedes or marks the beginning of vitellogenesis. The oocyte next goes through a lobate stage when the entire surface is covered with protuberances or lobes. After this stage, the chorion is fully formed and tightly packed around the oocyte and the cytoplasm is now only faintly basophilic. The mature oocyte then looses connection with the platelet and becomes free in the ovarian lumen. Although most oocyte development and maturation

takes place on the tissue platelets, some does take place in the interplatelet areas.

Analysis of Testes

Three main criteria were used in the analysis of the testes to assess the amount of spermatogenesis and the ripeness of the gonad: (1) relative amount of sperm production as judged by the area along the lamellae where mature sperm are present, (2) the shape of the enclosing epithelial cells and the relative extent of cells of each shape, and (3) the total width of the lamellae from epithelial layer to epithelial layer. The quantity of mature sperm in the lumen was also noted.

Central Oregon Coast--Yaquina Head. Results of the analysis are summarized in the following tables: Table 8, amount of sperm production; Table 9, shape of epithelial cells; and Table 10, width of lamellae. Figure 12 presents the information of Table 10 in graphic form.

At the time of the first sample (August, 1967), there was a small to medium amount of gametogenic activity. Sperm production was very patchy along the lamellae and did not exceed half of the lamellae area (Table 8). The epithelial cells were mixed in shape, being cuboidal over areas without sperm production and flat over the pockets of sperm production (Table 9). The spermatocyte layer was very dense and thick, indicating that sperm production would

increase in the near future.

By November, 1967, gametogenic activity had increased until sperm production was almost continuous along the lamellae in some individuals (Table 8 and Figures 13, 14). In other individuals, only about three-fourths of the lamellar surface was engaged in sperm production. The lumen was beginning to fill up with mature sperm.

Sperm production reached its peak in February, 1968, and stayed at a high level through the month of March, 1968. During this time sperm were produced in a continuous band along the lamellae with only a few small areas inactive (Table 8 and Figure 15). Besides the lumen being very full of sperm, other indications of high activity were the high lamellar width values obtained in February, 1968 (Table 10) and the flatness of the surrounding epithelial layer (Table 9).

Although sperm production was active in March and April, the lamellar width values were decreasing (Table 10). This indicates that sperm were escaping into the lumen and that no new spermatids were forming to replace the ones that matured into sperm. This latter point is confirmed by the sample taken at the first of April which showed a much thinner spermatocyte layer than the February or March samples.

Examination of testes from the May, 1968, samples showed spawning had taken place as the lumen contained only small amounts

of sperm (Figure 16). Sperm production had become patchy (Table 8) and the epithelial cells were mixed in shape (Table 9). The spermatid layer was absent except where small amounts of sperm production was still taking place.

The low point in spermatogenic activity for 1968 was reached in June when sperm production was represented by an occasional small pocket of activity (Table 8), the lowest lamellar width values were observed (Table 10), and the majority of epithelial cells were cuboidal in shape (Table 9). In July (1968) activity started to increase as the size of the pockets of sperm production increased (Table 8) and the lamellae widened (Table 10). The events described for the 1967-1968 breeding season were repeated between August 1968 and June 1969 (Tables 8, 9, and 10, and Figure 12). Testicular analysis was not done for the southern Oregon coast and San Juan Island collection because of the lack of specimens.

Analysis of Ovaries

Sections of ovaries were analyzed by estimating (counts per square unit of field were not made) the relative abundance of the following stages: Stage 1 - young oocyte with basophilic cytoplasm, Stage 2 - pear-shaped oocytes, Stage 3 - prelobate oocyte, past pear-shape stage but still with basophilic cytoplasm, Stage 4 - lobate stage, and Stage 5 - mature oocyte surrounded by chorion.

Central Oregon Coast. The ovarian analysis for the central Oregon coast is summarized in Table 11. The ovarian lumens of the September, 1967, sample did not contain Stage 5 oocytes except for a few residual ones not spawned out from the previous spawning season. The absence of any Stage 4 cells is an indication the mature oocytes were not being formed at this time. The most abundant stage found in this sample was Stage 1.

In the October, 1967, sample, Stage 4 cells began to appear, although no new mature oocyte formation was visible in any of the sections. Because of this, I classified the few mature oocytes present as residual. The number of Stage 2 and 3 cells also showed an increase over the September, 1967, sample, while the quantity of Stage 1 cells decreased.

The November, 1967, sample showed the first formation of Stage 5 cells. The most abundant stages in this sample were 3 and 4, although 1 and 2 were present (Figures 17 and 18). By the February, 1968, sample, the lumen of the ovary was beginning to fill up with mature oocytes and the quantity of Stage 4 was still quite high, indicating that more mature oocytes were to be formed. Only a few Stage 1, 2, and 3 cells were observed in February, 1968.

In March and the first part of April, 1968, the abundance of mature oocytes reached its highest point as the gonadal lumens were packed tightly with these cells (Figure 19). The low number of

Stage 4 cells indicated the formation of mature oocytes had almost stopped. The April, 1968, sample simultaneously showed lumens full of Stage 5 cells ready to be spawned and an increase of Stage 1 cells, the beginning of the next year's population of gametes (Figure 20).

After spawning took place between April 1 and 15, 1968, the lumen became almost empty of mature oocytes, as the low May, 1968, gonad index indicated (Figure 21). No lobate stages (Stage 4) appeared after the April, 1968, sample, indicating that mature oocyte production had ceased. The oocytes still present in the May sample were therefore residual or yet to be spawned. The dramatic decrease in the number of mature oocytes in the lumen found in the entire May sample as compared to the April sample is another indication of a single major period of spawning in April, 1968. Without additional oocyte formation, other spawning periods would release relatively few gametes.

During June, July, and August, 1968, the number of early stages progressively increased as the development of the next year's gametes began to take place at a very rapid rate (Figure 22).

The cycle for the year 1968-1969 was essentially the same as that for 1967-1968, as the data in Table 11 show.

San Juan Island. The ovarian analysis for San Juan Island is summarized in Table 12. Although only five samples were evaluated

for San Juan Island, they were sufficiently separated in time to demonstrate that the T. lineata in this area undergo an oogenic cycle with the same sequence of major events as the ones along the central Oregon coast. The only significant difference is in timing, as the San Juan population runs about one to one and a half months behind that of the central Oregon coast. This variation is also indicated by a comparison of gonad index data from the two regions (Figures 9 and 11). The San Juan chitons seemed to have a larger number of mature oocytes remaining in the ovary after the first spawning period than did the animals along the central Oregon coast, indicating that one major spawning may not be the rule in this area.

EMBRYOLOGY

Materials and Methods

Tonicella lineata were placed on rocks encrusted with coralline algae in a shallow tank with running sea water. During or after spawning occurred, eggs were collected with a pipette and placed in 1000 mls. of filtered sea water. Sperm was also collected with a pipette and concentrated in a 50 ml. beaker. Because males and females were kept together in the same tank, some eggs were already fertilized when collected. Both the fertilized and unfertilized eggs were cleaned of debris by pouring the egg suspension through two layers of clean cheese cloth several times. The eggs were then placed in 500 ml. beakers with about 400 ml. of filtered sea water. As the eggs do not float, their concentration was regulated so that only a thin layer was present on the bottom of the beaker.

Fertilization was carried out in 500 ml. beakers occasionally using naturally spawned gametes, but in the majority of cases naturally spawned eggs and artificially removed sperm were used. To obtain sperm, the testes was removed, cut with scissors, and then agitated in a 100 ml. beaker of filtered sea water. Three pipettes of sperm mixture were put into each 500 ml. beaker containing the unfertilized eggs and the mixture was gently agitated for 30 seconds. After the eggs had settled to the bottom, the sea water containing the

excess sperm was poured off and replaced with fresh filtered sea water. About 90 percent of the eggs were fertilized by this method. The beakers were then placed on the sea water table at local sea temperatures. The water was changed every day at first and every two or three days after the larvae hatched, depending on the cleanliness of the culture. Antibiotics were not used.

Microscopic observations of living larvae in sea water on slides were facilitated in the following ways: (1) more detail could be seen if the coverslip was supported on all four corners by small pieces of clay. This type of support permitted the application of pressure on the coverslip toallow maximum observation and (2) swimming larvae were slowed down with polyethylene oxide dissolved in sea water. A weak magnesium chloride sea water mixture was used before fixation to anesthetize the larvae. Photomicrographs were made using both live and fixed material.

Spawning Behavior

The spawning behavior of one female Tonicella lineata was observed in detail. This female was collected at San Juan Park on April 5, 1969, and brought back to the laboratory on a rock encrusted with coralline algae. At 1600 hours on the day collected, this chiton began moving around the rock it was on and finally stopped on the side of the rock with its right side downward. At 1820 the girdle on

the right side in the areas of valves 7 and 8 was lifted from the substrate in an arch shape. A few eggs were observed in the area of the mantle cavity exposed by the girdle lift. A moment later these eggs began to stream from the area of the posterior girdle cleft, directly behind the anus. Twenty minutes later the girdle was also lifted on the left side in the areas of valves 7 and 8 (Figure 23).

The eggs, green in color and covered with a frilly chorion (Figure 24), came out the area of the posterior cleft in groups of two or three surrounded by a mucus-like substance. They immediately settled to the bottom of the sea water tank, forming a clump. Spawning continued for one and one half hours but was intermittent, with short periods of time when no eggs would be released.

Whether or not the eggs were released in two strands, as reported for other chitons, could not be determined as the position of the spawning female under observation caused gametes from both sides to fuse into one strand.

Attempts to induce spawning in Tonicella with alternating periods of light and dark, heat and cold, by placing in calm water, in isolated bowls, and by electrical shock failed.

The following list summarizes observed spawning behavior for T. lineata at the Friday Harbor Laboratories:

20 June 1955	Specimens from Garrison Bay spawned in laboratory during afternoon.
1 August 1955	Male and female spawned in outside tank at 1600 hours. Male spawned first.
22 July 1958	Spawned in laboratories at 1020 - male first, then female.
28 July 1959	Both sexes spawned in laboratory.
23 May 1968	Observed spawning in field at Cattle Point.
5 April 1969	One female from San Juan Park spawned for one and a half hours in laboratory, starting at 1820 hours.
6 April 1969	Another female from San Juan Park started spawning at 2000 hours. The female that spawned on 5 April started spawning again at 2200 and spawned for one hour. Number of eggs spawned greatly reduced from number spawned previous night.
28 May 1969	Male spawned in laboratory during night.
29 May 1969	Isolated male spawned in laboratory during night.
30 May 1969	Eight females collected from MarVista area spawned between 1930 and 2100. Average spawning time one and a half hours.

In 1969 the majority of T. lineata on the west side of San Juan Island probably spawned during the last days in May and the first in June as evidenced by the high number of animals spawning in the

laboratory on 30 May. Gonad index data from the same area also supports this as there was a 3.52 drop in the gonad index between 18 May (7.59) and 1 June (4.07). This drop would seem to indicate a spawning had taken place between these samples.

No field or laboratory spawning observations have been made on T. lineata from the Oregon coast.

The differences in spawning time between the central Oregon coast and San Juan Island populations will be discussed later.

Table 13 lists the times of spawning for T. lineata in relation to the phase of the moon and tidal heights for Port Townsend, Washington. Since most of these animals are from the west side of San Juan Island, the tidal cycle and the times of exposure would be similar to those at Port Townsend located directly across the Straits of Juan De Fuca from San Juan Island. In 1968 and 1969 the majority of observations at Friday Harbor Laboratories indicate that T. lineata spawns near the period of full moon and early in the evening. This early evening time also coincides somewhat with the time of the low high tide at this time of year.

Viability of Gametes

Eggs

Fertilization in the laboratory was successful only with naturally spawned eggs. Attempts to fertilize eggs artificially removed

from females failed. The chorion is very frilly and fully expanded in naturally spawned eggs, whereas it never expands to its fullest in stripped eggs even after they are soaked in filtered sea water for 12 hours. Stripped eggs also retain their germinal vesicles, while naturally spawned eggs are released with the germinal vesicle broken down.

After spawning, the length of time the eggs will remain viable appears to be long. The eggs spawned from one female were stored in a beaker at 9-10° C for 14 hours and then fertilized. No differences between these embryos and ones produced from eggs fertilized immediately after spawning could be detected.

Sperm

Naturally spawned and stripped sperm seem to be equally viable and no differences in embryos from eggs fertilized with either could be detected.

Discussion

Johns (1960) made similar observations on the viability of eggs artificially removed from ovaries of Sypharochiton pelliserpentis. When artificially removed, the sperm of S. pelliserpentis were found to remain clumped and, due to rapid disintegration, gave very low fertilization success. By contrast, stripped or naturally spawned

sperm of T. lineata gave equally good fertilization success.

The only information found in the literature on the length of time chiton eggs will remain viable after spawning is given by Grave (1932) who comments that the eggs of Chaetopleura apiculata will remain viable for 24 to 40 hours after shedding.

It would appear that germinal vesicle breakdown occurs within the oviduct. Since there is some evidence (Cryptochiton, Tucker and Giese, 1962, and this study) that the ovary is not emptied of ripe eggs in one spawning, it would seem that breakdown of the germinal vesicle does not take place in the ovary, but instead occurs in the oviduct where only those eggs being spawned can be acted upon. The oviducal tissue is very glandular and secretes the mucus-like material associated with spawning.

In Cryptochiton stelleri (Lawrence et al., 1965), the oviduct increases in size as the ovary increases, and the oviduct reaches its maximum size at the height of the reproductive season.

Early Development

Fertilization, Cleavage, and Gastrulation

At the time of fertilization, a space develops between the egg proper and the surrounding membranes. Chiton eggs are considered to have two membranes, an inner vitelline membrane, and an outer,

frilly chorion (Hyman, 1967). Since there is no cortical reaction or raising of any new membranes at the time of fertilization (Anderson, 1969), this space probably develops by a pulling away of the egg cytoplasm from the membranes. One half hour after fertilization the first polar body is produced and the second polar body is formed one and a half to two hours after fertilization. They are found in the space between the fertilized egg and the chorion and their location marks the animal pole (Figure 24).

Cleavage and gastrulation do not differ from previous accounts for chitons (Heath, 1898; Christiansen, 1954; Hyman, 1967) and will not be described further. Cleavage and gastrulation stages are shown in Figures 25, 26, 27, 28, 29 and 30. The timing of these stages is given in Table 14.

Development Before Hatching

This period of development is defined as the time from the formation of the prototroch to the hatching of the trochophore. This usually takes one day, depending on the temperature. Developmental times of this period are given in Table 14.

Within a short time period, usually four to five hours after the start of gastrulation, the developing embryo, still within the chorion, starts to take on the shape of the trochophore larva. The blastopore, which originally formed at the posterior end of the embryo, begins an

anterior migration along the ventral side. At this point the opening of the blastopore is fairly large and a narrow furrow extends from its anterior edge (Figures 30 and 31). The blastopore contines to migrate forward and at the same time the opening decreases in size and the furrow disappears. At the time of hatching, the blastopore is represented by a very small opening in a mid-ventral position immediately behind the prototroch.

The development of the prototroch begins at about the same time as the beginning of the migration of the blastopore. The first indication of prototrochal formation is an indentation which develops completely around the embryo about one-third to one-half the distance back from the anterior end. The developing embryo can now be divided into a pretrochal and post-trochal region (Figure 31). The prototroch, which is fully developed when the larva hatches, is made up of two rows of cells extending completely around the larvae. Although both rows are of the same height, the cells of the anterior row are only half of the width of the posterior row of cells. The anterior row therefore contains twice the number of cells found in the posterior row. The cilia of the anterior row are shorter than those of the posterior row. While still within the chorion, the prototrochal cilia point anteriorly, surrounding the pretrochal region. They may show a slow beat which slowly rotates the developing trochophore within the egg membranes.

The other major external morphological developments before hatching take place at the anterior end of the pretrochal region. At the time of prototroch development, the extreme anterior portion of the pretrochal region flattens slightly and becomes the apical region (Figure 31). In the center of this plate, the apical tuft develops. Within a short time a group of very granular appearing cells becomes differentiated on the apical plate encircling the apical tuft. At the same time two cellular tracks with the same granular appearance as the circle of cells on the apical plate become differentiated in a ventrolateral position. They are attached on each side to the circle (Figures 32 and 33). Just before the trochophore hatches, the tracks become ridges elevated above the rest of the pretrochal region and lose their connection, at least externally, to the circle of cells around the apical tuft. These elevated ridges retain their very granular appearance and become covered with long motile cilia that are intermediate in length between the cilia of the prototroch and apical tuft and much longer than the general body ciliation that will develop later.

About the time of hatching, the elevated ridges begin a slow ventrolateral migration. At the same time, the circle of granular cells around the apical tuft differentiate into four knob-like structures which are also elevated above the general surface of the pretrochal region. These apical knobs are found close to the apical tuft, two in

a right lateral position and two in a left lateral position. They retain their granular appearance, as do the elevated ridges. The apical knobs also become covered with long motile cilia like those found on the elevated ridges.

At the time of hatching, short cilia cover the rest of the pretrochal region three-fourths of the way down to the prototroch. The short cilia covering the pretrochal region and the longer cilia of the apical knobs and elevated ridges beat continuously whereas the cilia of the prototroch do not. Also present at the time of hatching are two tufts of stereocilia at the extreme posterior of the postrochal region (Figure 34). These will be called anal tufts as they probably mark the site of the future anus.

Hatching

Hatching takes place at around two days (Table 14). At the beginning of hatching, a split develops in the chorion, usually in the region of the apical tuft. At this time the prototrochal cilia begin to beat very vigorously, pushing the anterior end of the larva out of the split in the chorion. The larva also uses rapid body flexions to escape from the chorion. Once the prototroch is outside of the chorionic membrane, it is used by the larva to swim free of the membrane.

Development of Free-Swimming Trochophore

For purposes of the following description, development of the trochophore larva from the time it hatches until it settles and metamorphoses has been divided into 10 to 15 hour intervals. Most of the observations were made on animals that developed at 10° C. Development times are given in Table 14.

60 to 70 Hours (Figure 35)

At 60 hours the free-swimming larvae are very active, although at times they may remain motionless near the bottom of the beaker for a few seconds. The pretrochal region is bulbous in shape. The four apical knobs have increased in size and the elevated ridges are continuing their ventro-lateral migration at a very slow rate. They will continue to migrate down towards the anterior lateral margin of the prototroch until about the 140 hour period, when they disappear. The apical knobs will also disappear at about the same time.

The post-trochal region begins to elongate and become more dorso-ventrally flattened. The larva is now completely covered with small cilia except for the dorsal area where the shell will form.

The blastopore is a small opening behind the prototroch.

85 Hours

There is little change in external appearance except for more elongation and flattening of the post-trochal region.

100-110 Hours (Figure 36)

Paired larval eyes first become distinct in this period as small, dark, circular areas, one on each side of the larva at the anterior lateral margin of the post-trochal region directly behind the prototroch (Figure 39). The apical knobs and the elevated ridges are still very evident (Figures 37 and 38).

120 Hours (100 Hours at 12 to 13° C)

Distinct lines across the dorsal area of the post-trochal region, demarking the shell glands, first appear in this period. The post-trochal region continues to elongate.

130 Hours

The musculature has developed and the larvae have the ability to change from an oval to an elongate shape. The elevated ridges have migrated close to the prototroch and are inconspicuous. The apical knobs have become reduced in size and the foot has sufficiently differentiated for the larvae to crawl on a glass slide or on the bottom

of the beaker. When crawling, the larva attaches only the posterior part of the foot and waves the pretrochal region from side to side.

140 Hours (Figure 39)

The elevated ridges become indistinct and cannot be followed externally past this time period. The function and fate of the ridges was not determined. The apical knobs are either very reduced or not visible at this time.

150 Hours

The shell glands become elevated and the girdle area becomes differentiated in the post-trochal region. Spicule formation begins all around the edge of the newly differentiated girdle area and also extends a short distance in front of the prototroch on each side, connecting with a band of spicules across the pretrochal region (see Figure 40). The spicules are small, clear, and usually in groups of three.

The majority of the larvae are now swimming near or attached on the bottom of the beaker.

160 Hours (Figures 40 and 41)

The foot gland becomes distinct as a clear, circular area in back of the prototroch. The opening of this gland is represented by

a crease just behind the anterior of the foot.

The larvae are ready to settle at this point and will not undergo further development until settling takes place on the proper substrate.

Other Observations

It was not determined whether or not the blastopore becomes closed. Once the anterior region of the foot becomes highly differentiated, an anterior flap covers the area where the blastopore had been previously visible.

Metamorphosis and Development after Metamorphosis

The remaining part of this developmental description will be based on time periods of half or full days. A metamorphosed larva will be considered as one that has lost its prototroch and apical tuft, has a flattening of the pretrochal region, and one in which the formation of the shell plates has begun. Metamorphosis is usually completed within 12 hours.

One-Half Day after Settlement (Figures 42 and 43)

During this time period, the prototroch and apical tuft are lost. The pretrochal region, bulbous in the pre-settled larvae, now begins to flatten out. The spicules and anterior shell plates move forward over the dorsal part of the pretrochal region.

Only after settlement does shell plate formation begin. The plates first form as very thin, rectangular-shaped plates at the site of the shell glands. The plates are seven in number and are only lightly calcified--as evidenced by their freely bending and flexing with the movements of the animal.

All locomotion is now carried out by the foot and the larva actively crawls over the substrate. The larval eyes are still present.

One to One-and-One-Half Days after Settlement

The seven shell plates have increased in size, but still have spaces between them. The valves have become more calcified and their flexibility is greatly reduced.

Two-and-One-Half Days after Settlement

The mouth is evident in the center of the semicircular head. The larvae are transparent and differentiating internal structures are visible.

Three-and-One-Half to Four-and-One-Half Days after Settlement (Figure 44)

The radula is present as a dark, straight line in the head region. The seven valves are now beginning to overlap and show a dorso-ventral curvature.

Five to Five-and-One-Half Days after Settlement

The eighth shell plate is now distinct as a small, slightly rectangular piece of shell posterior to the seventh shell plate.

By the sixth day after settlement, all of the major external morphological features, except the gills, are present.

Other Observations

One month after settlement the young chitons (Figure 45) actively grazed on encrusting coralline algae and formed copious amounts of fecal pellets. Figure 46 shows an unsettled larvae that is one month old.

At the 45th day the larval eyes were still present, but the gills had not yet appeared.

Development of the young chitons was not followed beyond 45 days.

LARVAL SETTLING

Materials and Methods

The substrate selection experiments were carried out with 100 to 125 larvae placed in beakers with 400 ml. of filtered sea water. The beakers were placed in the sea water tables. Control beakers contained only larvae and sea water. Algae used in the experiments were collected at San Juan Park, Mar Vista, and Minnesota Reef on San Juan Island. Rocks and small pieces of roofing tile were gathered from the intertidal beach in front of the Friday Harbor Laboratories. All these materials were cleaned in running sea water and left in the sea water table twelve hours before use.

Observations on settling and settling counts were made under a dissecting microscope. The pieces of rock, algae, or tile were removed from the test beakers with forceps and placed in shallow bowls of filtered sea water. After the observations were made, the substrates were returned to the original beakers and any swimming larvae remaining in the observation bowls were pipetted back into the experimental beaker.

Photographs of settled larvae were made through a dissecting microscope using transmitted light and an electronic flash. Settled larvae were also removed from the substrate and photographed with a photomicrographic apparatus.

Settling Experiment 1A--Coralline Substrate

This experiment was carried out using 230 hour old larvae raised at 10° C. These larvae had not shown any external developmental changes since the 160th hour (Figure 40). At 230 hours the larvae were observed crawling on the bottom of the culture beakers and only occasionally would they leave the bottom for short periods of swimming.

To test their substrate response, a piece of encrusting coralline algae was placed in three beakers each containing 100 to 125 larvae. Settled larvae were found on the encrusting coralline within 8 hours. Twenty-four hours after the beginning of the experiment 68 larvae had settled on the encrusting coralline algae. Figure 47 shows 24 hour settled larvae on encrusting coralline algae. These larvae had metamorphosed and were now oval in shape, white in color, and had seven shell plates. The apical tuft and prototroch had also been lost. Larvae, with prototrochs still present, which were crawling on the substrate were not considered settled in this or subsequent experiments as they could still swim away.

After three days the external appearance of the larvae in the control beakers remained the same as when the experiment started. No larvae settled on the glass of the control experimental beakers. The results of this experiment are summarized in Table 15.

Settling Experiment 1B--Coralline and Rock Substrate

Following the 24 hour observation of Experiment 1a, seven additional beakers each containing 100-125 larvae (254 hours old and raised at 10° C) were used for substrate choice observations. Two control beakers, two beakers with only rock and three choice beakers with both rock and encrusting coralline were used. The results of this experiment are summarized in Table 16. After six hours larvae were crawling in large numbers over the coralline and some had already lost the prototroch and apical tuft. During the entire experiment only a small number of animals were observed crawling on plain rock and none of these metamorphosed on it.

Observations on the number of settled larvae after the third day yielded count differences of only three or four, so the number of larvae settling in each case is given in Table 16 only through day three. It is likely that more larvae settled than indicated as it was difficult to make an accurate count of those settling in cracks and depressions of the coralline algae. Larvae swim to the coralline in their normal spiral manner and touch it with the anterior end. At this point they usually back off a short distance and swim parallel to the algae surface briefly before settling down on it with their foot. Occasionally a larva follows this procedure of touching and swimming two or three times before settlement. When crawling on the alga starts, the

prototroch immediately stops beating and the foot takes over as the organ of locomotion. At this time the larvae may detach its foot and swim off using the prototroch. All Experiment 1a and 1b cultures were observed for 15 days. During this time no metamorphosed larvae were observed in the control beakers. These control animals remained at the 160 hour development stage. From about the fifth day of the experiment on, most larvae remained motionless on the bottom of the beaker or crawled slowly although they still had a prototroch. There was no evidence of shell formation in any of these larvae. By the fourteenth and fifteenth day, about half of the unsettled animals had lost their apical tuft and a few their prototroch. Such larvae would remain motionless and many died.

By the fifteenth day the settled larvae were beginning to resemble adult animals and were actively crawling over the substrate. Observations were continued on the development of these settled larvae.

Settling Experiment 2--Rock, Tile and Two Coralline Algal Species as Substrates

In this experiment 100 to 125 one hundred and fifty six hour (raised at 11 to 12° C) larvae were placed in the following beakers: 6 control beakers, 3 beakers with only pieces of one of three encrusting coralline species, 2 beakers with choices between one of

two encrusting coralline algal species and rock or tile, and 2 beakers containing only rock or tile. The coralline algae used were an unidentified form and one species each of the genera Lithophyllum and Lithothamnion.

Observations were made at 15 hours and 40 hours after the start of the experiment. The results of this experiment are summarized in Table 17. A total of 247 settled larvae were counted, none of which were observed on the tile or rock or in the control beakers. Larvae settled on all three species of encrusting coralline algae used, but in greatest numbers on Lithophyllum. Settled animals were observed in clumps or patches of 27, 18, 12, and 22. The non-settled larvae in the control and rock and tile containing beakers remained in the same stage of development as when the experiment began.

Settling Experiment 3- Coralline Extracts on Substrates

The purpose of this experiment was to determine if the larvae are attracted to the coralline algae by a chemical stimulus. The larvae used were 200 hours old when the experiment began and had been in contact only with the glass beakers.

Separate cold sea water extracts of Lithothamnion and another undetermined species of encrusting coralline were made following the method of Gee, 1965. Small pieces of old roofing tile were

soaked in each extract for twelve hours. The control tile pieces were soaked in sea water for twelve hours. The control and experimental tile pieces were then placed in beakers each containing 100 to 125 larvae. The results of this experiment are summarized in Table 18.

Settling under these conditions took place at a much slower rate than in the previous two experiments. Only after 24 to 36 hours did settling begin. At each observation several larvae were crawling on the extract-soaked tiles, while never more than one or two were observed crawling on the untreated tile. The developmental stage of the control beaker larvae did not change during the experiment.

Settling Experiment 4--Effect of Boiling

The purpose of this experiment was intended to determine the effect of boiling on the attractiveness of coralline algae to larvae. Pieces of tile used in Experiment No. 3, which had been soaked in algal extract and on which larvae had settled, were also boiled. The pieces of algae and tile were boiled for two hours in sea water. The boiled algae were pieces used in Experiment No. 3 upon which larvae had settled in Experiment 2. Larvae used were 144 hours old (raised at 12.0° C) and 100 to 125 were placed in each beaker. The results of this experiment are summarized in Table 19. No settled larvae were observed on the boiled substrates.

Settling Experiment 5--Other Algal and Coralline Substrates

The purpose of this experiment was to test the receptiveness of the larvae to other algae found in and around the habitat of adult *T. lineata* in comparison to coralline substrates. Larvae used were 144 hours old (raised at 12.0° C) and 100 to 125 were placed in each beaker. The substrates presented were: *Hedophyllum sessile* holdfast, *Hedophyllum sessile* frond, *Laminaria* sp. holdfast, *Fucus gardneri*, *Odonthalia floccosa*, branched coralline alga (sp. unknown), and *Lithothamnion* sp. All of the above listed algae were placed singly in bowls and presented to the larvae. Also, two bowls, one containing *Lithothamnion* and *Hedophyllum* holdfast and the other containing *Lithothamnion* and *Hedophyllum* frond, were presented to the larvae. Two control beakers were set up.

The results of this experiment are summarized in Table 20. After 12 hours a total of 311 settled larvae were observed. All of these were found on the encrusting *Lithothamnion* sp., while none were observed on any of the other algae presented including the branched coralline. As in the other experiments, the larvae in the control beakers did not alter their stage of development during the course of the experiment.

DISCUSSION

Ecology

Encrusting Coralline Association

Tonicella lineata is one of the most abundant chitons in the lower intertidal and upper subtidal along the Oregon coast and on San Juan Island. They are found only in association with a specific substrate, encrusting coralline algae, which makes up the major part of their diet and which is required for successful larval metamorphosis. Similar specific associations have been found for other members of the genus Tonicella. Yakovleva (1952) reports that T. submarmorea, T. marmorea, T. granulata, and T. rubra are all found associated with coralline algae and T. lineata is common on the red alga Delesseria. T. marmorea, T. submarmorea, and T. granulata are also reported by Yakovleva (1952) to be common among Lamanaria holdfasts. Dur- this study T. lineata was found only rarely on a lamanarian holdfast. Other chitons such as Katharina tunicata, Cryptochiton stelleri, Mopalia muscosa, and Mopalia lignosa, which were also common at the sampling areas, do not show a specific substrate association as does T. lineata. Within their intertidal ranges these other chitons may be found on any substrate available, including encrusting coralline algae. The feeding habits of these species also differ from that of T. lineata; their diet is not as restricted and they do not limit themselves to one particular group

of algae. Barnawell (1960) found the chitons of the genus Mopalia to be omnivorous scrapers feeding on all types of algae and some sessile animals such as sponges, hydrozoans, and barnacles. Katharina tunicata feeds on most of the foliose red and brown algae found within its intertidal range and also takes in some encrusting corallines (R. I. Caplan, personal communication).

I have observed only one other animal at the study areas, the limpet Acmaea mitra, to use encrusting coralline algae as its major food source. This limpet is abundant in the lower intertidal and always is associated with encrusting coralline algae with which its shell is usually covered. While T. lineata grazes only the upper layers of the algae, A. mitra scrape the coralline off to bare rock.

Coralline algae had the lowest caloric values (1.03 to 1.65 kcal/g dry weight) of any of the Pacific Coast intertidal algae studied by Paine and Vadas (1969). Because of the quantity of nonusable calcium carbonate taken in, animals like T. lineata and A. mitra probably have to process large amounts of encrusting coralline to provide enough energy to meet their metabolic requirements. The observation that T. lineata feeds continually when immersed in the laboratory and the copious amounts of fecal pellets at the bottom of urchin burrows found in the field support this hypothesis.

Size Variations and Growth Rate

No reference was found in the literature concerning chitons having a size difference in relation to intertidal level. Size difference at various levels in T. lineata is probably attributable to differences in amount of time available for feeding. It is interesting to note that the subtidal T. lineata taken in the Shady Cove transect were rather small, although at these depths there is plenty of coralline and since they are never exposed feeding can be continuous. Their small size may be due to a paucity of epiphytic diatoms available at that depth to augment the coralline diet. This distribution places complications on growth studies.

The size characteristics of a population sample of T. lineata would depend on the intertidal level the sample was taken from. Also, the characteristics of a population sample would probably depend upon the size of the predator population present at the study site. Landenberger (1968), in his work on selective feeding by Pisaster, found that there is a good relationship between the size of the predator and the size of their prey; Menge (1970) reports a similar relationship in Leptasterias. The small number of T. lineata found above 3.1 mm in the tide pool sample at Yaquina Head may have been due to the presence of medium size Pisaster in the pool. As Landenberger (1968) points out, selective behavior by the predator

can have important effects on the age structure and spatial distribution of a prey population.

Using size as the parameter, year class determination for T. lineata would be valid only if data from a single tidal level was used, since large animals at one level could be the same age as smaller animals at another level. Growth data would be subject to the same limitations. Because of this, the growth data collected on Tonicella was from a tide pool where feeding conditions and feeding time would presumably be the same for all individuals in the pool/ The growth data presented in this paper, although small in quantity, is included because of the lack of growth data on chitons in the literature. From the data presented one can see that there is great variation among individual animals within a size class even in a situation where feeding conditions are presumably uniform.

Homing

Thorne (1967) reported homing in the chiton Acanthozostera gemmata. This chiton returns to a definite homesite after each feeding excursion by following its outgoing trail. In a later study Thorne (1968) points out that cues from old trails and a topographical memory are two possible factors involved in the homing mechanism. Since animals in the Tonicella population exhibiting homing behavior were always in their "homes" during exposed conditions, it would

seem that they too return after each feeding excursion. Because the coralline algal food of Tonicella surrounds the home, feeding excursions would probably not require the covering of great distances.

Reproductive Cycle

This study shows that Tonicella lineata has an annual reproductive cycle fitting the pattern described for most benthic marine invertebrates, with alternating periods of gamete maturation and restricted periods of spawning (Hedgpeth and Gonor, 1969). In the case of T. lineata, a long period of gamete maturation is followed by a short, restricted period of spawning. Similar annual cycles have been observed in the following chitons found along the North American Pacific Coast: Katharina tunicata, Monterey Bay, California (Giese et al., 1959, and Nimitz, 1964), central Oregon coast and San Juan Islands (Caplan, personal communication); Cryptochiton stelleri, Monterey Bay, California (Tucker and Giese, 1962); Mopalia hindsii, Monterey Bay, California (Giese et al., 1959); Mopalia muscosa, Santa Monica Bay, California (Monroe and Boolootian, 1965). Monthly gonad indices were determined in all of the above studies, but only in Nimitz' study (1964) of Katharina and Tucker and Giese's study (1962) of Cryptochiton are histological data included. Information on spawning observations of chitons from the Pacific Coast of North America is given by Heath (1898, 1905), Hewatt (1938), and Thorpe

(1962).

Three studies on chiton reproduction from areas other than the Pacific Coast of North America have used histological examination of gonads to determine the reproductive cycle (Johns, 1960; Christiansen, 1954; Stephenson, 1934), while others have used only spawning observations (Grave, 1922; Brewin, 1942; Thorson, 1949; Costello et al., 1957). In a discussion of methods for determining annual reproductive cycles, Giese (1959) points out the drawbacks in using spawning as a lone criterion and why additional measures such as gonad indices and histological examination must be included for accurate determination of annual reproductive cycles. If the causative factors are also being considered, a complete knowledge of gametogenesis and gonadal growth characteristics is requisite since the elements that induce spawning may not be those controlling the timing of gametogenesis (Giese, 1959).

The wide gonad index ranges observed during the months of gonad buildup are due to individual variation in gonad ripeness and the amount of food within the gut when the gonad index was determined. Concurrent appearance of small ranges and low gonad index mean values probably is a result of having only one major variable, food quantities within the gut, since most of the gonads are fairly empty of mature gametes due to spawning.

The size of the animals within a sample may also play a role

in the gonad index range and mean values obtained. In this study the medium to large size animals available at the collecting sites were deliberately gathered since gonad indices are much easier to determine on larger animals, particularly when the gonads have just been spawned out. The mean length of the samples used for determination of the gonad index values was kept fairly uniform (Tables 5, 6, and 7) and it is felt that any effect on the obtained values due to size differences has been kept at a minimum. Giese and Hart (1967) feel that selective avoidance of small animals will not affect determination of the general cycle pattern if all sizes within the population are in synchrony. Pearse (1965) found in his study on the asteroid Odontaster validus that for animals weighing between five and ten grams, the smaller animals had the smaller index values and the larger animals had the larger values. In animals above ten grams, the gonad indices were independent of animal size. Gonor (personal communication) has shown in the purple urchin S. purpuratus that gonad index samples of mixed sizes will affect the mean values obtained.

There seems to be some correlation between the body size and the gonad index value in T. lineata as shown by two samples taken 15 days apart in July, 1968, at Yaquina Head (Table 2). One sample contained ten large Tonicella from the normal bench collecting site; the other consisted of ten of the largest T. lineata ever

collected at Yaquina Head. These were obtained from the lower intertidal during one of the lowest tides of the year. Although histological examination of the gonads in both samples revealed close synchrony, the mean gonad index value of the larger sample was 1.5 points above that of the smaller sample. This difference in values can be attributed to the fact that the proportion of gonad weight to body weight increases with the size of the animals, thus giving a higher gonad index. This phenomenon is further demonstrated in the March, 1968, San Juan Island data. The intertidal sample had a mean gonad index value of 6.89, but a sample collected subtidally at 15 to 20 feet contained animals that were significantly smaller and with lower gonad index values. The smaller animals had a lower gonad weight to body weight proportion. Again, histological comparison of the gonads of both samples showed them to be in close synchrony.

The gonad index curves obtained for the three main regional study areas show essentially similar cycles with a period of long gonadal buildup preceding a rapid decline indicating a major spawning, followed possibly by smaller spawnings. The comparatively small San Juan Island drop in mean gonad index value during spawning in June of 1969 was probably due to the time of sampling. That the sample was taken during the major spawning season and not after is demonstrated by the wide range of mean values and histological

findings of some gonads empty and others full of mature gametes. A sample taken two weeks later would almost certainly have shown a lower mean value, possibly of the same magnitude observed in the central Oregon coast animals.

Whether or not a single large spawning season occurs on the southern Oregon coast cannot be determined because of the lack of samples.

Comparison to Other Species- Gonad Index Curves

In comparing the gonad index curves for various chitons, there is wide variation in curve characteristics between the various species and also from year to year for individual populations of the same species. To accurately understand any gonad index curve, there must be concurrent histological examination of the gonads. For example, a drop in gonad index may indicate either spawning or resorption of old gametes. This can be resolved only by histological examination.

Comparison of the annual gonad index curves of Katharina tunicata (Giese and Hart, 1967; Nimitz, 1964) and Cryptochiton stelleri (Tucker and Giese, 1962; Lawrence et al., 1965) with that of Tonicella lineata points out some interesting differences between these chitons. These three chitons may be compared because histological studies have been carried out on gametogenesis (Nimitz, 1964;

Tucker and Giese, 1962; this study).

Gonad index data has been collected for nine years on Katharina from the Monterey Bay, California, area by Giese and his associates (Giese and Hart, 1967). At that location, K. tunicata has exhibited a consistent pattern of only one annual spawning period except for the 1959-60 cycle when several periods of spawning were observed.

The gametogenic cycle in Katharina tunicata is characterized by a regular pattern of low gonad indices from August to December and then a rise to the high in late spring. Production of gametes in this case takes only five to six months since Nimitz (1964) has shown that little gametogenic activity is present during the low index period. The decline in gonad index due to spawning takes place in May, June, or July, with some years showing very rapid declines from high to low values in one month (Giese et al., 1959) but in other years the decline covers three months (Giese and Hart, 1967).

Cryptochiton stelleri also shows a very fast buildup of gametes (Tucker and Giese, 1962). Although Tucker and Giese (1962) found some gametogenic stages at all times in the gonads of C. stelleri, the gonad index reaches its high point in a period of less than four months "from the beginning of the regenerative processes." The time period involved in the drop from high to low mean value was four to five months, which may indicate many spawning periods.

Lawrence et al. (1965) found the drop from high to low in 1964 took only two months, but this was followed by a five month period when the gonad index mean value rose only one and a half points, followed by a rise of about six points in four and a half months.

In T. lineata, the gonad does not go through a resting stage as in Katharina, nor is there a fast buildup of gametes as in Katharina and Cryptochiton. Immediately after or right before spawning, the gonads in Tonicella show active gametogenesis, with increased formation of young oocytes in the ovary and the thickening of the spermatocyte layer. This is borne out by a gonad index curve which gradually rises from its lowest to its highest point over a period of ten to eleven months. The drop in the Tonicella curve from high to low varied for the two years that populations on the central Oregon coast were observed. The slope of the dropping curve might indicate the number and length of spawning periods, a rapid drop indicating one major spawning and a slow drop many smaller spawnings, since reabsorption of gametes was not observed in any of the histological studies.

In all three species the drop appears to be the most variable part of the curve, while the buildup characteristics are almost constant from year to year for each. The situation in Tonicella is similar to that found in Haliotis cracheroidii, the black abalone, in which gametogenesis also starts immediately after spawning

(Webber and Giese, 1969), while in Katharina the situation is more like that found in certain lamellibranchs (Loosanoff, 1942) and the prosobranch gastropod Patella (Orton et al., 1956) in which the gonad goes through a resting stage after spawning.

Comparison to Other Species--Histology

Histological analysis of the gonad was important because it showed that gametogenesis in Tonicella does not differ from other chitons (Gabe and Prenant, 1949; Cowden, 1961; Johns, 1960; Nimitz, 1964; Tucker and Giese, 1962; Selwood, 1968; Anderson, 1969) and that the gonad index data accurately reflected the buildup of gametes. In T. lineata gametogenesis is a continual process and the gonads do not go through a rest period. The next year's gametes make their appearance in the gonads immediately after spawning. Although the gonad indices showed wide ranges in some cases, the sections confirmed that the populations within each of the three regional study areas were quite synchronous in the timing of gametogenic events. This synchrony produces the well defined annual reproductive cycle found in T. lineata.

The lobate stage seen during oogenesis in the gonads of T. lineata has also been reported for Cryptochiton stelleri (Tucker and Giese, 1962), Katharina tunicata (Nimitz, 1964), and Mopalia muscosa (Anderson, 1969). Anderson (1969) has shown that the protuberances

or lobes are made up of cytoplasm from the egg which are covered by flattened follicle cells. The lobes probably play a role in chorion formation as eggs at the end of the lobate stage are enclosed with a fully-formed chorion.

Nimitz (1964) has found that in Katharina oogenesis is a two year process with the oocytes reaching a diameter of 35 to 50 μ in the first season and approximately 175 μ at the end of the second year. It is not clear whether or not the same phenomena occurs in T. lineata. In T. lineata some of the next year's oocytes are discernible about the time of spawning, although it could not be determined whether or not these newly differentiated oocytes have been present for some time but are too small to be detected by the methods of this study. Also at this time the majority of the germinal epithelial cells are very small and the ones that will form oogonia are indistinguishable from those that will form follicle cells. Differentiation of the next year's oocytes becomes quite evident immediately after the present year's are spawned.

Latitudinal Variation in Reproductive Activity

As shown by this study, the timing of the reproductive activity of Tonicella lineata varies with latitude, a phenomenon that is quite common in marine invertebrates (Giese, 1959). At 44.39° N (Newport, Oregon) spawning took place about 1 April in 1968 and in 1969 and at

48.33° N (Friday Harbor, Washington) spawning started at the end of May and continued into June in 1969. Histological comparisons also showed that gametogenic events in the central Oregon coast animals were 30 to 45 days ahead of those in the San Juan Island animals.

The reason why T. lineata breeds earlier in the southern end of its range is not known. Orton (1920) proposed that temperature was the most important factor in regulating breeding in marine animals and that under normal conditions each species has a rather constant temperature that triggers breeding throughout its range. Since temperature data for the San Juan Island study sites is not available for the time period of this study, a valid comparison of the temperature regimes of the central Oregon coast and San Juan Island cannot be made.

Orton's hypothesis of constant breeding temperature throughout a latitudinal range does not hold up for all species and has been criticized by Korringa (1957) as being too general. What environmental or genetic factors cause this latitudinal variation within Tonicella are undetermined. As Sastry (1970) found, populations of the bay scallop Aequipecten irradians from Woods Hole, Massachusetts, and Beaufort, North Carolina, vary in their timing of reproductive events. He pointed out that further information will have to be obtained to determine whether variation in annual

reproductive activity is due to environmental or genetic acclimation. Korringa (1957) and Loosanoff (1969) have shown that there are distinct groups of physiological races throughout the range of the American oyster Crassostriea virginica, each requiring different temperatures for gonad development and successful spawning.

Control of Annual Reproductive Cycle

According to Giese (1959) control of the annual reproductive cycle events in marine invertebrates is probably explainable in three ways:

> It could be that the result of a series of endogenous or internal events which build up inside of the organism, or it could be the result of the operation of various exogenous factors of the environment such as temperature, light, salinity, foods available, etc. acting upon a rather plastic organism, or it could be a combination of both.

Although the control of the annual cycle of T. lineata is probably by one of the above listed methods, the exact one is not known. We can, however, speculate on how the known exogenous factors of temperature and food availability might affect the reproductive cycle of T. lineata.

Temperature

Of all the exogenous factors that might possibly affect an annual reproductive cycle, temperature is the one most studies have centered

upon (Giese, 1959). In their review of the literature on temperature and annual reproductive cycles, Hedgpeth and Gonor (1969) cite studies showing that temperature is probably the most important factor "in synchronizing reproductive periodicity with the seasons." Studies on the effects of temperature fell into two main categories:

1. Experimental--where the temperature is manipulated in the laboratory or by transplantation of animals along latitudinal differences in temperature.
2. Correlation of reproductive activity data (gonad index, etc.) with environmental temperatures.

All data found in the literature on chitons fall into the latter category (Tucker and Giese, 1962, and Webber and Giese, 1969). The temperatures listed in these studies, like those found for most studies of this type, are monthly or weekly shore station surface temperatures--which are frequently collected from areas other than the study site (Hedgpeth and Gonor, 1969).

In studying Cryptochiton stelleri in the Monterey, California area, Tucker and Giese (1962) report that rapid growth of the gonads is correlated with declining autumn temperatures, but make no other statement concerning the effect of temperature on the reproductive cycle. Webber and Giese (1969) compared ten years of gonad index data (1956-1966) for Katharina tunicata with water temperatures for possible correlation. The hypothesis they examined was that a

continual rise in water temperature controls gonad development. They concluded that:

> From the relationship between water temperature and gonad growth for Katharina tunicata, there is little evidence to support the hypothesis that seasonal increase (or decrease) in water temperature controls gonad growth.

Hedgpeth and Gonor (1969) recommend that such conclusions based on periodic temperature measurements be rejected. They also point out that the only meaningful temperature curve for use in correlation with reproductive cycles is the one for the intertidal level at which any given organism occurs. The type of correlation made by Webber and Giese (1969) does not consider the fact that Katharina is an intertidal animal and is exposed to air temperatures part of the time. Along the central Oregon coast, Katharina is very abundant at a level four feet above MLLW. This level at the Oregon State University dock at Newport, Oregon, is exposed 41 percent of the time. Gonor (1968) reported on June 14, 1968, that 30 Katharina were found to have a mean internal temperature of 26.9° C (range 21.5 to 30.2). The sea water temperature at this time was 14.4° C. Comparing water temperatures with reproductive activity would only be meaningful for subtidal and lower intertidal animals that are rarely uncovered. The temperature curves presented here with the T. lineata gonad index data are probably more applicable and meaningful than those for Katharina because T. lineata occurs in the

lower intertidal. However, T. lineata occurs high enough in the intertidal to be exposed up to 20 percent of the time and reach internal temperatures of 18 to 26° C in summer. On some days, probably in the spring and summer, these animals would experience in a few hours a temperature range of 16° C if the sea water temperature at the time was 10° C. Webber and Giese (1969) say:

> It is possible that on the Pacific Coast annual temperature fluctuations are too small to control gametogenesis (for example at the study site the seasonal temperature fluctuation was 5° C).

Although 5° C represents the measured fluctuation of the water temperature, this does not represent the internal temperature fluctuation within Katharina tunicata.

In examining water temperature and gonad index data of T. lineata for the central Oregon coast (Figure 9), it is interesting to note that gametogenic activity for both years was increasing while the water temperature was dropping and continued to increase while the water temperature increased. Gametogenic activity then takes place at both high and low water temperatures. Mitosis, initiating the production of the next year's gametes, and spawning took place at still higher temperatures. Some sort of system might be in effect in which a high temperature triggers mitosis but yolk synthesis requires a lower temperature.

Although water temperature probably plays a role in regulating

the annual reproductive cycle of T. lineata, its exact role can be found only through temperature control experiments in the laboratory.

Nutrition

It would seem that a constant source of food is available to T. lineata throughout the year, even though its caloric value is low. Paine and Vadas (1969) found no significant shifts in seasonal caloric values for the coralline algae they examined. The amount of diatom intake of T. lineata varies due to seasonal variations in diatom abundance and may be an impostant factor in providing nutrients for use in the annual reproductive cycle. This effect may be quantitative, since Nitzschia paradoxa, an intertidal diatom, has a caloric value of 3.28 kcal/g dry weight (Paine and Vadas, 1969), or qualitative, providing factors absent or in low supply in coralline algae.

Although diatoms are probably present throughout the year, the greatest diatom cover in the intertidal range of T. lineata occurs during the spring and summer. This increase in available energy comes at a time when gametes are being produced and presumably much of the energy provided by the diatoms would be used for gamete formation. Lawrence et al. (1965) reported that the digestive gland of Katharina tunicata probably functions as a nutrient storage organ and attains maximum size in the fall, decreasing to its minimum size in the spring when the gonad index reaches its highest values. The

buildup of nutrients within the Katharina digestive gland takes place when the gonad is in an essentially resting phase (Nimitz, 1964). Whether or not a similar relationship between gonad growth and digestive gland growth exists in T. lineata is not known. The gonad of T. lineata might require a more constant source of energy throughout the year since there is no resting phase in the production of gametes as found in Katharina.

In examining the effect of starvation on gametogenesis in Katharina, Nimitz (1964) found that starved animals had smaller gonads than field animals and thus produced fewer gametes. She could not distinguish histologically and histochemically between the gonads of starved and non-starved animals. Her histochemical studies indicated that foot and mantle nutrient reserves were used extensively during starvation.

Since food is continuously available to T. lineata and the guts were always full, the amount of food taken in would not seem to be important in regulation of the reproductive cycle. The qualitative changes in food as diatoms become a major food item may be important, however. This sudden increase in available energy might act as a trigger to gamete production. It would be interesting to examine gamete production in T. lineata that have eaten only coralline algae and compare it with production in animals on a normal diet of coralline and diatoms to see if a pattern emerged similar to that for

starved and non-starved Katharina.

Spawning Behavior

The spawning behavior of Tonicella lineata conforms to the pattern described for other chiton species (Hyman, 1967) except, of course, those that brood their eggs in the mantle cavity. Brooding of eggs by the genus Tonicella has been reported by Yakovleva (1952), but was not observed for T. lineata during this study. Thorson (1949) has reported that Ischnochitron cinereus has been found to both spawn gametes freely into the water and also to retain eggs in the mantle cavity where they develop. The period of movement prior to spawning that I observed in one female has also been reported for other chitons (Metcalf, 1892; Brewin, 1942). The duration of spawning, the clumping of the eggs by a mucus secretion, and the characteristic girdle lift have all been described for other chitons (Hyman, 1967).

The stimulus which initiated spawning in T. lineata is not known. A lunar periodicity of spawning for some chitons has been reported (Grave, 1922; Stephenson, 1934; Brewin, 1942; Glynn, 1970). Grave (1922) reports that Chaetopleura apiculata shows its greatest spawning activity at the approach of full moon during the months of June, July, and August and continues until two to three days after new moon. Between these peak periods, low spawning

activity was also exhibited. Acanthozostera gemmata, found on the Great Barrier Reef, were placed in jars anchored in the field by Stephenson (1934), who observed that on seven of eight times in the morning following the night of the full moon the animals had spawn in with them. This species was observed to spawn at the time of full moon between August and April with the maximum spawning in December. Brewin (1942), working with Cryptoconchus porosus in New Zealand and Chatham Island, observed spawning five times in two and a half months with spawning always starting at full moon. The average time between spawning was 15 days--the time between full and new moon. Glynn (1970) has shown that spawning in Acanthopleura granulata occurs in phase with the new and full moon.

Whether or not T. lineata shows a lunar cycle cannot be resolved from the small amount of data I have collected (Table 13). Although the data for 1968 and 1969 show some correlation between spawning and the lunar phase and tidal height, they are not definite and only indicate that further investigation might be worthwhile.

If the height of the tide plays a role in the time that T. lineata spawns, it would be advantageous for it to spawn at the time of a high tide. Chitons which spawn eggs in thick mucus strings, such as Ischnochiton heathiana (Heath, 1898), may not require a water covering at the time of spawning as the strings would provide some

protection for the eggs, but chitons like T. lineata which enclose their eggs in little mucus would benefit by a water covering at the time of spawning. Also, it is questionable if the spawning process could take place out of water.

A tidal rhythm has been reported for two chitons (Johns, 1960; Thrope, 1962). In 1959 Sypharochiton pelliserpentis, a New Zealand species, spawned three times during February, March, and April (Johns, 1960). Laboratory spawnings coincided with field spawnings. The time of spawning was found to occur on a rising or falling tide near the time of high water, usually on high spring tides when these occurred during the early evening. Johns also feels the lunar phase may play a role and that the trigger of spawning is due to an interaction between the lunar phase and the tidal cycle. Thorpe (1962) presents data to show that both sexes of Mopalia ciliata along the central California coast release their gametes during the low high tide of the day.

It has been stated many times that spawning in female chitons is preceded by the spawning of the males and that the seminal fluid initiates the spawning of the female (Hyman, 1967). Heath (1898, 1905) was the first to report this from his work with Ischnochiton heathiana, I. mertensii, I. cooperi, Mopalia lignosa, M. muscosa, and Katharina tunicata. Other chitons reported to show this phenomenon are Lepidopleurus asellus (Christiansen, 1954), Sypharochiton

pelliserpentis (Johns, 1960), and Cryptochiton stelleri (Okuda, 1947).

My observations on T. lineata show that it is not necessary for females of this species to be in contact with seminal fluid of the male before they will spawn. Females of Cryptoconchus porosus (Brewin, 1942) and Chaetopleura apiculata (Grave, 1922) also do not require the presence of seminal fluid. Females of seven species of the genus Mopalia (including the two listed above observed by Heath) and Ischnochiton mertensii, also observed by Heath as requiring the male to spawn first, will spawn when isolated from males (Thorpe, 1962). The other species reported in which the male is reported to spawn first should also be reinvestigated.

Development

The development of Tonicella lineata is similar to that described for other chitons (Kowalvesky, 1892; Heath, 1898; Christiansen, 1944; Thorpe, 1962). The major new information provided by this study is on the differentiation of the pretrochal region and the division of development into distinct periods before and after settling.

The developmental rate in T. lineata is temperature dependent, as has been found for other chiton species by Costello et al. (1957), Hoffman (1931), and Thorpe (1962). Development times of T. lineata cannot be compared with those given for other chitons because of the lack of time-temperature data in the literature.

Events at fertilization have never been described in detail for any chiton. The space which forms between the chorion and the egg proper at the time of fertilization was said by Southwick (1939) to be caused by shrinking of the egg cytoplasm. Since there does not seem to be any membrane elevation or new membrane formation at the time of fertilization, shrinking of the egg cytoplasm seems to be the most plausible explanation. Anderson (1969) found that the eggs of Chaetopleura apiculata do not show a cortical reaction at the time of fertilization. He found cortical granule-like bodies in the outer cytoplasm, but could not determine their function.

Cleavage, gastrulation, and blastopore migration in T. lineata is the same as has been found in other chitons (Heath, 1898; Hammerstam and Runnstrom, 1925; Hoffman, 1931). The pointing of the prototrochal cilia anteriorly while the larvae is still enclosed within the chorion and the observation that the prototrochal ring is composed of two cell sizes have not been reported previously in the literature. Okuda (1947) mentions that the anterior row of prototrochal cilia in Cryptochiton larvae is shorter than the posterior row, as is the case in the larvae of T. lineata.

The formation of ciliated apical knobs and ciliated elevated ridges at the apical plate area of the pretrochal region has not been observed for any other chiton. Heath (1898) shows a similar looking granular region of cells on the apical region of the larvae of Ischnochiton heathiana as found in the larvae T. lineata before the knobs and ridges develop. He interpreted that region to be the anlagen of the cerebral ganglia and did not mention anything about this area

differentiating into structures resembling the apical knobs and the elevated ridges of T. lineata. It is not known whether these knobs and ridges contribute to the formation of the nervous system in T. lineata as the fate of these bodies could not be followed after they disappeared externally. Hyman (1967) summarizes what is known concerning the formation of the apical sensory organs and the nervous system in chitons:

> The four apical cells give rise to the apical sensory organ bearing the apical tuft. The definitive nerve cells are proliferated from the internal surface of the four rosette cells, products of the first quartet. The cerebral part of the nervous system is thus also quadripartite at its inception but the cells soon rearrange to become the cerebrobuccal ring. Continued proliferations from the same source produce the lateral and pedal cords.

If the knobs and ridges do contribute to the nervous system, their mode of doing so, an external proliferation and then a sinking, would be different from that described for any other chiton.

The knobs and ridges may be larval organs only. Smith (1935) describes structures similar to the apical knobs of Tonicella in the apical region of the larvae of the limpet Patella where two occur, one on each side of the apical tuft and each made of one cell. They bear fine, stiff hairs and have an optically refractive character. Smith states: "There can be little doubt that they carry out some sensory function in the larvae, but they do not persist for more than a day after larval torsion." These bodies do not contribute to the nervous system of Patella. Boutan (1899) found similar bodies present on the apical region of the gastropod limpet Acmaea. The bodies

described for Patella are fewer in number and differ from those in Tonicella in having stiff, fine hairs instead of motile cilia. Although the elevated ridges migrate down towards the position of the larval eyes, the eyes are usually distinct before the ridges disappear.

The two anal tufts of cilia cannot be considered a telotroch as they are nonmotile stereocilia and do not encircle the embryo.

The developmental events that take place after hatching, elongation of the post-trochal region, larval eye and shell gland formation, differentiation of the foot and girdle, and spicule formation, are similar to the accounts already in the literature and summarized by Hyman (1967). After settling, the larvae of T. lineata undergo a rapid and drastic metamorphosis. Christiansen (1954) feels that chitons do not undergo a metamorphosis but instead have only a gradual change from the free-swimming stage to a young adult animal.

The timing and nature of shell formation in Tonicella lineata differs somewhat from that reported for Ischnochiton heathiana (Heath, 1898), Chaetopleura apiculata (Grave, 1932), Cryptochiton stelleri (Okuda, 1947), and Mopalia ciliata (Thorpe, 1962) where shell plate formation starts while the larvae is still in the free-swimming stage. Christiansen (1954) does not make it clear if shell formation starts in the free-swimming stage of Leipidopleurus asellus or not. In T. lineata shell formation does not begin until

after settling takes place and not in the free-swimming stages as reported for other chitons. Even in those chitons reported to have short free-swimming stages (Acanthochiton discrepans, 12-24 hrs, Hammerstam and Runnstrom, 1925; Trachydermon cinercus, at least 6 hrs, Hoffmann, 1931; Cryptochiton stelleri, 12-20 hrs, Okuda, 1947; Ischnochiton heathiana, 15 minutes to 2 hrs, Heath, 1898) shell formation was observed to take place in this period.

Basically, the development of T. lineata closely follows the developmental patterns reported for other chitons except for the differentiation of the apical region of the early trochophore larva and the stopping of further development until settling takes place. Settling in T. lineata appears to initiate a separate set of developmental events including sequential loss of the prototroch and apical tuft, the flattening of the pretrochal region, and finally formation of the shell. In chitons which do not show a settling preference, the sequence of developmental events is different. Shell formation takes place in the free-swimming stages before the prototroch and apical tufts are lost. Development in Tonicella can then be divided into distinct before settling and after settling periods.

Larval Settling

T. lineata larvae show a selective settling response. Of all substrates tested, settling and metamorphosis took place only on

encrusting coralline algae and most readily on the genera Lithothamnion and Lithophyllum. The specific stimulus is probably chemical rather than tactile. The settling is accompanied by a relatively swift metamorphosis which includes change in shape and color, development of shell plates and loss of apical tuft and prototroch. These changes take place within six hours after the larvae are placed in with encrusting coralline. A clumping pattern of settled larvae was observed in many cases although many were randomly scattered. Once the animals reach the stage of development which permits settling, no further external developmental changes take place until the settling stimulus is received. The time period of larval responsiveness to the settling stimulus may be as early as 110 hours or four and a half days for larvae raised at 12 to 13° C. Absence of the stimulus for several days (approximately 18 to 20 days at 10° C) results in larval deterioration and death.

These observations on a selective settling response and the delay of metamorphosis by the larvae of T. lineata are the first to be reported for an amphineuran. The larvae of Ischnochiton heathiana may have a similar specific response since Heath (1898) reported that the larvae would metamorphose only on Ulva or "water worn shells" of Mytilus.

Specific settling responses and delay of metamorphosis had been described for other molluscs, particularly bivalves, as

discussed by Bayne (1964). There have been only five observations of this phenomenon in gastropods. Thompson (1964), Tardy (1962), and Swennen (1961) report that the veligers of certain nudibranchs will metamorphose only when they come in contact with the preferred food of the adult. Scheltema (1961) reported that the veliger larvae of Nassarius obsoletus show the highest percentage of settling when substratum taken from where the adults live is available. Hadl et al. (1970) have found that the opisthobranch Microhedyle milaschewitchii requires an optimum sand grain size (0.5 to 2 mm) and a micro-organism fauna on the grains for settlement to take place.

The specific settling response of the T. lineata larvae provides immediate access to the preferred food and substrate upon which the adults live. As Crisp (1965) points out, the larvae of many species of marine invertebrates have been shown to settle in response to highly specific materials, many of which are the preferred food of the adult. The adaptation to obligatory feeding on encrusting coralline algae, which has a wide range and commonly covers large intertidal areas provides T. lineata with an almost unlimited source of food which even though it may be low in calories in comparison to other algae (Paine and Vadas, 1970) does not fluctuate in quantity as much as soft species which show strong seasonal changes in growth and which are torn by winter storms.

Because the larvae of T. lineata showed a strong all or none

preference in their settling response in early experiments, only simple choice experiments were used. Crisp (1965) points out that when a preference is strong, it may be unnecessary to take precautions to eliminate extraneous environmental factors (Crisp and Meadows, 1962, and Ryland, 1959).

The nature of the settlement stimulus that Tonicella larvae receive from the coralline was not conclusively determined but the results indicate that it is probably a chemical stimulus. Gee (1965) found polychaete Spirobis rupestris larvae will settle only on the encrusting coralline Lithothamnion polymorphum, but would settle on plain roofing tile soaked in aqueous extracts of L. polymorphum. Similarly, the larvae of T. lineata would settle on roofing tile only after it had been soaked in coralline extract. Gee (1965) found, as I did, that the factor from the algae was made inoperable by boiling.

As with other larvae (Crisp, 1965) it is probably necessary for the T. lineata to come into direct contact with the substrate to receive the settling stimulus. This contact is facilitated by development during the pelagic phase of a positive response to gravity and a searching activity of alternate periods of swimming and crawling. A similar behavioral pattern for Spirorbis has been described by Knight-Jones (1959). Heath (1898) reports a similar stage for Ischnochiton heathiana just prior to settling. As pointed out by Bayne (1964), this stage is usually associated with exploratory

behavior which facilitates larval contact with the settlement initiating stimulus. A physiological basis for chemical attraction of small larvae at a distance is hard to envision (Crisp, 1965). As the boundary layer theory states, "When water flows over a surface, such as currents over coralline algae, a substance diffusing from the surface forms only a very thin layer in which there is an appreciable concentration" (Crisp, 1965). As the apical tuft and cells are the first area of T. lineata larvae to contact the coralline, these might play a role in the reception and recognition of a chemical stimulus which promotes settling. This study of Tonicella confirms Wilson's (1952) comment that it has been easier to demonstrate that metamorphosis can be delayed and that larvae can choose a particular substratum than to determine the environmental stimulus to which the larvae respond or the mechanism by which this recognition takes place.

The settling stimulus also plays an important role in controlling developmental timing of T. lineata and other marine animals (Thorson, 1949, 1950; Bayne, 1964, and Reese, 1964). In T. lineata, development ceases from the time the larvae start seeking the settling stimulus until settling occurs. All other accounts of chiton embryology, except that of Heath (1898), record that metamorphosis and further development take place without benefit of a settling stimulus.

The clumping phenomenon observed for some T. lineata at the time of settling has also been observed by Heath (1898) for

Ischnochiton heathiana. He mentions that certain places on Mytilus shells and Ulva were closely packed with settled larvae while some areas nearby were entirely devoid of them. Whether or not T. lineata exhibit a true gregariousness is undetermined as some areas of the coralline may provide a stronger stimulus than others. Also it is possible that crawling activity brings the larvae together after settlement. Since adults are not sessile and do not have internal fertilization, the significance of a clustered distribution at settlement is not clear.

T. lineata larvae show responsiveness to the coralline settling stimulus in laboratory cultures for 14 to 15 days at 10 to 12° C, after which time they begin to show signs of degeneration. The period of responsiveness in nature is not known.

The ecological significance of a long period of settling stimulus receptivity has been discussed by Thorson (1950). He points out that if currents carry the larvae over wide areas, there would be little wastage of larvae. Bayne (1965) considers that an ecological advantage of this prolonged period of receptivity is that this ability must greatly enhance the chance of the larvae encountering suitable substrates for metamorphosis and future development.

Since lecithotrophic larvae such as Tonicella do not feed, they can remain active seekers only as long as yolk reserves were present. Thus planktotrophic larvae of other molluscs such as the veligers of

gastropods and bivalves would probably be able to maintain longer periods of receptivity in nature. Bayne (1964) has shown that the planktotrophic veliger larvae of Mytilus edulis are capable of metamorphosis any time during a five to six week period at a temperature of 10° C, but for only two days at 20° C. After this time the larvae begin to evidence signs of degenerating, the major sign being the gradual degeneration of the velum. Temperature would probably have a similar effect upon the length of time during which larvae of T. lineata are capable of being receptive to a settling stimulus.

The actual time when the larvae of T. lineata become ready to settle is undetermined, but is probably at that point when no further external development can be observed in the free-swimming larvae. At 10° C this point was reached at around 160 hours, whereas at 12° to 13° C it was reached at 110 to 120 hours. This stage of development is accompanied by a behavioral change in locomotor habits. Originally the larvae spend much of their time swimming throughout the beaker, but after development stops they begin alternating between crawling or swimming near the bottom of the beaker. This stage when the larvae can both swim and crawl would be similar to the pediveliger stage of bivalves (Carriker, 1961). The larvae of Mytilus edulis first become capable of metamorphosis at this pediveliger stage (Bayne, 1964) and the same would probably hold true for the larvae of T. lineata.

Although the early pelagic stage of T. lineata closely follows the major larval settling behavior pattern found in many phyla (Reese, 1964), most pelagic phase larvae have been reported as being photo-positive. Preliminary observations on T. lineata are inconclusive, but suggested no response to light in their early pelagic phase. The larvae of Cryptochiton stelleri were found to show no response to light during their pelagic phase (Okuda, 1947).

SUMMARY

1. Along the central and southern Oregon coast and on San Juan Island, Washington, the lined chiton Tonicella lineata is very abundant in the lower intertidal levels on rocky shores.

2. T. lineata is usually found on encrusting coralline algae which, with epiphytic diatoms, make up the major portion of its diet.

3. On the central and southern Oregon coast, T. lineata are closely associated with the purple urchin Strongylocentrotus purpuratus and are often found in the burrow of the urchin. This association, together with algal cover, protects T. lineata from desiccation during periods of tidal exposure, whereas on San Juan Island the lack of an intertidal urchin reduces the diversity of habitats available to Tonicella.

4. The asteroids Pisaster ochraceous and Leptasterias hexactis are the most common predators of T. lineata.

5. The average growth of twelve T. lineata studied for ten and one-half months was 0.40 cm (range 0.06 to 0.88 cm) with great variation among animals 2.0 to 3.0 cm in body length.

6. Some T. lineata exhibit a homing behavior.

7. The reproductive cycle was studied by determination of a monthly gonad index and examination of gonad histology. In 1968 and 1969 Tonicella lineata showed a distinct annual

reproductive cycle along the central Oregon coast, spawning between 1 April and 15 April in 1968 and during the middle of April in 1969. Southern Oregon coast data is inconclusive, but spawning in 1968 and 1969 probably occurred in February or March. In 1968 and 1969 spawning took place during May and June on San Juan Island. Tonicella lineata thus shows a latitudinal difference in the timing of its annual reproductive cycle.

8. Histological examination of the gonads revealed a close correlation of gamete buildup and release with gonad index changes. Gamete production starts immediately after spawning. The gonads of T. lineata do not go through a resting period as has been observed for the chitons Katharina tunicata, Mopalia hindsii and Cryptochiton stelleri.

9. Comparison of gonad index and histological data with sea surface temperature data shows that gametogenic activity increases while the water temperatures are dropping.

10. Spawning behavior is described and data are presented which suggest that there may be a correlation between the time of spawning and the lunar phase.

11. The development of Tonicella lineata is described from the time of fertilization through metamorphosis. The features described follow closely those which have been described for

other chitons except for the description of previously unnoted structures on the pretrochal region of the trochophore larva.

12. In laboratory cultures the development of the trochophore stops between 110 and 160 hours post-fertilization, depending upon the temperature. Further development will take place only when the larvae are presented with the proper settling substrate of encrusting coralline algae.

13. In substrate choice experiments, T. lineata larvae settle only on encrusting coralline algae and on pieces of tile previously soaked in an encrusting coralline algae extract.

14. After settling, the trochophore larvae go through a drastic metamorphosis within twelve hours, losing the apical tuft and prototroch, taking on the shape of a small chiton, and developing seven shell plates. When 30 days old the young chitons have a fully developed radula and feed on encrusting coralline algae.

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APPENDICES

Table 1. Results of growth studies on marked Tonicella lineata from a high tidepool, Yaquina Head, Oregon. Forty eight animals were marked on 30 April, 1968 and 12 were recovered on 13 March, 1969.

Initial total length in centimeters	Final total length in centimeters	Total growth in centimeters
2.17	2.76	.59
2.33	2.79	.46
2.38	2.70	.32
3.16	3.47	.31
1.96	2.84	.88
2.37	3.03	.66
2.50	2.70	.20
2.57	2.98	.41
1.44	1.75	.31
2.75	2.81	.06
2.46	2.85	.39
2.14	2.36	.22

Mean Growth = .40 centimeters/11 months.

Table 2. Gonad index data for T. lineata from the Central Oregon Coast, Yaquina Head.

Year	Date	Place	Sample size	Mean Gonad Index	± 2 S. E.	Gonad Index Range
1967	Aug. 23	YH	30	3.03	3.38-2.67	4.0-0.7
1967	Sept. 5	YH	18	4.15	4.69-3.62	5.8-2.3
1967	Oct. 3	YH	20	4.98	5.56-4.40	8.0-2.8
1967	Nov. 4	YH	20	6.31	7.19-5.42	9.0-2.3
1967	Dec.	NO SAMPLE				
1968	Jan. 12	YH	14	7.52	8.47-6.56	9.8-4.7
1968	Feb. 13	YH	15	9.88	11.25-8.50	12.3-4.5
1968	Mar. 14	YH	12	9.00	10.31-7.68	12.8-5.5
1968	Apr. 1	YH	15	10.89	12.21-9.57	13.8-7.3
1968	Apr. 15	YH	24	1.74	2.03-1.44	3.5-0.6
1968	Apr. 19	SH	15	1.10	1.47-0.73	2.2-0.2
1968	Apr. 29	YH	15	0.96	1.26-0.65	2.2-0.4
1968	May 16	BB	15	1.12	1.43-0.81	2.0-0.2
1968	May 28	YH	20	2.09	2.38-1.79	3.7-0.9
1968	June 29	YH	15	2.14	2.56-1.71	3.9-1.0
1968	July 10	YH-LA	10	4.37	4.82-3.92	5.5-3.1
1968	July 25	YH	15	2.99	3.49-2.48	4.9-1.5
1968	Aug. 22	YH	15	3.74	4.69-2.78	7.2-1.2
1968	Sept	NO SAMPLE				
1968	Oct. 21	YH	14	3.95	4.75-3.16	6.7-1.6
1968	Nov. 20	YH	15	6.53	7.49-5.57	10.3-3.2
1968	Dec.	NO SAMPLE				
1969	Jan. 17	YH	20	9.21	10.18-8.24	13.2-4.0
1969	Feb. 15	YH	16	8.78	10.22-7.35	14.0-4.2
1969	Mar. 15	YH	10	10.60	13.12-8.07	14.1-6.1
1969	Apr. 19	YH	11	2.90	3.56-2.23	4.5-1.4
1969	May 18	YH	14	2.32	3.01-1.64	4.9-0.6
1969	June 28	YH	15	1.90	2.32-1.48	3.4-0.9

YH = Yaquina Head; YH-LA = Yaquina Head-Low Animals; BB = Boiler Bay; SH = Strawberry Hill

Table 3. Gonad index data for T. lineata from Southern Oregon Coast, Cape Arago, Oregon.

Year	Date	Place	Sample size	Mean Gonad Index	± 2 S. E.	Gonad Index Range
1968	Apr. 17	CA	30	3.83	4.46-3.19	7.0-2.0
1968	May 14	CA	18	2.50	3.02-1.97	5.8-1.2
1968	June 11	CA	17	1.48	1.90-1.06	2.8-0.5
1968	July 12	CA	10	2.11	2.78-1.43	3.3-0.8
1968	Aug. 7	CA	15	3.88	4.33-3.42	5.0-2.0
1968	Nov. 24	CA	15	8.34	9.47-7.20	11.3-2.7
1969	Jan. 5	CA	13	8.51	9.55-7.47	11.8-6.4

CA = Cape Arago

Table 4. Gonad index data for T. lineata from San Juan Island, Washington.

Year	Date	Place	Sample size	Mean Gonad Index	± 2 S. E.	Gonad Index Range
1968	Mar. 20	DMB	22	6.89	8.27-5.50	12.0-3.7
1968	Mar. 20	ER-SUB	11	5.22	6.65-3.79	8.0-2.0
1968	May 23	CP	14	5.22	7.12-3.32	11.8-1.3
1968	Sept. 4	DMB	15	3.44	4.18-2.70	5.9-1.9
1968	Nov. 19	TI	15	4.18	4.89-3.46	7.5-2.0
1968	Nov. 20	CP	22	3.37	4.15-2.58	7.1-1.1
1968	Nov. 20	SJP	21	3.90	4.49-3.32	6.5-1.3
1969	Mar. 2	SJP	15	5.80	6.74-4.85	9.3-1.3
1969	Apr. 5	SJP	13	7.53	8.67-6.39	10.0-4.8
1969	May 18	SJP	15	7.59	8.57-6.60	10.9-5.2
1969	June 1	SJP	15	4.07	5.00-3.13	9.6-2.7

DMB = Deadman's Bay; ER-SUB = Edward's Reef - subtidal; CP = Cattle Point; TI = Turn Island; SJP = San Juan Park

Table 5. Mean length of T. lineata used in gonad index samples from the Central Oregon Coast.

Year	Date	Place	Sample size	Mean length of sample in centimeters	Standard Deviation
1967	Aug. 23	YH	30	2.88	.378
1967	Sept. 5	YH	18	2.82	.389
1967	Oct. 3	YH	20	2.87	.306
1967	Nov. 4	YH	NO MEASUREMENTS		
1967	Dec.	NO SAMPLE			
1968	Jan. 12	YH	14	2.82	.367
1968	Feb. 13	YH	NO MEASUREMENTS		
1968	Mar. 14	YH	12	3.13	.242
1968	Apr. 1	YH	NO MEASUREMENTS		
1968	Apr. 15	YH	24	2.76	.490
1968	Apr. 19	SH	15	2.47	.314
1968	Apr. 29	YH	15	2.54	.387
1968	May 16	BB	15	2.23	.196
1968	May 28	YH	20	2.67	.367
1968	June 29	YH	15	2.61	.186
1968	July 10	YH-LA*	10	3.94	.451
1968	July 25	YH	15	2.84	.208
1968	Aug. 22	YH	15	3.00	.381
1968	Sept	NO SAMPLE			
1968	Oct. 21	YH	14	2.76	.153
1968	Nov. 20	YH	15	2.82	.221
1968	Dec.	NO SAMPLE			
1969	Jan. 17	YH	20	2.88	.401
1969	Feb. 15	YH	16	3.04	.494
1969	Mar. 15	YH	10	3.42	.489
1969	Apr. 19	YH	NO MEASUREMENTS		
1969	May 18	YH	NO MEASUREMENTS		
1969	June 28	YH	15	2.99	.324

Total Samples = 20
Mean Length = 2.81
Standard Error = .0576

YH = Yaquina Head
BB = Boiler Bay
SH = Strawberry Hill
*LA = Lower Sample

Table 6. Mean length of T. lineata used in gonad index samples from Southern Oregon, Cape Arago.

Year	Date	Place	Sample size	Mean length of sample in centimeters	Standard Deviation
1968	Apr. 17	CA	30	2.85	.343
1968	May 14	CA	18	2.98	.306
1968	June 11	CA	17	2.67	.297
1968	July 12	CA	10	2.85	.585
1968	Aug. 7	CA	15	3.37	.355
1968	Nov. 24	CA	15	3.28	.278
1969	Jan. 5	CA	NO MEASUREMENTS		

Total Samples = 5

Mean Length = 3.00

Standard Error = .0972

Table 7. Mean length of T. lineata used in gonad index samples from San Juan Island, Washington.

Year	Date	Place	Sample size	Mean length of sample in centimeters	Standard Deviation
1968	Mar. 20	DMB	NO MEASUREMENTS		
1968	Mar. 20	ER-SUB	NO MEASUREMENTS		
1968	May 23	CP	NO MEASUREMENTS		
1968	Sept. 4	DMB	15	3.45	.423
1968	Nov. 19	TI	15	2.99	.377
1968	Nov. 20	CP	22	3.66	399
1968	Nov. 20	SJP	21	3.31	.399
1969	Apr. 5	SJP	NO MEASUREMENTS		
1969	Apr. 5	SJP	13	3.08	.330
1969	May 18	SJP	15	3.62	.302
1969	June 1	SJP	15	3,53	3.64

Total samples = 7
Mean length = 3.37
Standard Error = .0881

DMB = Deadman's Bay
ER-SUB = Edward's Reef - subtidal
CP = Cattle Point
TI = Turn Island
SJP = San Juan Park

Table 8. Analysis of sperm production from histological sections for Yaquina Head T. lineata.

Date	Amount of Sperm Production
Aug. 1967	Patchy along lamellae, equal areas without
Sept.	Same as August, 1967
Oct.	Large areas along lamellae, all have areas without
Nov.	Almost continuous along lamellae, areas without are rare
Dec.	----------No Sample----------
Jan. 1968	Same as November 1967, Lumen of testes full of sperm
Feb.	Continuous sperm production along lamellae, areas without very rare
Mar.	Same as February 1968
Apr.	Same as March 1968
May	Patchy along lamellae, Lumen only partially full
June	Occasional small pockets, large areas without
July	Same as June 1968
Aug	Medium size pockets quite common
Sept	----------No Sample----------
Oct	----------No Sample----------
Nov	Same as November 1967
Dec	----------No Sample----------
Jan. 1969	Same as November 1967
Feb.	Continuous sperm production along lamellae, areas without are very rare
Mar.	Same as February 1969. Lumen of testes full of sperm
Apr	Same as February 1969. Lumen as in March, 1969
May	Very patchy, long continuous areas without--Lumen with little sperm
June	Same as May 1969

Table 9. Analysis of sperm production using shape of epithelia cells for Yaquina Head T. lineata. As sperm production increases epithelial cells change shape from cuboidal to flat.

Date	Shape of Epithelial Cells	Approximate amount of each shape	
Aug. 1967	Cuboidal and flat	1/2 Cuboidal	1/2 Flat
Sep.	Cuboidal and flat	1/2 Cuboidal	1/2 Flat
Oct.	Cuboidal and flat	1/2 Cuboidal	1/2 Flat
Nov.	Cuboidal and flat	1/4 Cuboidal	3/4 Flat
Dec.	No Sample		
Jan. 1968	Flat	All Flat	
Feb.	Flat	All Flat	
Mar.	Flat	All Flat	
Apr.	Flat	All Flat	
May	Cuboidal and flat	1/2 Cuboidal	1/2 Flat
June	Cuboidal and flat	3/4 Cuboidal	1/4 Flat
July	Cuboidal and flat	3/4 Cuboidal	1/4 Flat
Aug.	Cuboidal and flat	1/2 Cuboidal	1/2 Flat
Sep.	No Sample		
Oct.	No Sample		
Nov.	Flat	All Flat	
Dec.	No Sample		
Jan. 1969	Flat	All Flat	
Feb.	Flat	All Flat	
Mar.	Flat	All Flat	
Apr.	Flat	All Flat	
May	Cuboidal and flat	3/4 Cuboidal	1/4 Flat
June	Cuboidal and flat	3/4 Cuboidal	1/4 Flat

Table 10. Analysis of sperm production using lamellar widths as an indication of spermatogenic activity for Yaquina Head T. lineata. Lamellar widths are expressed in millimeters.

Date	Mean Width	Range	Number Measurements
Aug. 1967	1.73	12.8-2.48	40
Sep.	1.85	1.60-2.20	15
Oct.	2.13	1.80-2.40	15
Nov.	2.16	1.48-3.20	40
Dec.	----------	No Sample	----------
Jan. 1968	----------	Not Measured	----------
Feb.	2.25	1.56-3.20	40
Mar.	1.89	1.56-2.40	25
Apr.	1.68	1.20-2.40	20
May	----------	Not Measured	----------
June	1.33	1.00-2.00	40
July	1.59	1.20-2.20	31
Aug.	1.96	1.40-2.60	20
Sept.	----------	No Sample	----------
Oct.	----------	Not Measured	----------
Nov.	1.78	1.20-2.40	30
Dec.	----------	No Sample	----------
Jan. 1969	1.93	1.52-2.60	20
Feb.	2.00	1.28-2.80	32
Mar.	2.17	1.28-3.00	30
Apr.	----------	Not Measured	----------
May	1.54	1.00-2.40	18
June	1.69	.92-2.80	30

Table 11. Analysis of oocyte production from histological slides for Yaquina Head T. lineata.

Oocyte stages	Relative amount
1 = Young oocyte, basophylic cytoplasm	O = absent
2 = Pear Shaped oocyte	R = Residual
3 = Prelobate oocyte	⊞ = Present few
4 = Lobate oocyte	▨ = Common
5 = Mature oocyte	■ = Abundant

Date	Stages 1	2	3	4	5
Sep. 1967	▨	⊞	▨	O	R
Oct.	▨	▨	■	⊞	R
Nov.	▨	⊞	■	■	⊞
Dec.	No Sample				
Jan. 1968	⊞	⊞	▨	■	▨
Feb.	⊞	⊞	⊞	■	■
Mar.	⊞	⊞	⊞	⊞	■
Apr	▨	⊞	⊞	⊞	■
May	■	▨	⊞	O	R
June	■	■	⊞	O	R
July	■	■	⊞	O	R
Aug.	■	■	▨	O	R
Sep.	No Sample				
Oct.	No Sample				
Nov.	▨	▨	■	▨	▨
Dec.	No Sample				
Jan. 1969	⊞	⊞	▨	■	▨
Feb.	⊞	⊞	▨	■	■
Mar.	▨	⊞	▨		
Apr.					
May	■	⊞	⊞	O	R
June	■	▨	⊞	O	R

Table 12. Analysis of oocyte production from histological slides for San Juan Island T. lineata. For an explanation of oocyte stages and relative amounts see Table 11.

Date	Stages 1 2 3 4 5
Mar. 1968	▨ ▨ ▨ ■ ▨
Apr.	-------- No Sample ----------
May	⊞ ⊞ ⊞ ⊞ ■
June	-------- No Sample ----------
July	-------- No Sample ----------
Aug.	-------- No Sample ----------
Sep.	■ ▨ ⊞ 0 R
Oct.	-------- No Sample ----------
Nov.	■ ■ ▨ ▨ ⊞
Dec.	-------- No Sample ----------
Jan. 1969	-------- No Sample ----------
Feb.	-------- No Sample ----------
Mar.	-------- No Sample ----------
Apr.	⊞ ⊞ ⊞ ⊞ ■

Table 13. Spawning times of Tonicella lineata in relation to the lunar phase and tidal height.

Location	Date	Time (PDT)	Date of nearest full or new moon	Approximate tidal height at time of spawning
Friday Harbor[1] Laboratories	20 June 1955	----	20 June (new)	----
Friday Harbor Laboratories	1 August 1955	----	3 August (full)	----
Friday Harbor Laboratories	22 July 1958	----	half-way between new and full	----
Friday Harbor Laboratories	28 July 1959	----	half-way between new and full	----
Cattle Point San Juan Island	23 May 1968	----	27 May (full)	----
Friday Harbor Laboratories	5 April 1969	1820	2 April (full)	+7.0
Friday Harbor Laboratories	6 April 1969	2000, 2200	2 April (full)	+7.8
Friday Harbor Laboratories	28 May 1969	early evening	31 May (full)	+6.0 to 7.0
Friday Harbor Laboratories	29 May 1969	early evening	31 May (full)	+7.0
Friday Harbor Laboratories	30 May 1969	early evening	31 May (full)	+7.0

[1]All spawning observations listed as Friday Harbor Laboratories were made on animals held in running seawater tables.

Table 14. Developmental times of Tonicella lineata at 10°C and 12-13°C.

Stage	Developmental times at 10°C	Developmental times at 12-13°C
Pulling away of egg cytoplasm	2-3 minutes	2-3 minutes
Polar body formation		
First	1/2 hr	1/2 hr
Second	1 1/2 hrs	1 1/4-1 1/2 hrs
Cleavage		
First (2 cells)	2 hrs	2 hrs
4 cell	--	4-4 1/2 hrs
8 cell	--	5-5 1/2 hrs
16 cell	--	6-6 1/2 hrs
32 cell	--	7 1/2 hrs
64-72 cell	--	12 hrs
Gastrula		
Blastopore becomes evident	18 hrs	15-16 hrs
Trochophore		
Prototroch development	23-24 hrs	16-18 hrs
Hatching	48 hrs	43-44 hrs
Foot starting to differentiate; posttrochal region starting to elongate; larvae now completely covered with cilia, except shell gland area.	60-70 hrs	60 hrs
Larval eyes present	110 hrs	100 hrs
Shell glands present	120 hrs	100 hrs (also some spicules)
Apical knobs reduced in size or absent; elevated ridges absent	140 hrs	110 hrs
Shell gland area	150-160 hrs	110 hrs
	Days After Settlement	
Prototroch and apical tuft lost; pretrochal region starting to flatten out and small plate formation begins	1/2 day	
Pretrochal region continues to flatten; head distinct	1-1 1/2 days	
Mouth evident	2 1/2 days	
Radula present	3 1/2-4 1/2 days	
Eighth shell plate present	5-5 1/2 days	

Table 15. Results--settling experiment 1A. Larvae used were 230 hours old.

Content of Beaker	Total number of settled larvae	
	1 day	3 days
Control	0	0
Coralline alga	27	42
Coralline alga	12	35
Coralline alga	29	40

Table 16. Results--settling experiment 1B. Larvae used were 254 hours old.

Content of Beaker	Total number of settled larvae	
	1/2 day	3 days
Control	0	0
Control	0	0
Coralline and Rock	22.0	22.0
Coralline and Rock	48.0	48.0
Coralline and Rock	14.0	44.0
Rock	0	0
Rock	0	0

Table 17. Results--settling experiment 2. Larvae used were 156 hours old.

Contents of Beaker	Total number of settled larvae	
	15 hrs	40 hrs
Control	0	0
Control	0	0
Control	0	0
Control	0	0
Control	0	0
Control	0	0
Coralline (Not Identified)	17	19
Lithophyllum sp.	41	50
Lithothamnion sp.	73	75
Lithophyllum sp. & Tile	21.0	21.0
Lithophyllum sp. & Rock	4.0	22.0
Tile	0	0
Rock	0	0

Table 18. Results--settling experiment 3. Larvae used were 200 hours old.

Contents of Beaker	Total number of settled larvae			
	14 hrs	1 day	1 1/2 days	2 1/2 days
Control - tile soaked in seawater	0	0	0	0
Tile soaked in extract of undetermined coralline species	0	2	3	5
Tile soaked in Lithothamnion extract	0	4	32	35

Table 19. Results--settling experiment 4. The effect of boiling on the attractiveness of the settling substrate. Larvae used were 144 hours old.

Contents of Beaker	Total number of settled larvae 12 hrs
Boiled Lithothamnion	0
Boiled Lithothamnion	0
Boiled Lithothamnion	0
Boiled Tile, Previously soaked in Lithothamnion extract--Expt. 3	0
Boiled Tile, Previously soaked in Lithothamnion extract--Expt. 3	0

Table 20. Results--settling experiment 5. Larvae used were 144 hours old.

Contents of Beaker	Total number of settled larvae 12 hrs
Control	0
Control	0
Hedophyllum sessile holdfast	0
Hedophyllum sessile frond	0
Laminaria sp. holdfast	0
Fucus	0
Odonthalia floccosa	0
Branched coralline alga (sp. unknown)	0
Lithothamnion	92
Lithothamnion	81
Lithothamnion & Hedophyllum holdfast	75.0
Lithothamnion & Hedophyllum frond	63.0

Figure 1. Two individuals of Tonicella lineata in the typical habitat. Encrusting coralline algae almost entirely cover the rock.

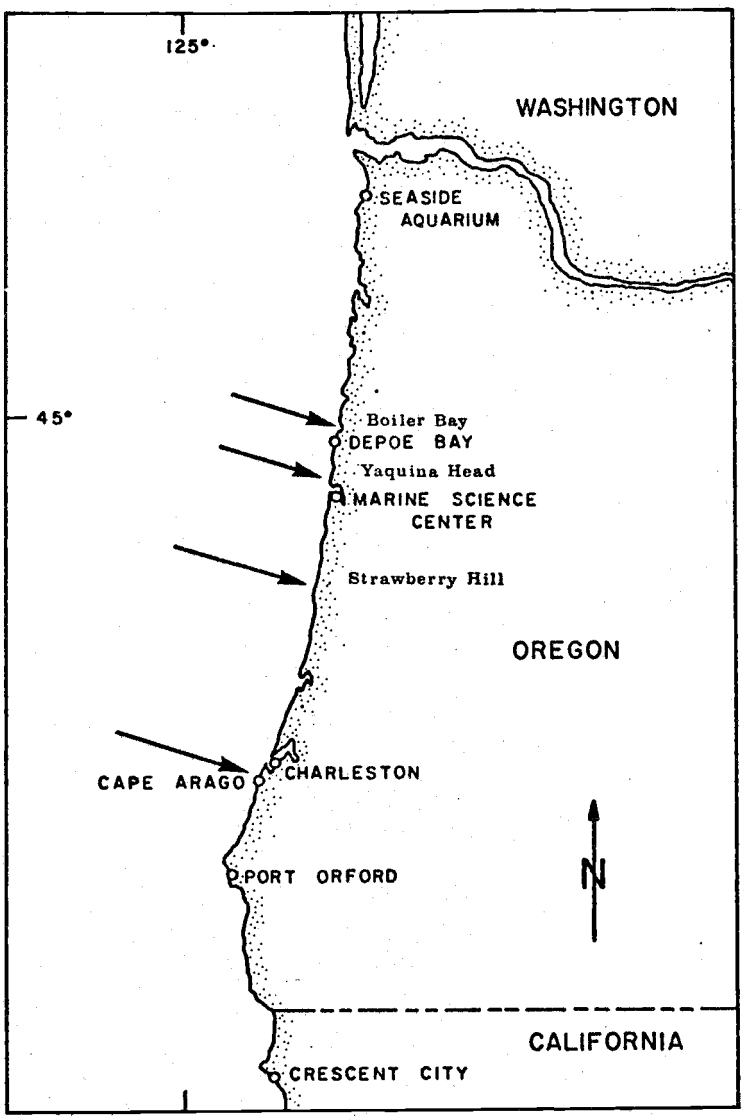

Figure 2. Map of the Oregon Coast. Arrows indicate collection sites.

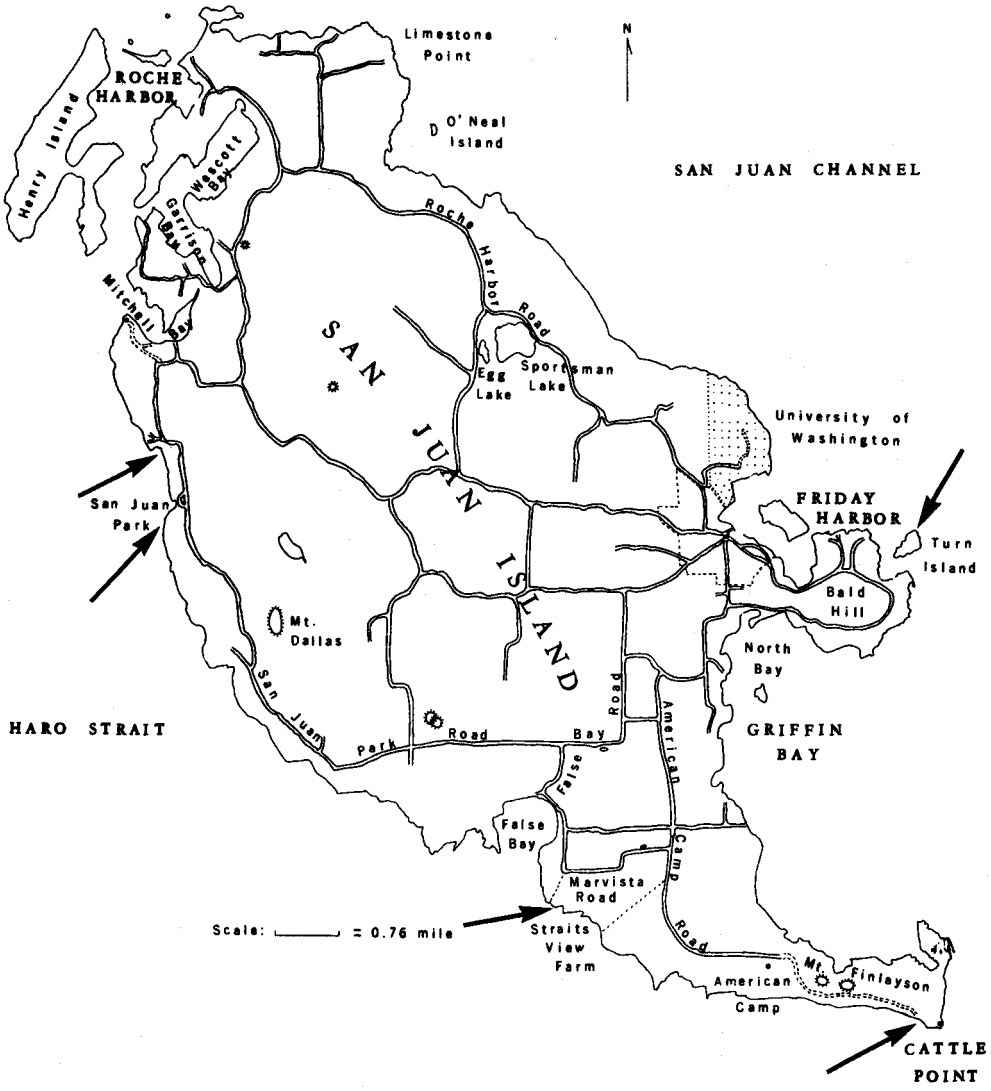

Figure 3. Map of San Juan Island. Arrows indicate collection sites.

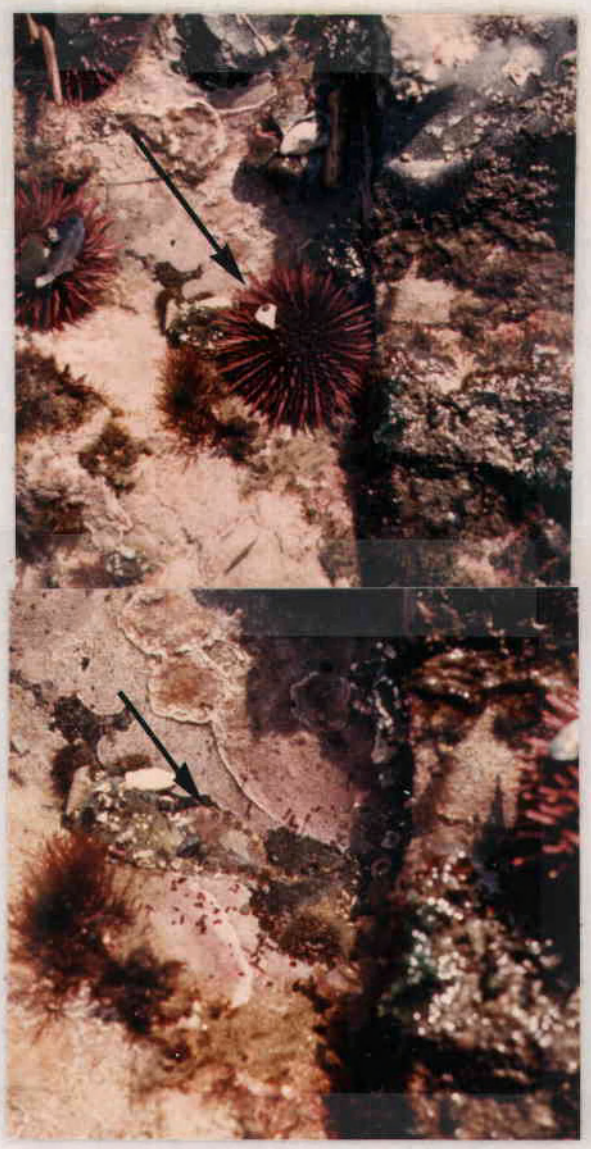

Figure 4. Typical habitat of Tonicella lineata along the Central and Southern Oregon Coast.
Top. Urchin, Strongylocentrotus purpuratus in center at arrow concealing a T. lineata underneath
Bottom. Urchin removed to show chiton (arrow)

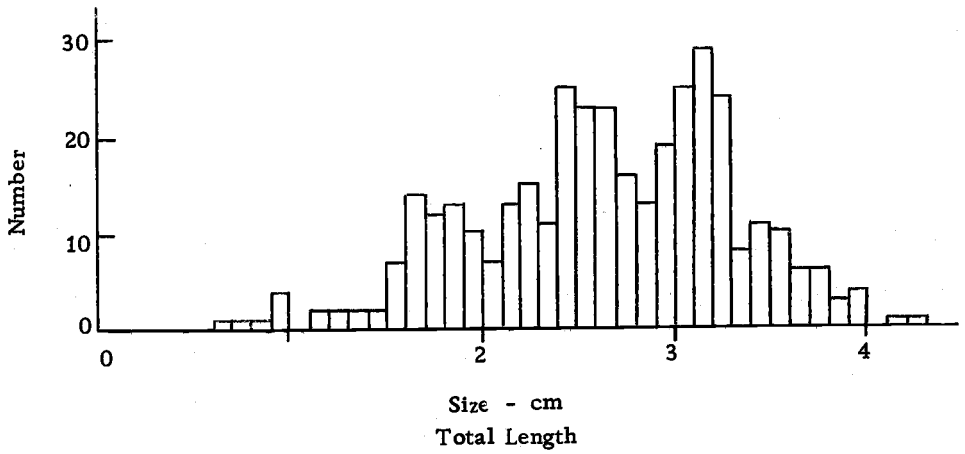

Figure 5. Histogram showing size-frequency distribution of an intertidal population sample of T. lineata from Cape Arago, Oregon, May 15 and 16, 1968. N = 363, mean length 2.58 cm, range 0.6 cm-4.2 cm.

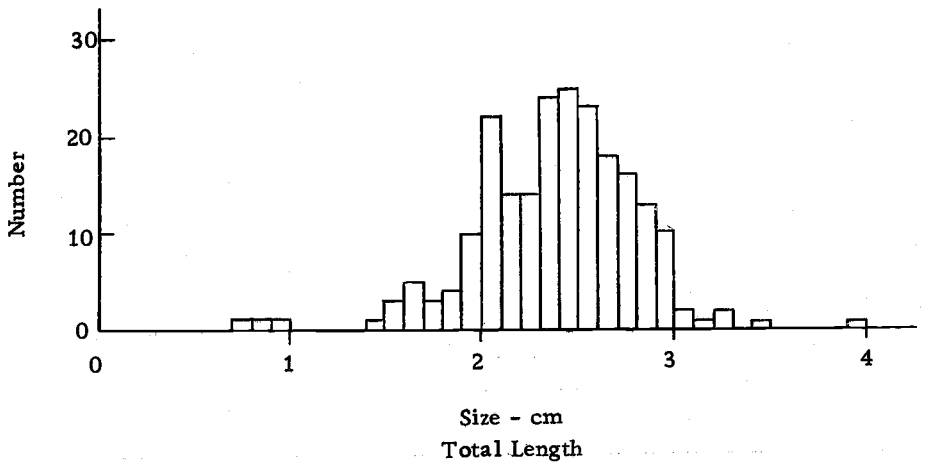

Figure 6. Histogram showing size-frequency distribution of a high tidepool population sample from Yaquina Head, Oregon, May 1, 1968. N = 216, mean length 2.33 cm, range 0.7-3.9 cm.

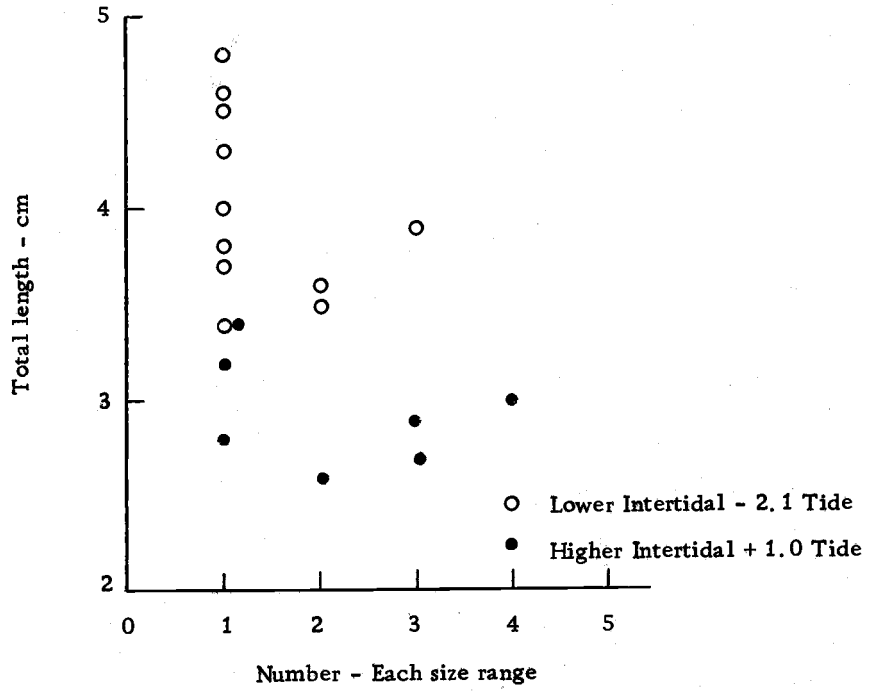

Figure 7. Size comparison of the 15 largest T. lineata collected on -2.1 and +1.0 tides at Yaquina Head, Oregon.

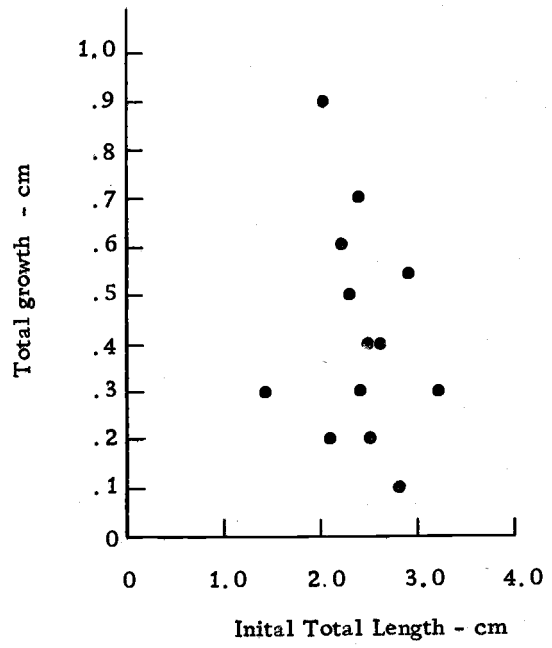

Figure 8. Size-growth relationship of marked T. lineata, Yaquina Head, Oregon.

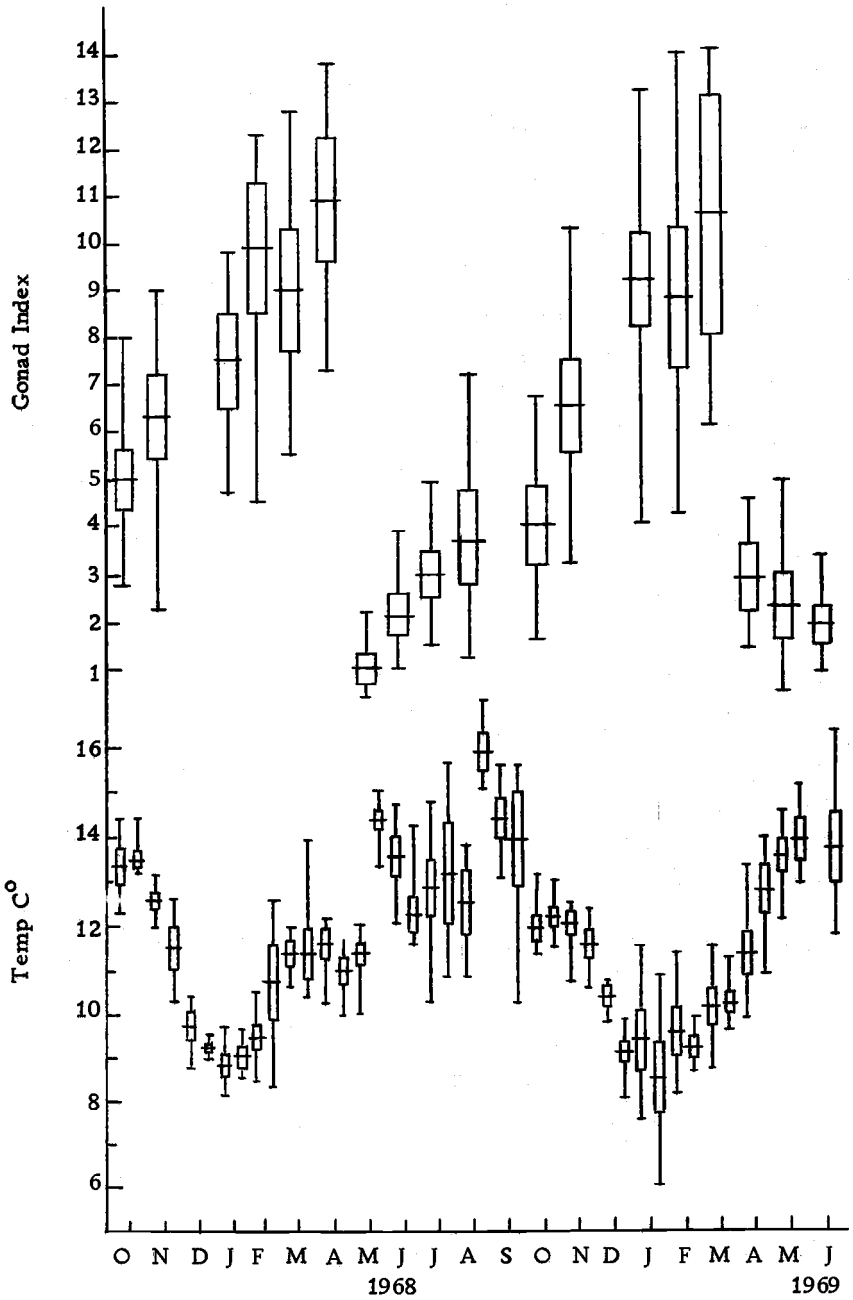

Figure 9. Comparison of gonad index values from Yaquina Head, Oregon with inshore sea surface temperatures from Agate Beach, Oregon. The mean range and ±2 standard error are shown for monthly gonad index values and 15 day temperature periods.

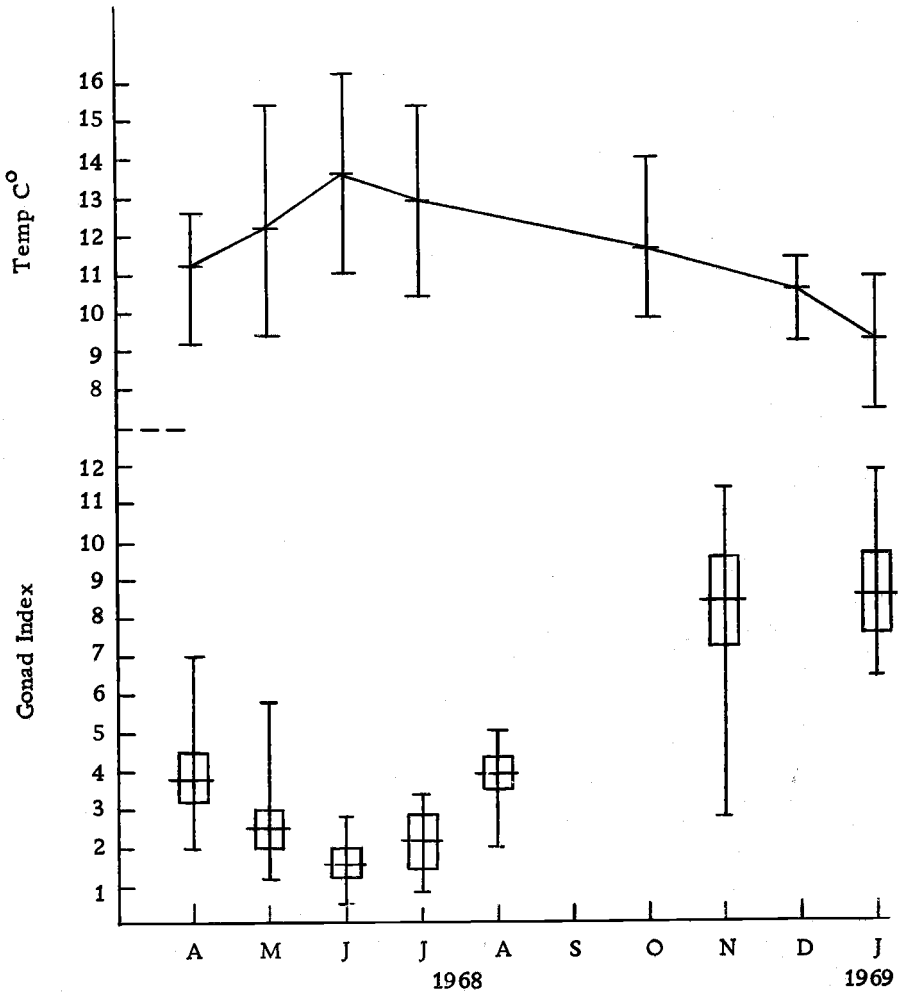

Figure 10. Comparison of gonad index values with inshore sea surface temperatures from Cape Arago, Oregon. The mean, range and ±2 standard error are shown for monthly gonad index values. The mean and range are shown for monthly temperature periods.

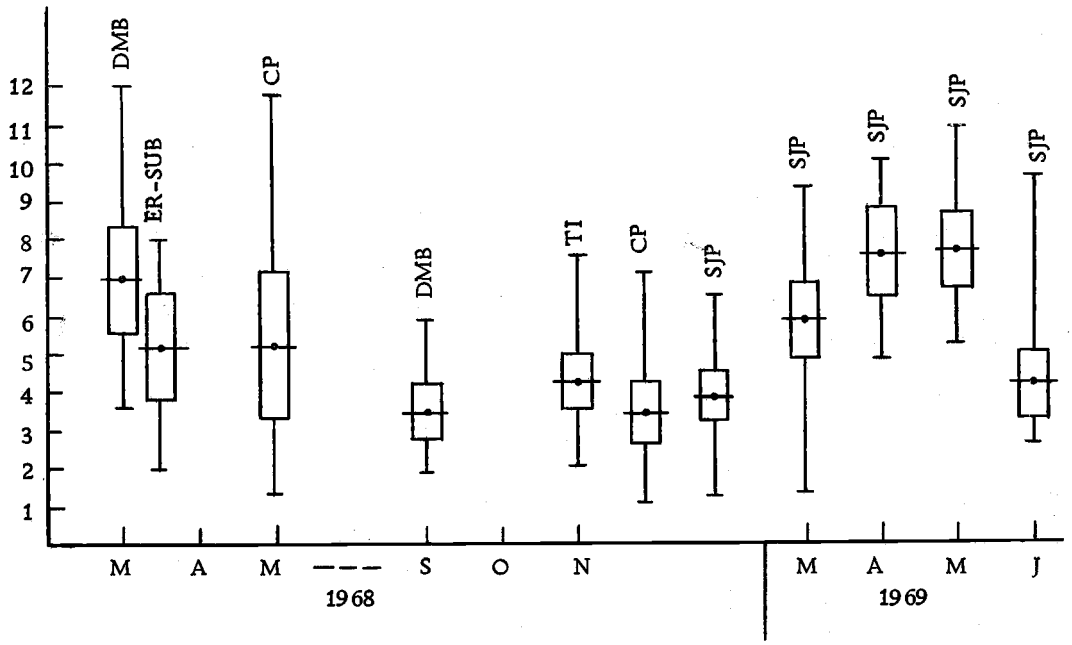

Figure 11. Gonad index data for T. lineata from San Juan Island, Washington. The range, mean and ±2 standard errors are shown for each sample. DMB = Deadman's Bay, ER-SUB = Edwards Reef Subtidal, CP = Cattle Point, TI = Turn Island, SJP = San Juan Park.

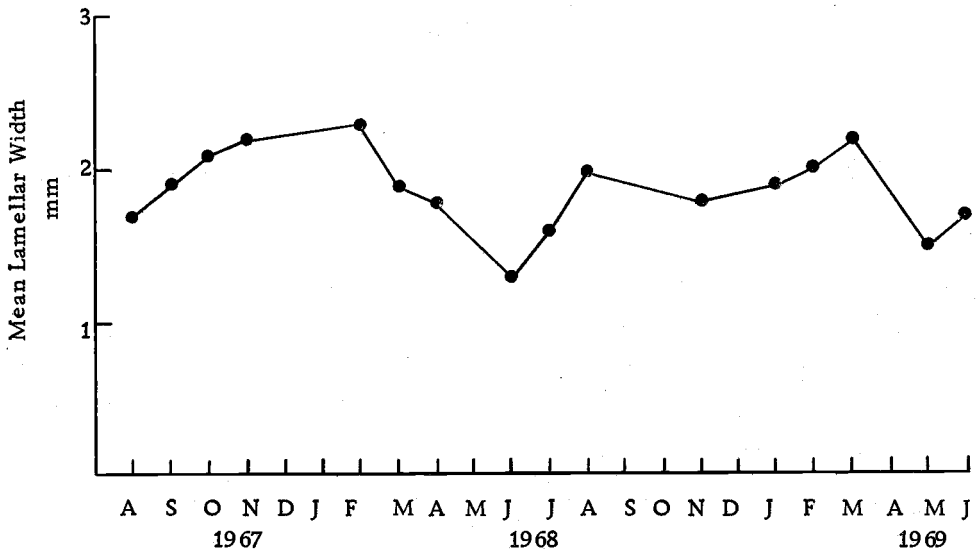

Figure 12. Graphic representation of T. lineata testicular lamellar widths.

Figure 13. Cross-section of testes (November, 1968) showing lamellae and lumen. Lumen is full of sperm. lu = lumen, la = lamella. Line is equal to .1mm.

Figure 14. Detail of Figure 13 showing sperm production along left side of lamella. lu = lumen, s = mature sperm, sp = other stages of spermatogenesis. Line is equal to .05 mm.

Figure 15. Cross-section of testes (February, 1968). Lumen is full of mature sperm. Sperm production is continuous along lamellae. Line is equal to .1 mm.

Figure 16. Cross-section of testes (April, 1968) after spawning. Lamellae are close together as most of the sperm have been spawned from the lumen. Line is equal to .1 mm.

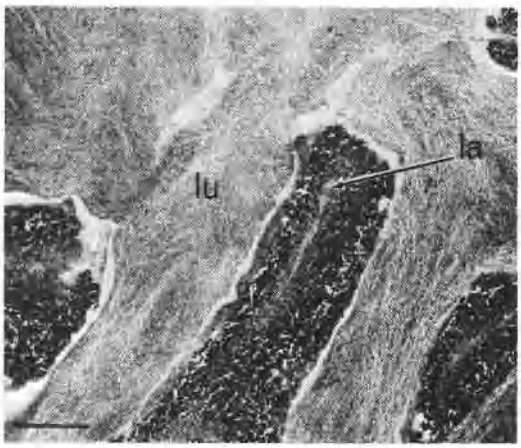

Fig.13

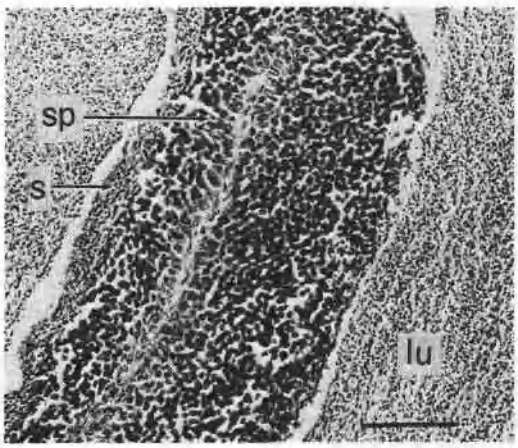

Fig.14

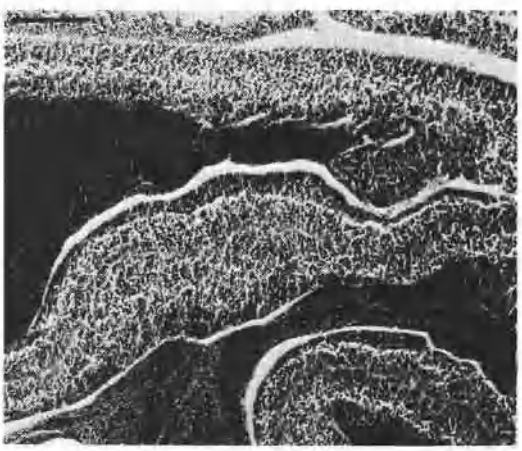

Fig.15

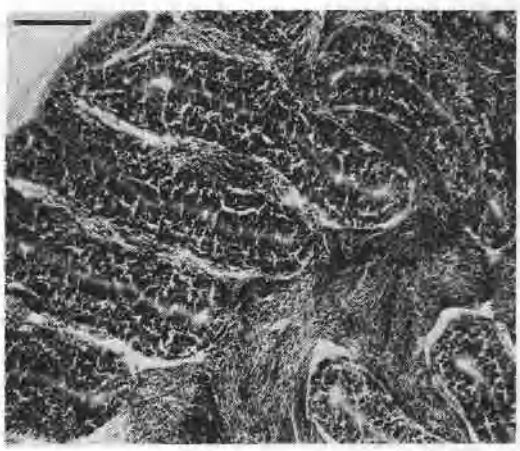

Fig.16

Figure 17. Cross-section of ovary (November, 1968). mo = mature oocyte, ls = lobate stage, io = immature oocytes, la = lamella. Line is equal to .1 mm.

Figure 18. Detail of Figure 17, lobate stage (November, 1968). Line is equal to .05 mm.

Figure 19. Cross-section of ovary (March, 1969). Lumen is full of mature oocytes. Line is equal to .1 mm.

Figure 20. Cross-section of ovary (April, 1968) just before spawning. Lumen is full of mature oocytes. Area of germinal epithelium shows production of immature oocytes which will be spawned next year. ge = germinal epithelium. Line is equal to .1 mm.

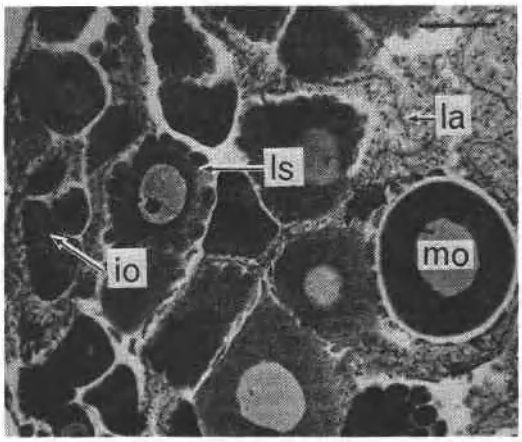

Fig.17

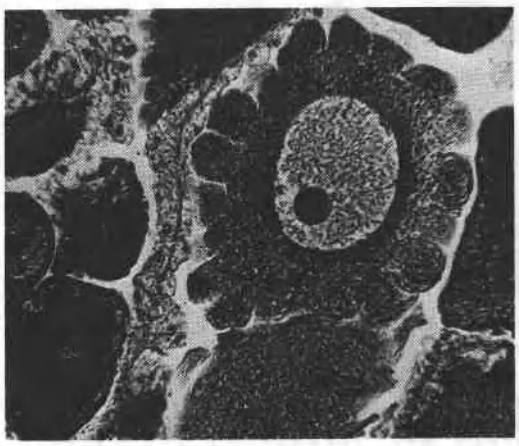

Fig.18

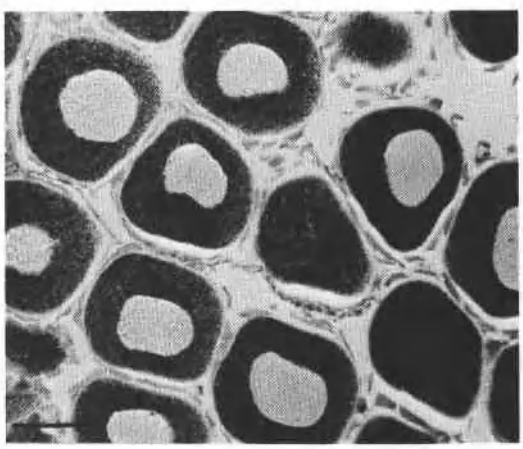

Fig.19

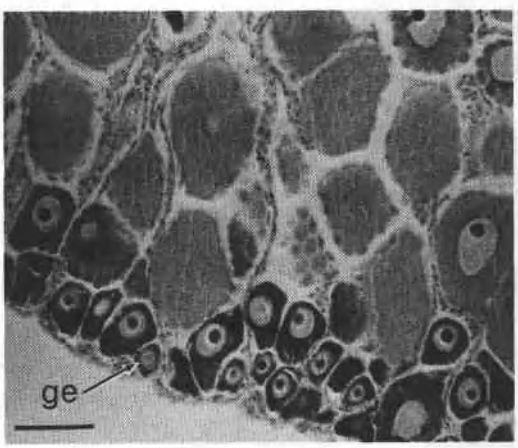

Fig. 20

Figure 21. Cross-section of ovary (May, 1968) after spawning. A few residual mature oocytes remain in the lumen. Next year's oocytes are also present. Line is equal to .1 mm.

Figure 22. Cross-section of ovary (June, 1968). Only next year's oocytes are present. Line is equal to .1 mm.

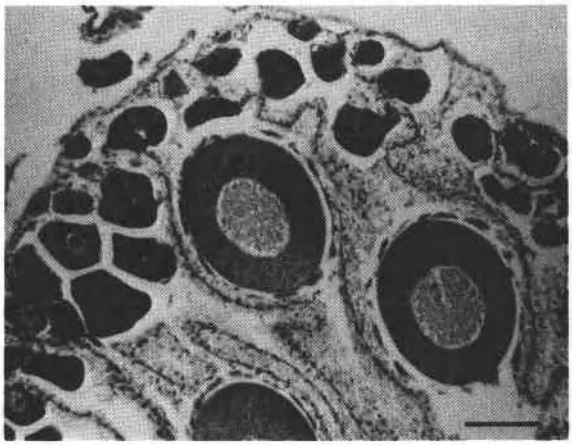

Fig. 21

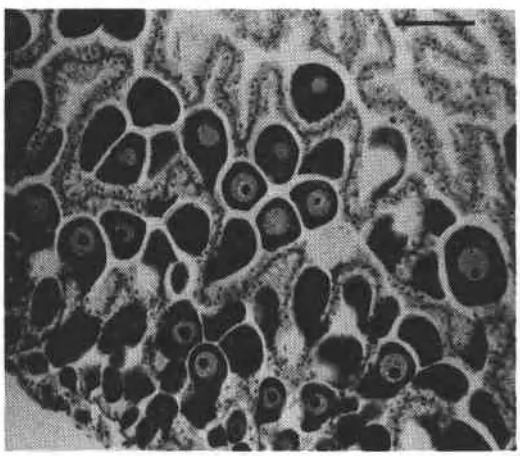

Fig. 22

Figure 23. Female Tonicella lineata spawning in seawater table at Friday Harbor Laboratories. Note girdle lift. Arrow indicates eggs.

Figure 24. T. lineata fertilized egg showing chorion (c), space (s) between chorion and the egg proper, and first polar body (pb). X150. Line is equal to .1 mm.

Figure 25. First cleavage, 2 cell stage. X150. Line is equal to .1 mm.

Figure 26. Vegetal pole view, about 32 cell stage, showing position of macromeres. Note large D cell (d). X150, slightly flattened. Line is equal to .1 mm.

Figure 27. Animal pole view, about 60 cell stage. X150, slightly flattened. Line is equal to .1 mm.

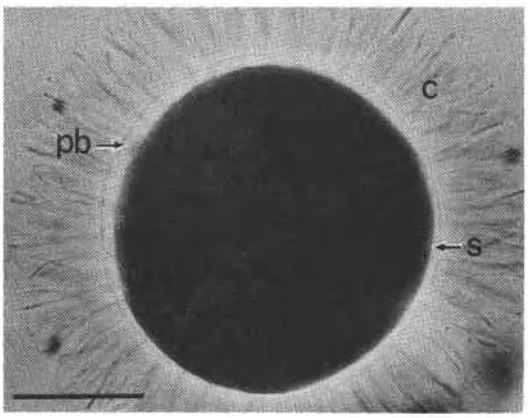

Fig. 24

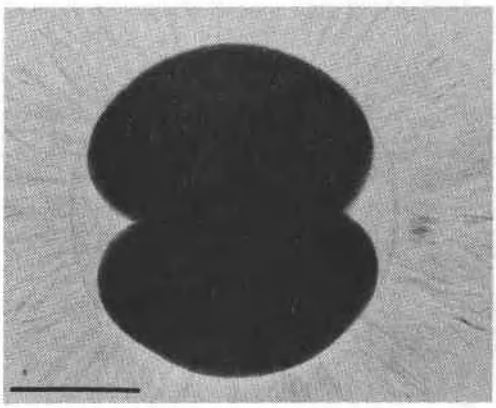

Fig. 25

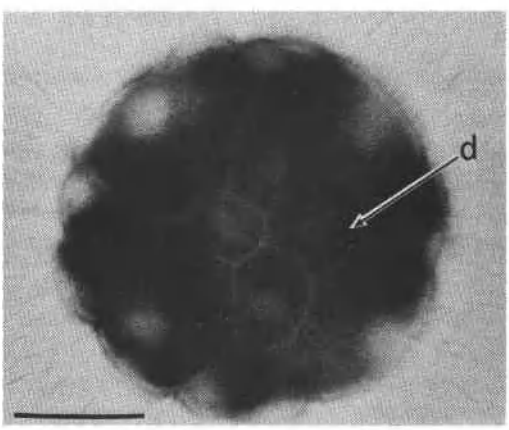

Fig. 26

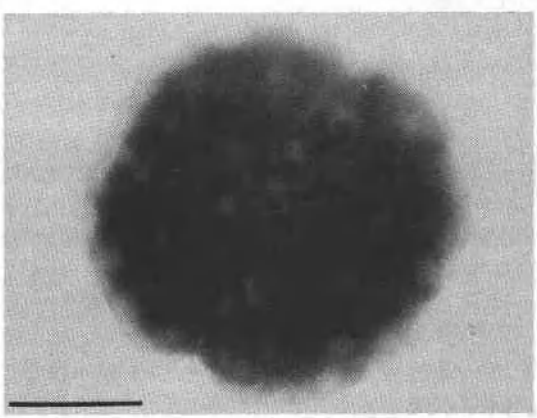

Fig. 27

Figure 28. Vegetal pole view, about 72 cell stage, showing division of D cell (d) into D and 4d mesoblast cell. X150, slightly flattened. Line is equal to .1 mm.

Figure 29. Vegetal pole view, 18 1/2 hour embryo, showing blastopore (bp). X150, slightly flattened. Line is equal to .1 mm.

Figure 30. Vegetal pole view, 20 hour embryo, showing blastopore (bp) and anterior directed furrow (f). X150, slightly flattened. Line is equal to .1 mm.

Figure 31. Side view, early trochophore, about 24-26 hours, showing blastopore (bp) and furrow (f); furrow around animal where prototroch (p) is forming. This early trochophore can now be divided into pretrochal and postrochal regions. Apical region (ar) where apical tuft is forming is slightly flattened. X150, slightly flattened. Line is equal to .1 mm.

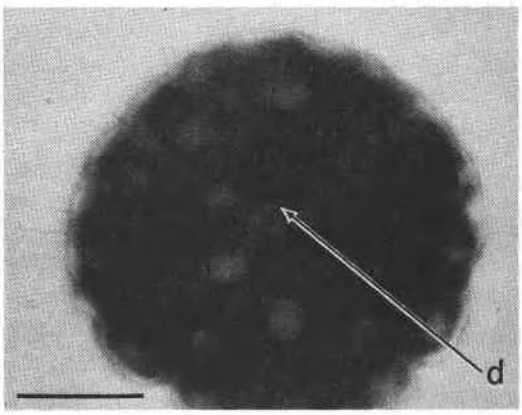

Fig. 28

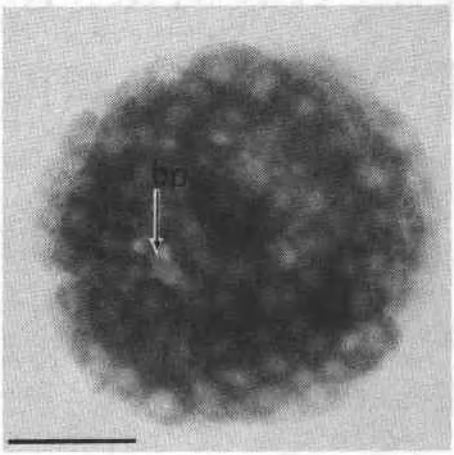

Fig. 29

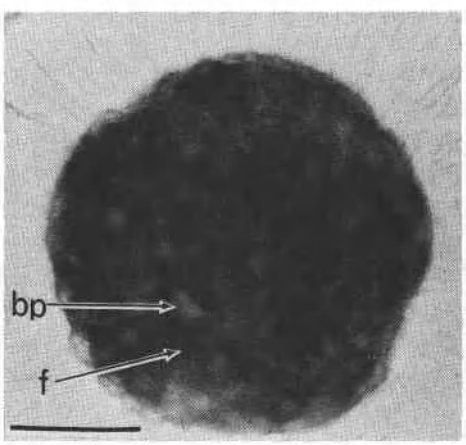

Fig. 30

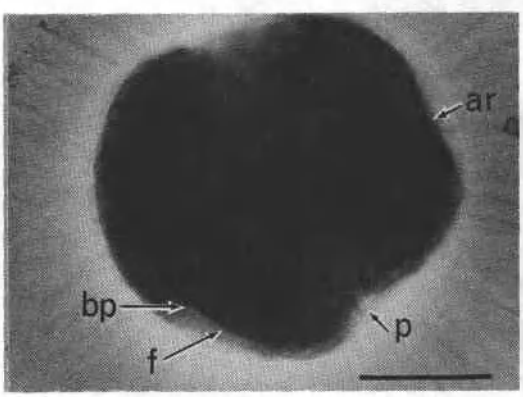

Fig. 31

Figure 32. Apical view of artificially hatched embryo, about 40 hours. Note granular area (ga) on each side of apical tuft (at). This granular area represents the circle of granular area around the apical tuft. X240, slightly flattened. Line is equal to . 1 mm.

Figure 33. Apical view of artificially hatched embryo, about 40 hours. Note the two granular tracks (gt) extending down from the apical region. These will become the elevated ridges. Note also the granular appearance of the prototroch cells (pc). X240, slightly flattened. Line is equal to . 1 mm.

Figure 34. View showing anal tufts (al) at posterior end of hatched trochophore. X240, slightly flattened. Line is equal to . 1 mm.

Figure 35. Dorsal view trochophore larvae, 66 hours, showing apical tuft (at), apical knobs (ak), and prototroch (p). X60, slightly flattened. Line is equal to . 1 mm.

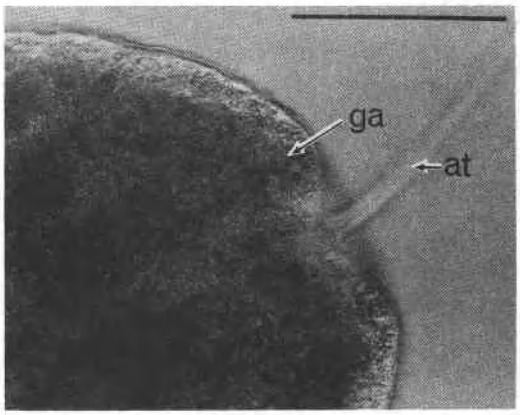

Fig. 32

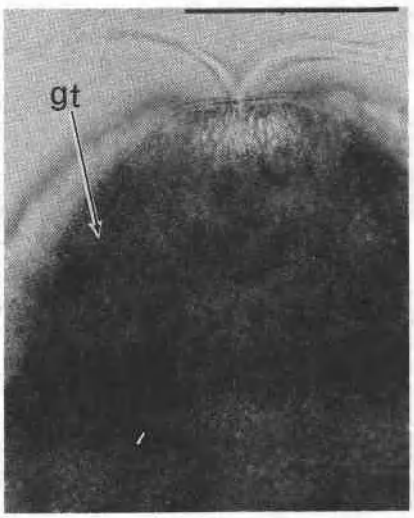

Fig. 33

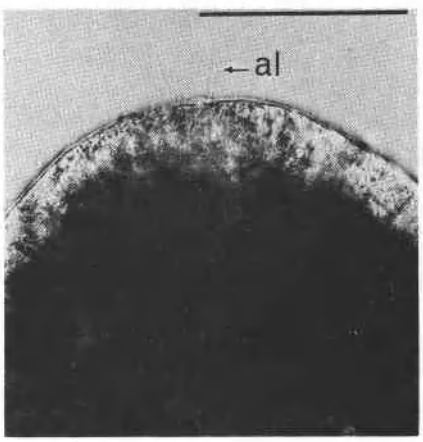

Fig. 34

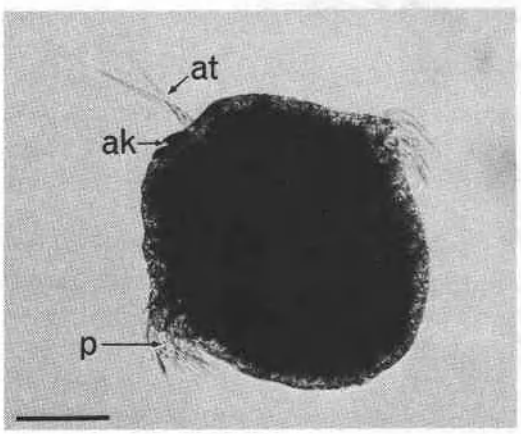

Fig. 35

Figure 36. Free swimming trochophore, 112 hours. Note change of shape over 66 hours (Figure 35). X60, slightly flattened. For description of symbols see Figure 35. Line is equal to .1 mm.

Figure 37. Dorsal view of apical region of trochophore, about 105-110 hours, showing development of apical knobs (ak). Note also the apical tuft (at). X240, slightly flattened. Line is equal to .1 mm.

Figure 38. Ventral view of apical region of trochophore, about 105-110 hours, showing development of elevated ridges (er). Note also the apical tuft (at). X400, slightly flattened. Line is equal to .05 mm.

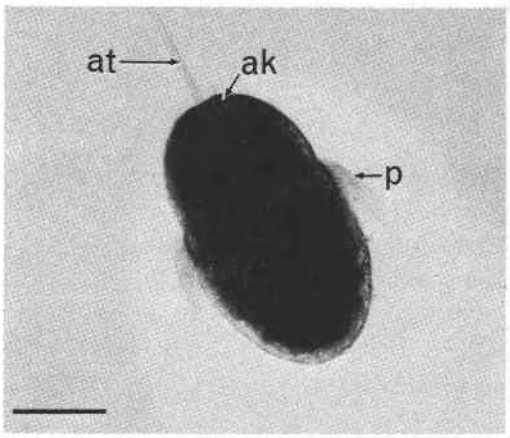

Fig. 36

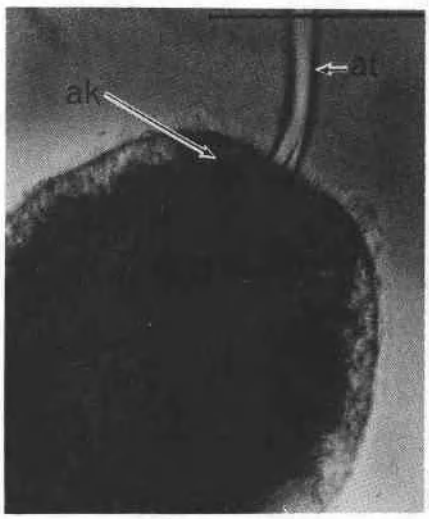

Fig. 37

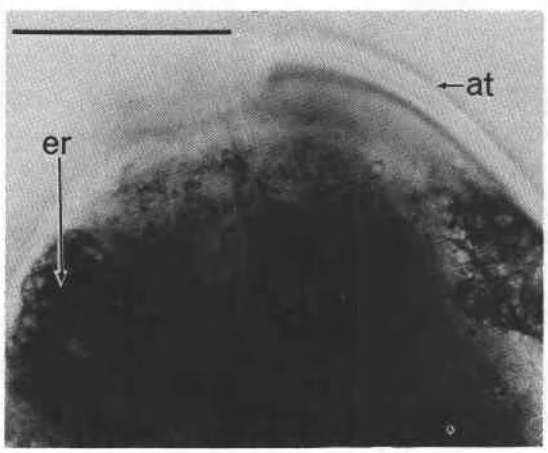

Fig. 38

Figure 39. Dorsal view of trochophore, 144 hours. Note the presence of larval eyes (le) and shell glands (sg). Apical knobs are absent. X60. Line is equal to .1 mm.

Figure 40. Dorsal view of trochophore larvae, 216 hours (used to represent 160 hour stage since stage of development is exactly the same), flattened slightly to show spicules (s) and area of shell glands (sg). Larval eyes (le), prototroch (p), and apical tuft (at). X60. Line is equal to .1 mm.

Figure 41. Ventral view of same larvae as in Figure 40 to show differentiation of foot (f), larval eyes (le) and protrochal region (pt). X60. Line is equal to .1 mm.

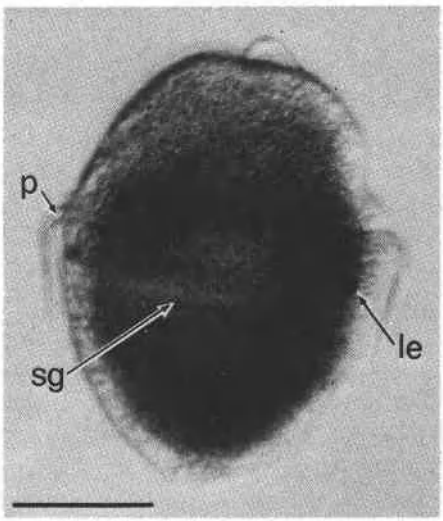

Fig. 39

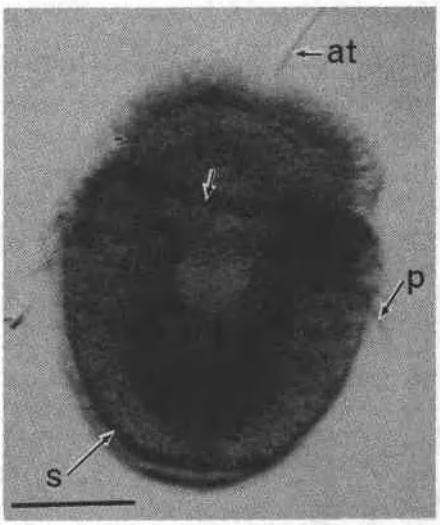

Fig. 40

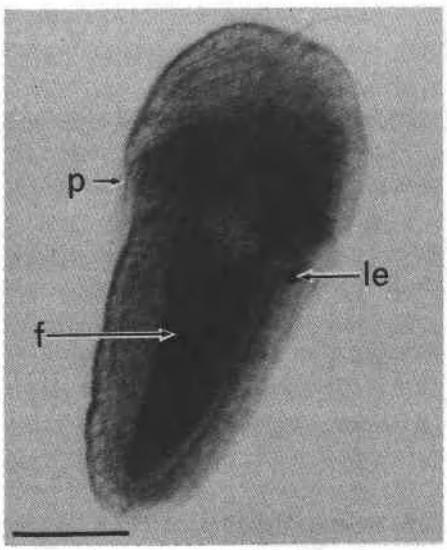

Fig. 41

Figure 42. Dorsal view of metamorphosed chiton one half day after settling. Apical tuft and prototroch have been lost, pretrochal region still somewhat bulbous. Shell plates (sp), larval eyes (le) present. X60. Line is equal to .1 mm.

Figure 43. Ventral view of same chiton as, in Figure 42. Foot (f), larval eyes (le). Note head region not very distinct. X60. Line is equal to .1 mm.

Figure 44. Dorsal view of young chiton, four days after settlement. The radula (r) is present. Larval eyes (le), shell plates (sp), and spicules (s). X60. Line is equal to .1 mm.

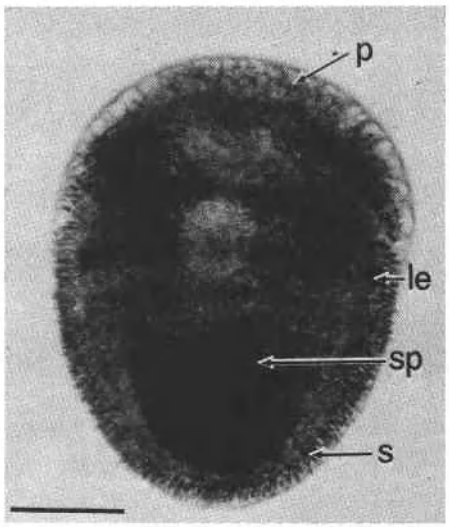

Fig. 42

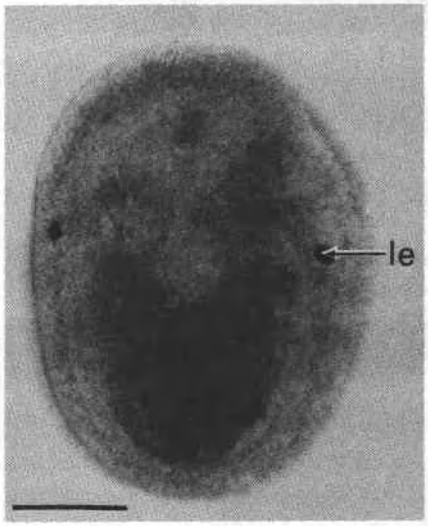

Fig. 43

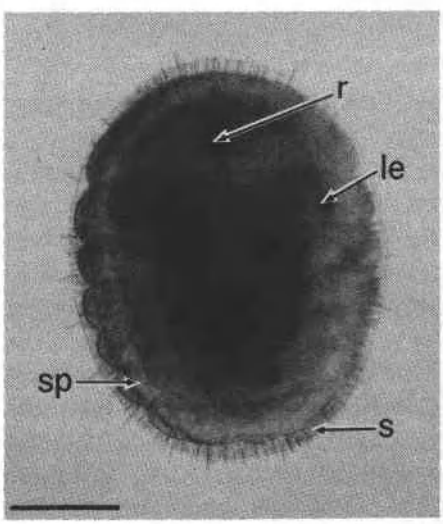

Fig. 44

Figure 45. Young chiton one month after settlement. Radula (r), shell plate (sp), larval eyes (le). X60. Line is equal to .1 mm.

Figure 46. Unsettled larvae same age as young settled chiton in Figure 45. Note the presence of the apical tuft (at) and prototroch (p). X60. Line is equal to .1 mm.

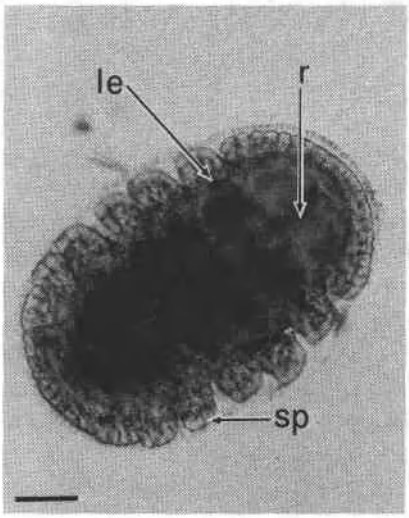

Fig. 45

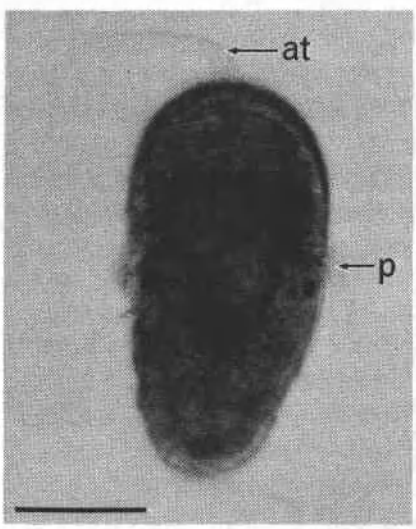

Fig. 46

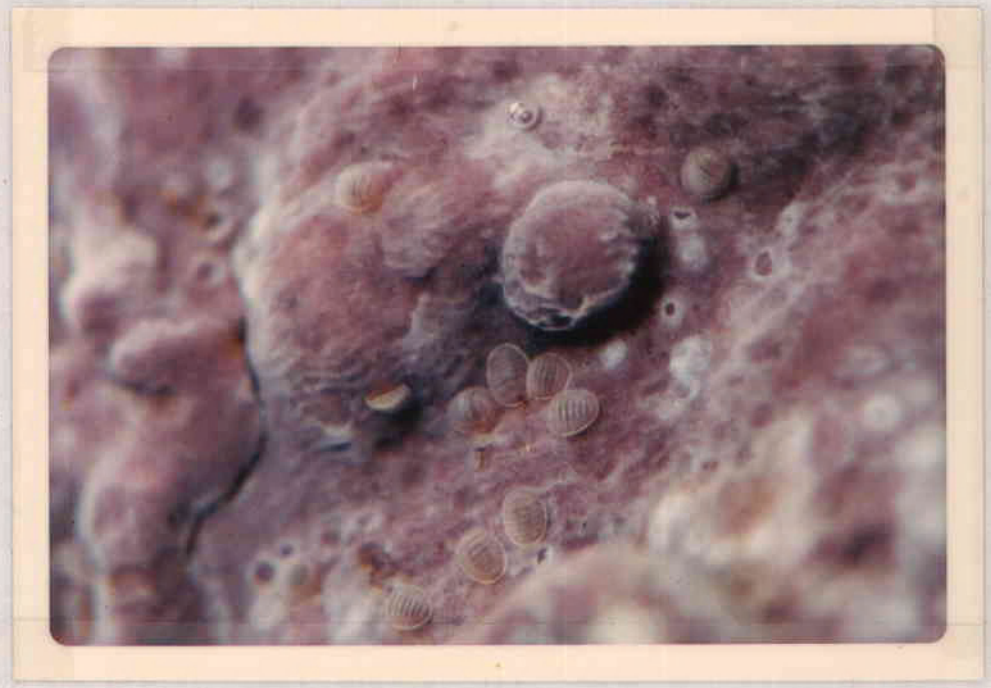

Figure 47. Newly settled T. lineata, 24 hours after settling on encrusting coralline algae.