REPRODUCTION, DEVELOPMENT, PROCESSES OF FEEDING AND NOTES ON THE EARLY LIFE HISTORY OF THE COMMERCIAL SEA CUCUMBER *PARASTICHOPUS CALIFORNICUS* (STIMPSON)

by

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Reproduction, development, processes of feeding and notes on the early life history of the commercial sea cucumber *Parastichopus californicus* (Stimpson)

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(date)

ABSTRACT

The commercial sea cucumber *Parastichopus californicus* (Stimpson) exhibits an annual reproductive cycle with spawning occurring in the late spring through summer. The sexes are separate, and occur at an approximately 1:1 ratio. Spawning is partially synchronous, and may be mediated by an increase in the duration of insolation.

Development of the planktotrophic larvae of *P. californicus* was followed from fertilization through settlement of the pentactula. Asynchronization of development within simultaneously fertilized cohorts resulted in a variable pelagic period for the larvae within any particular cohort. Differentiation of the larval gut occurred via the formation of two cavities, an anterior spherical vesicle that eventually becomes the larval esophagus and a posterior elongate archenteron that becomes the stomach and intestine of the auricularia. The larval coelom forms from a vesicle that originates on the left side of the anterior vesicle-archenteron junction and within the blastocoel. An unusual sequence of metamorphosis was noted in one cohort wherein metamorphosis to the pentactula was without the intervening doliolaria stage.

Tentacle structure and function in the pentactula larva, juvenile, and adult life stages were examined via light and electron microscopy. Food particle adherence to the tentacle surface is mediated by an adhesive material in the case of the pentactula larva and additionally by mechanical entrapment in juvenile and adult animals. Mechanical entrapment is of secondary importance to adhesion during feeding.

Juvenile *P. californicus*, ≃ 6 - 8 months post-settlement were commonly found associated with various red algae within "nursery" locales which were notably free of predatory sea stars of the genus *Solaster*. The swimming behavior noted in adult *P. californicus*, whenever contact is established with the sunflower star *Pycnopodia helianthoides*, does not occur with the same regularity or intensity in juvenile animals. *Solaster endeca* was shown to prey upon *P. californicus* ≤ 2 years of age in laboratory aquaria. Sea cucumbers > 2 years of age were able to avoid predation by swimming.

Except for size, juvenile animals are morphologically indistinguishable from adult animals. Skeletal ossicles from newly settled pentactula larvae and juvenile animals, though, are distinctly different from those of the adult. Visceral atrophy occurs in 100% of the juvenile population in the late fall and winter of each year. The polynoid worm *Arctonoë pulchra* and the endoparasitic gastropod *Enteroxenous parastichopoli*, common symbionts of adult *P. californicus*, were also noted on or within juvenile animals.

ACKNOWLEDGEMENTS

Numerous individuals assisted throughout the course of this study, and to these I express sincere appreciation. A few, who contributed significantly, are deserving of special mention. Timothy B. Smith, Jack da Silva, Bruce J. Leighton, David A. Fyfe, and Laurie Mitchell were regular and reliable diving partners. Victor Bourne of the Electron Microscope Laboratory at Simon Fraser University was a constant source of expertise and advice. To Peter V. Fankboner, my committee supervisor and friend, I am especially grateful for his unwavering demand for excellence, and his support both professionally and personally.

I would like to thank Dr. A.O.D. Willows, Director of the Friday Harbor Laboratories, and his staff for allowing me to use the facilities of the laboratory for a considerable portion of my tenure in this program. I am especially indebted to my parents, Jereld H. Cameron and Genevieve M. Cameron, for both financial and moral support throughout my graduate studies, I am also grateful to a mother for whom knowledge and education are especially important, and who sacrificed many of her own opportunities for self improvement so that her children could better themselves.

I must also mention my wife Janka P. Cameron, and my children Aaron Lane, Rebecca Jean, and Andrew Thomas who have patiently given up many of the necessities of life so that I could pursue my studies.

Drs. M.J. Smith and E.B. Hartwick kindly provided the sea water temperature data for the Woodlands study site as reported in Part A. Financial support was provided in part by awards to Dr. P.V. Fankboner from the Science Council of British Columbia and the Natural Sciences and Engineering Research Council of Canada.

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A. REPRODUCTIVE PERIODICITY AND SPAWNING BEHAVIOR

I. Introduction

Bakus (1973) indicated that the bulk of information available on holoturid echinoderms was generally contained within the taxonomic literature, and that little ecological, evolutionary or experimental work had been done on this group. Recently though, interest in the biology and ecology of sea cucumbers has increased significantly. For instance the feeding biology and/or tentacle function in holothurians has been examined by Fankboner (1978, 1981), Roberts (1979), Massin (1980a), Bouland *et al*. (1982), Roberts and Bryce (1982), Hammond (1983), Cameron and Fankboner (1984), and Costello and Keegan (1985). Some aspects of the ecology of this diverse group have been reported by Massin and Jangoux (1976), Yingst (1976, 1982), Sloan (1979, 1982), Sloan and von Bodungen (1980), and Hammond (1982). While, Sibuet and Lawrence (1981), Filimonova and Tokin (1980), and Massin (1980b) have examined the structure and function of the digestive system in holothurians.

One significant exception to Bakus' rule has been in the area of reproductive biology where a considerable amount of information has accumulated over the years. Boolootian (1966) states that specific breeding seasons are either established or have been indicated for many sea cucumbers and suggests that environmental parameters are the entrainment mechanisms facilitating these patterns. The factors that specifically regulate reproductive seasonality or stimulate spawning in invertebrates, however, are generally unknown (Giese and Pearse 1974; Barnes 1975; Todd and Doyle 1981).

Recently the reproductive biology of aspidochirote holothurians has been the focus of an increasing amount of scientific attention. The majority of this effort, however, has centered on sea cucumbers from tropical areas, ie. *Holothuria floridana*, *H. mexicana* (Engstrom, 1980), *Thelenota ananas*, *Microthele nobilis*, *M. fuscogilva* (Conand, 1981), *Actinopyga echinites* (Conand, 1982), *H. atra* (Harriott, 1982), and *Bohadschia marmorata* (Hendler and Meyer, 1982), where the effects of seasonality are not as extreme as are found in temperate or arctic marine systems (Thorson 1950, 1966). Sea cucumbers also form the basis of limited fisheries in countries bordering Indo-Pacific waters (Mottet, 1976), and knowledge of their reproductive processes is obligatory for the proper management of these commercially exploitable species (Conand, 1981).

Considering these factors, and the paucity of information on reproduction by aspidochirote holothurians from temperate waters, this study was undertaken to determine the processes of reproduction, and to assess the influence of environmental parameters on reproduction and spawning in *Parastichopus californicus* (Stimpson) a common epibenthic aspidochirote holothurian of potential commercial importance from the waters of the North-eastern Pacific Ocean.

II. Materials and Methods

Collection of Specimens

From May 1982 through November 1983 adult *P. californicus* were collected at monthly intervals via SCUBA at Woodlands Bay, Indian Arm fjord, British Columbia (49° 21' N, 122° 55' W). Ten sea cucumbers were selected randomly from the first 40 animals encountered while swimming a predetermined route over the bottom at a depth of between 6 and 15 m.

Sex Determination and Gonad Index

Within 5 hrs of collection, specimens of *P. californicus* were sexed, the volumetric displacement of the gonad was measured, and the damp weight of the body wall with its associated longitudinal muscle complex and buccal bulb (devoid of viscera and coelomic fluid) was determined. From these measurements a gonad index was calculated by the relationship of gonad volume/body weight x 100 (Giese, 1959, p. 550).

Histology of Gonadal Tissues

To ascertain the seasonal development of *P. californicus* reproductive organs, portions of the gonad tubules from each animal collected over the 19 months of this study were fixed in 4% glutaraldehyde buffered with 1% calcium carbonate. The tubules were then dehydrated in an alcohol series to 70% and embedded in JB-4 embedding medium (Polysciences, Inc.). Sections 1 - 2 µm thick from each preparation were stained according to the method of Richardson *et al.* (1960) and examined with the light microscope.

Oöcytes freely leak from mature ovarian tubules during the collection, fixation, and embedding process rendering size frequency data unreliable. Size measurements, which were taken on oöcytes in which the germinal vesicle and nucleolus were visible in section, were therefore used to determine the general developmental stage of the ovary and not to quantify the female reproductive effort.

Spawning

Observations on spawning were made *in situ* via SCUBA at least monthly or more frequently from October 1980 through November 1983 at Woodlands Bay, Kelvin Grove, Howe Sound (49° 25' N, 123° 14' W); and less frequently at Croker Island, Indian Arm (49° 27' N, 122° 53' W), but especially during an intensive effort of 41 dives from June 1, through August 31, 1982. In each of these locations systematic surveys were repeatedly made from within two populations of sea cucumbers by swimming predetermined routes and examining all animals within a two meter wide corridor, during quantitative counts from parallel transect lines perpendicular to the surface, or during quantitative examination of the bottom with circular transects.

Sea Water Temperature

To coordinate reproductive pattern with seasonal temperature pattern, surface and bottom (≥ 3 ≤ 5m in depth) sea water temperatures were taken at the Woodlands Bay site for the years 1978 through 1980.

III. Results

Morphology of Sexes & Gonad Description

The sexes are separate in *P. californicus*, but there is no external evidence of sexual dimorphism. Internally, the testis and ovary are similar anatomically and consist of two tufts of elongate bifurcating tubules. The tufts are separated by the dorsal mesentery and lie within the perivisceral coelom. Mature tubules may fill the perivisceral coelom in gravid adult sea cucumbers intertwining distally amongst the cloacal suspensor muscles. Differentiated gonadal tubules are cream-white in the male and bright orange in the female.

Gonadal tufts arise from a saddle-like structure, the gonad basis that is contiguous with the dorsal wall of the esophagus at the point where the esophagus passes through the water ring of the buccal bulb. The gonoduct is supported by the dorsal mesentery and opens to the exterior of the animal via the gonopore on the anterior mid-dorsal body wall. There is no obvious papilla or specialized protrusion of the body wall associated with the gonopore.

Sex Ratio

The proportions of adult male and female animals collected between May 1982 and November 1983 are represented in Figure A.1. An expected frequency distribution of male to female animals was calculated based on the binomial expansion of an assumed 1:1 relationship of sexes with a sample size of ten, $(p + q)^{10}$, where $p = 0.5$ and $q = 0.5$. By chi-square analysis it was determined that the observed distribution of sex ratios was not significantly different from the expected distribution ($P > 0.05$, df = 10, $\chi^2 = 16.886$).

Body Weight & Gonad Index

The mean damp weight of the body wall stripped of viscera and devoid of coelomic fluid was 312.9 gm, S.E. = 76.6, n = 19. The degree of correlation between the mean body weight and the mean gonad volume was not significantly different from zero (Spearman rank correlation coefficient $r = 0.4246$, df = 17, $P > 0.05$). At the height of the reproductive season the mean female gonad index was significantly larger than the mean male gonad index (Kruskal-Wallis $H = 6.9563$, df = 1, $P < 0.01$) (Fig. A.2).

Figure A.1. Relative proportion of male and female *Parastichopus californicus* in each monthly sample collected at Woodlands, Indian Arm, B.C. from May 1982 through November 1983.

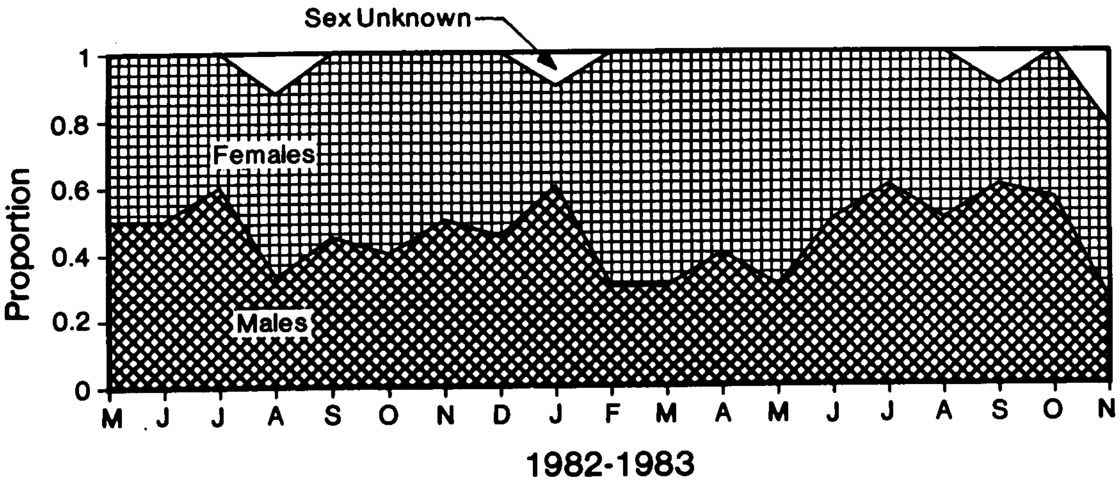
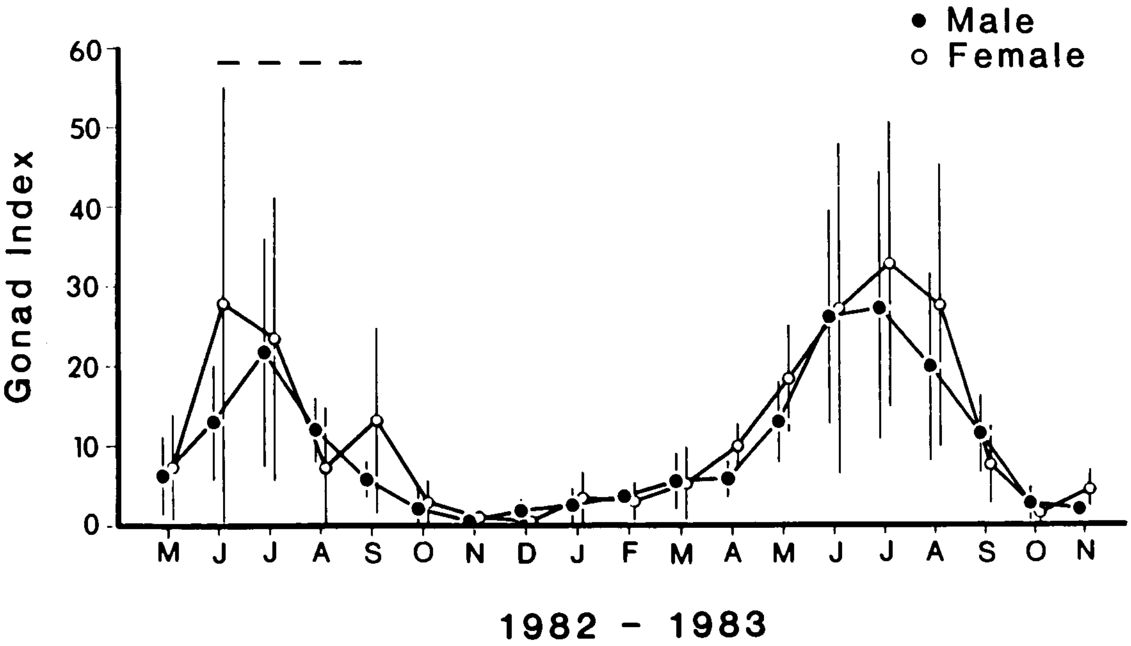

Sex Unknown
Females
Males
Proportion
1
0.8
0.6
0.4
0.2
0
M
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
O
N
1982-1983

Figure A.2. Mean monthly gonad index for adult male and female *Parastichopus californicus* collected from Woodlands, Indian Arm, British Columbia. Generally 10 animals were examined each month. Figure 1 gives the proportion of animals by sex so examined. The span of *in situ* spawning observations (dashed line) for the 1982 reproductive season is also plotted. Error bars represent one standard deviation about the mean. The points on the graph are slightly offset to better visualize individual values and to eliminate overlapping of error bars.



● Male
○ Female
Gonad Index
60
50
40
30
20
10
0
M
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
O
N
1982 - 1983

Reproductive Pattern

The gonad index begins to rise in January or February when most animals within the population have regenerated viscera and are actively feeding (Fankboner and Cameron, In Press). Maximum gonad size occurs in June and July which is also the period when most spawning activity is observed. The gonad begins to decrease in size in August, and is reduced to its smallest size in November and December (Fig. A.2). During this time visual determination of sex may be difficult as the coloring of both male and female gonad tubules have darkened to a uniform brownish-grey. Length and diameter of the tubules are markedly reduced at this time.

Vitellogenesis

Vitellogenesis occurs seasonally, and may be divided into four phases (Tanaka 1958). First there is a resting phase that is manifested within small ovarian tubules located anteriorly on the gonad basis. This phase is most obvious in October when small oöcytes ≃ 45 μm diameter are present within small ovarian tubules ≃ 0.5 mm in diameter (Fig. A.3). From November to the beginning of summer (June) there is a growth phase in which the oöcytes increase in size to ≃ 180 μm diameter. The length and branching complexity of ovarian tubules increases also with a concomitant expansion of the tubule diameter to ≥ 1 mm.

Vitellogenic activity decreases at the end of the growth phase as evidenced by the presence of few small oöcytes possessing the characteristic germinal vesicle and prominant nucleolus. The ovarian tubule reaches its greatest diameter (2.5 to 3.0 mm) in mid-summer during the ripe phase (Fig. A.4). At this stage spawning may occur at any time and spent ovarian tubules are common late in the ripe phase (Fig. A.5). After spawning has ended a degeneration phase occurs (cf. Fankboner and Cameron, in press) in which the fecund tubules degenerate and are lost. This phase may overlap the resting and early growth phases of newly maturing ovarian tubules.

Spermatogenesis

The spermatogenic cycle in *P. californicus* may be characterized by four phases of activity similar to those proposed by Buckland-Nicks *et al*. (1984) for the brittle star *Amphipholis squamata*. First there is a proliferation phase which may begin as early as November. Spermatogenesis begins and evidence of active cell division frequently can be seen in sections from testes collected during this phase of activity (Fig. A.6). The tubules of early proliferating testes are approximately 250 μm in diameter. Proliferation, as a distinct phase, lasts until late December or January at which time differentiation begins. During this period spermatogenesis proceeds towards the center of the tubule lumen. Spermatogonia are generally positioned at the tubule wall with spermatocytes, spermatids and spermatozoa stratified inwards toward the center of the tubule (Fig. A.7) (cf. Atwood, 1975). Convolutions of the tubule wall increase the surface area of the germ layer within the tubules (Fig. A.8). This latter phase extends well into June and possibly early July with a great deal of overlap observed between these two phases. During proliferation and differentiation the testicular tubules increase in overall length, branching complexity, and diameter.

Differentiation is followed by an evacuative phase wherein active spermatogenesis has terminated, and the testicular tubule wall is very thin. The lumen of the tubule is densely packed with mature spermatozoa, and the testicular tubules are at their maximum diameter ≃ 1.5 - 2.0 mm (Fig. A.9). Finally there is an aspermatogenic phase occurring in September and October, in which residual spermatozoa fill the lumina of very thin walled testicular tubules approximately 1 mm in diameter. The evacuative phase may be separated from the aspermatogenic phase in that within the tubule lumen of the latter residual spermatozoa appear to condense or coalesce and stain very intensely with Richardson's stain. Aspermatogenic tubules atrophy and are lost at the end of the reproductive season. Often fragments of atrophied tubules were observed within the coelomic fluid of males collected in October or later. Occasionally aspermatogenic tubules were noted from late November and early December.

Figure A.3. Growth phase of oöcytes within an ovarian tubule from October. Vitellogenesis is beginning and these oöcytes will be ready to spawn at the end of the current reproductive season. Scale bar, 380 µm.

Figure A.4. Mature ovarian tubule in the ripe phase ready to spawn. Those oöcytes that have been sectioned through the nucleolus are nearly all the same diameter and the ovarian wall has become very thin. Vitellogenic activity has all but stopped. Mature oöcytes from the center of the tubule lumen were lost during the collection, fixation, and embedding process. Scale bar, 760 µm.

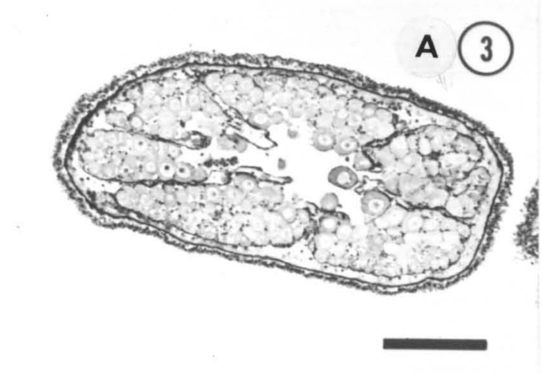
Figure A.5. Cross section of an ovarian tubule from September. A few residual oöcytes left after spawning may be observed within the tubule lumen. Scale bar, 840 µm.

Figure A.6. Apparent metaphase arrangement of chromosomes (arrow) within a spermatogonium or spermatocyte of a proliferating testicular tubule from January. Scale bar, 17 µm.

Figure A.7. Cross-section of a differentiating testicular tubule. Note columnar nature of the spermatogenic process with spermatogonia and primary spermatocytes located at the wall of a tubule convolution. Secondary spermatocytes, spermatids and spermatozoa occur nearly sequentially out to the center of the tubule lumen. Note the flagella of spermatozoa within the tubule lumen. Scale bar, 45 µm.

Figure A.8. Cross-section of a proliferating testicular tubule. Note the folding of the tubule wall which increases the surface area of the germ layer within the tubule. Scale bar, 180 µm.

Figure A.9. Mature testis prior to spawning. Tubule wall convolutions have disappeared, and the tubule lumen is full of mature spermatozoa. Scale bar, 500 µm.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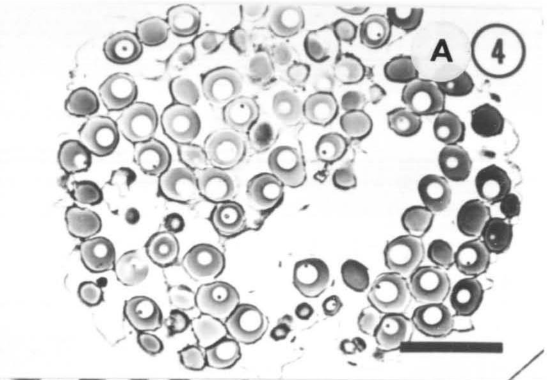
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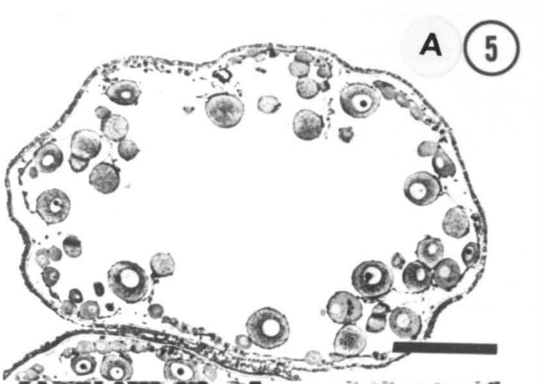
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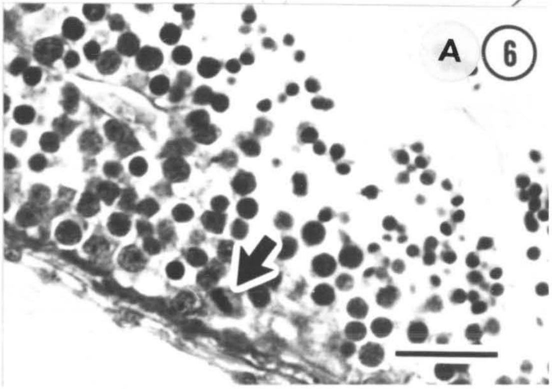
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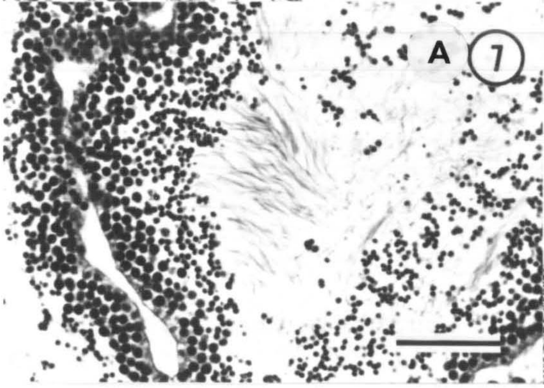
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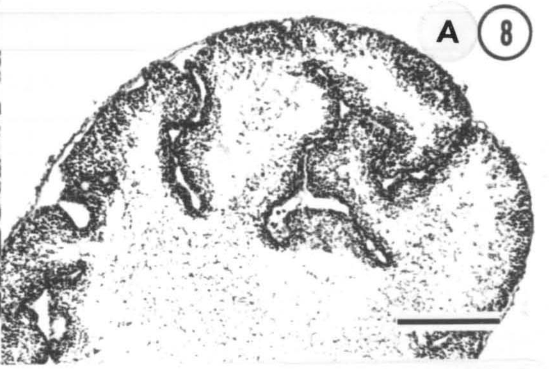
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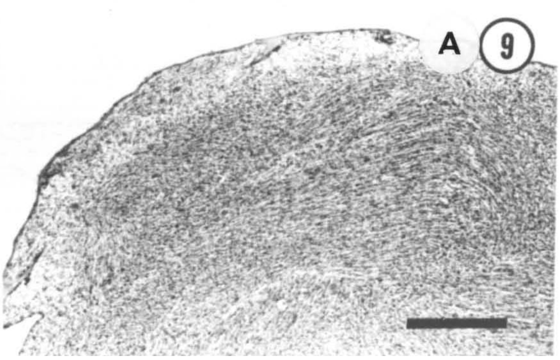
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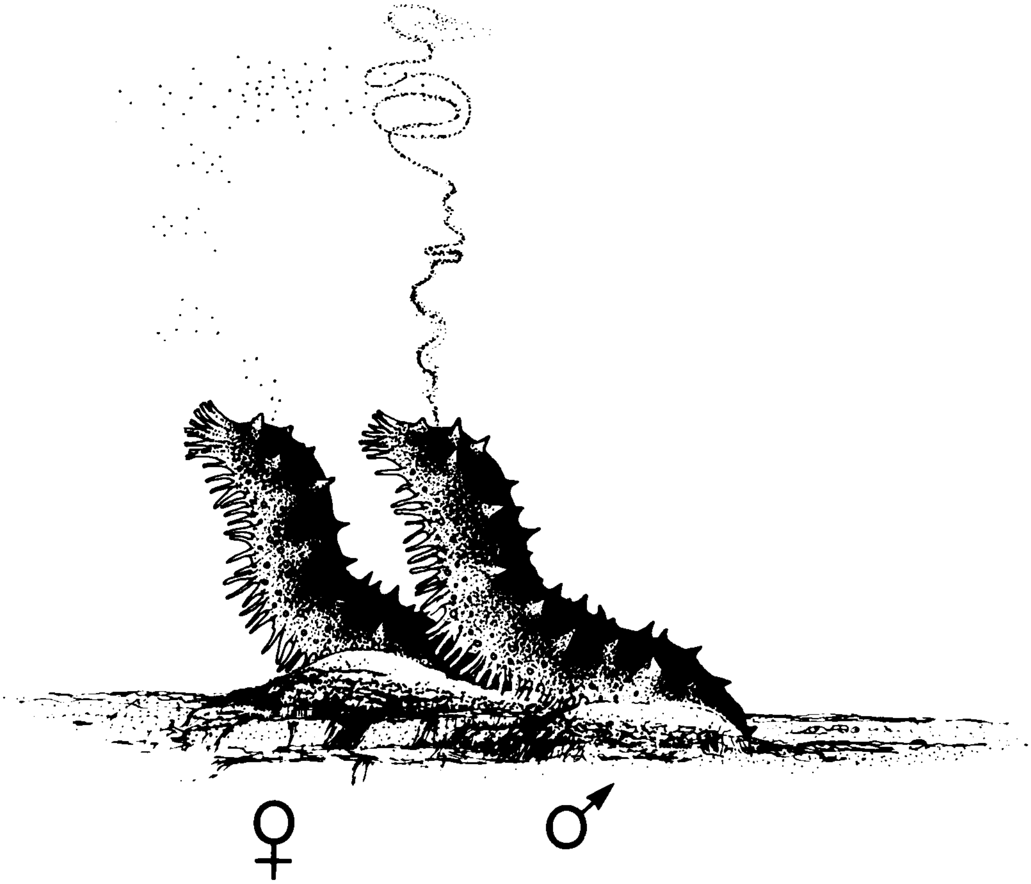
Spawning

Behavior

Spawning *P. californicus* assume a distinctive posture as they release their gametes into the water column (Fig. A.10). The anterior one-third to one-half of the animal is raised vertically from the substratum with the "head" (tentacular crown) curved forward toward the substratum. In profile, the trunk of *P. californicus* has a sigmoid shape which Mortensen (1937) has described as being similar to the attack postion of a cobra (cf. Hendler and Meyer 1982; Mosher 1982). The gonopore opens at the point of maximum elevation above the substratum on the anterior dorsal surface of the animal. Spawning occurs simultaneously amongst a portion of the population, but epidemic spawning by a whole population was never observed.

When released from the gonopore, sperm form a continuous streaming thread. The white cream color of the sperm rendered it extremely visible, and spawning males could be detected from a few meters away by both their characteristic spawning posture and the presence of sperm. Spawned oöcytes, which are a light orange color, are nearly invisible *in situ* and disperse within the water immediately upon release. Spawning females were initially detected by their characteristic spawning posture and then confirmed by observing regular spurts of oöcytes exiting

Figure A.10. Spawning posture and representation of spawned gametes in *Parastichopus californicus*. Juxtaposition of the male and female in the drawing is for illustrative convenience, and not indicative of spawning behavior *in situ*.



the gonopore (Fig. A.10). No aggregation of animals during spawning was noted, and often relatively great distances (≃ 10 m) separated spawning individuals.

Environmental Factors

During the 1982 reproductive season SCUBA dives were made to observe or collect *P. californicus* on 41 of the 92 days from June 1 through August 31. Individual spawning observations were subjectively correlated with surface weather conditions, and a pattern was noted wherein spawning occurred during periods of bright sunshine. The minimum number of days with five hours or more of bright sunshine (chosen arbitrarily after examination of the data) preceding a spawning observation was four ($\bar{x}$ = 11.6, S.D. 3.3 hours of sunshine per day) (Fig. A.11 and Table A.1). All observed spawning events during the period occurred on days of bright sunshine ($\bar{x}$ = 12.9, S.D. 1.7 hours of sunshine per day, n = 7) (Table A.1). Spawning animals were observed at between 5 and 12 m below 0 chart datum (4 to 11 m below MLLW).

On March 26, 1982 a single male *P. californicus* was observed spawning while diving at Woodlands Bay, and although this spawning occurred on a cloudy day, the previous 10 consecutive days were bright and sunny ($\bar{x}$ = 8.14, S.D. 1.82 hours of bright sunshine per day, nearly maximum for that time of year) (Table A.1). In addition, this spawning event was associated with a localized phytoplankton bloom of such

Figure A.11. Daily hours of bright sunshine (compiled from official Environment Canada daily weather summaries for the Vancouver, B.C. area) compared with spawning or no spawning observations from 41 SCUBA dives between June 1 and August 31, 1982. Spawning events were generally preceded by a minumum of two days with less than five hours of bright sunshine (dashed line).

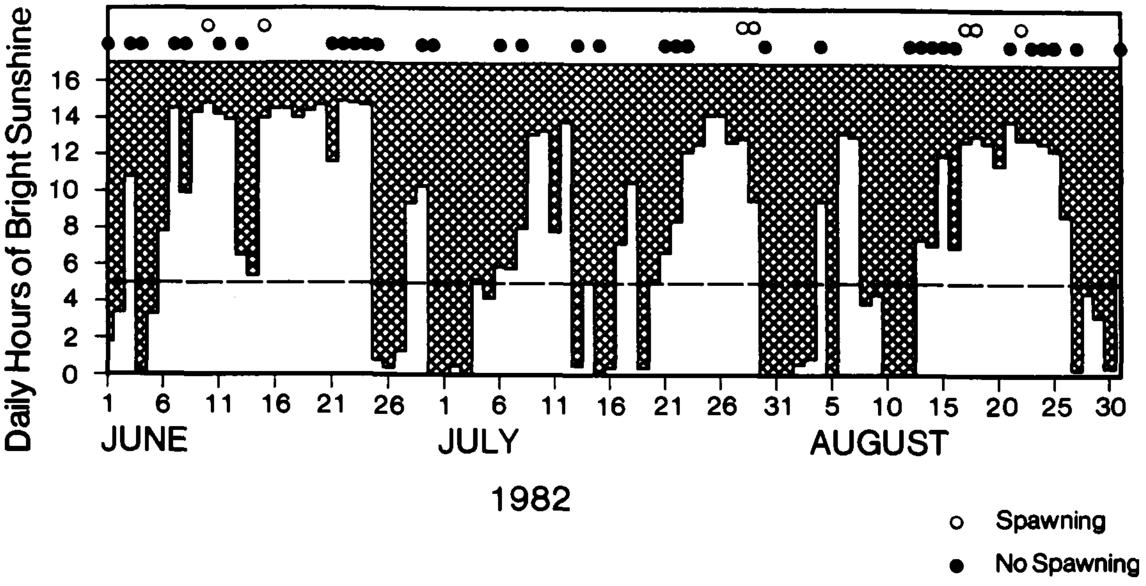

Daily Hours of Bright Sunshine
0
2
4
6
8
10
12
14
16
1
6
11
16
21
26
1
6
11
16
21
26
31
5
10
15
20
25
30
JUNE
JULY
AUGUST
1982
○ Spawning
● No Spawning

Table A.1. 1982 *in situ* spawning observations.

Date	Location	Weather & hours of sunshine†	Number of days previous to observation having > 5 hours bright sunshine, and $\bar{x}$ hours ± SD†	Sea Cucumbers Observed
Mar 26	Woodlands	Cloudy-rain	10, 8.1 ± 1.82	1 male
Jun 10	Croker Is.	Sunny-14.2	4, 11.6 ± 3.32	Spawning observed‡
Jun 15	Woodlands	Sunny-12.9	9, 11.3 ± 3.85	Spawning observed‡
Jul 28	Kelvin Grove	Sunny-12.9	8, 10.8 ± 3.50	3 males; 4 females
Jul 29	Woodlands	Sunny- 9.5	9, 11.0 ± 3.35	1 male; 3 in posture
Aug 17	Woodlands	Sunny-12.8	4, 8.4 ± 2.42	5 males; 1 female
Aug 22	Kelvin Grove	Sunny-12.9	9, 10.7 ± 2.73	1 female

†These data were compiled from official Environment Canada weather summaries for the Vancouver, B.C. area.

‡Animals in spawning posture as well as spawning were observed, but no exact count of individuals by sex was taken.

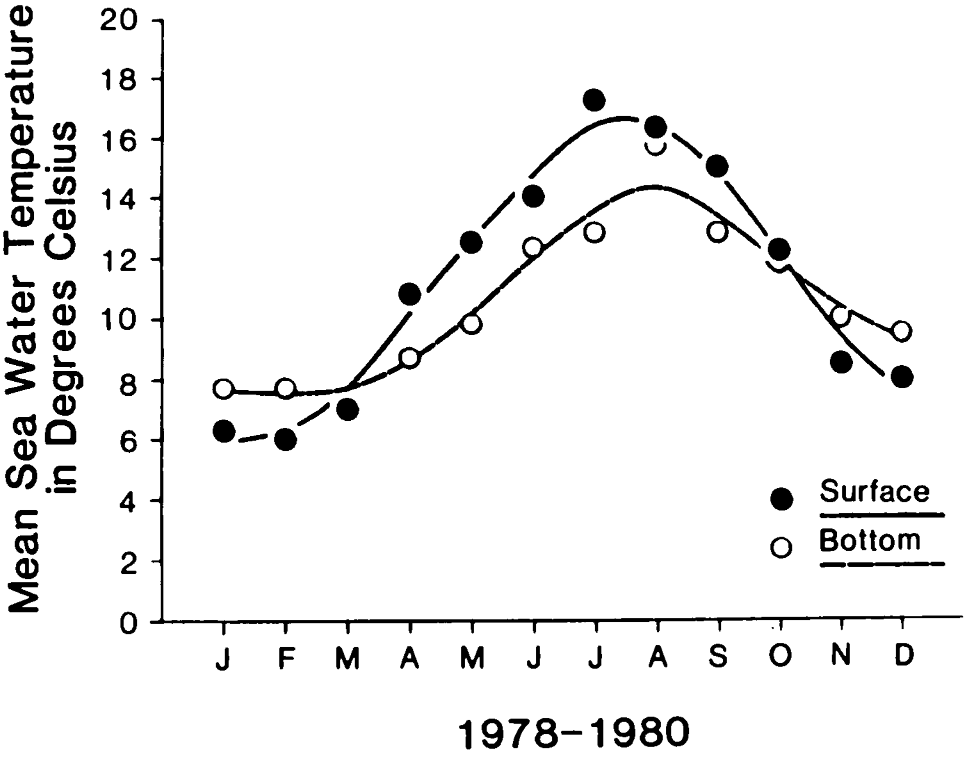
intensity that the top five meters of water were like a thin "pea soup", with the ambient visibility reduced to less than one meter within this layer. Under this layer of phytoplankton the visibility increased dramatically and the major limiting factor to horizontal visibility seemed to be the reduction in light penetration caused by the surface concentration of phytoplankton.

Sea Water Temperature

Ambient sea water temperature at Woodlands Bay fluctuates seasonally with a single maximum and minimum each year. During the spring and summer average sea water temperature for 1978 through 1980 was higher on the surface than at a depth of 3 - 5 m (Fig. A.12), and significantly higher than throughout the majority of the remainder of the year. The temperature at the surface showed a seasonal range of ≃ 12° C, while the temperature at a depth of 3 - 5 m spanned an ≃ 6° range.

Temperature was not directly measured for the time period of the study, but thermal stratification with warm water on the surface was noted during the spring and summer months when this study was being conducted. This thermal stratification was most intense during the period of maximum gonad size and observed spawning, with maximum water temperature, both at the surface and at depth, coinciding with maximum gonad index (Figs. A.2 and A.12).

Figure A.12. Mean monthly sea water temperatures taken from the surface and at a depth of > 3 m at Woodlands, Indian Arm, B.C. for the years 1978 through 1980.


Mean Sea Water Temperature in Degrees Celsius
20
18
16
14
12
10
8
6
4
2
0
J
F
M
A
M
J
J
A
S
O
N
D
1978–1980
Surface
Bottom

IV. Discussion

P. californicus exhibits an annual reproductive cycle that is characteristic of most temperate shallow water invertebrates, but is apparently atypical of animals living in the waters of British Columbia in that many species from this locality spawn in the winter or early spring (Giese and Pearse 1974). The gonad cycle in both male and female *P. californicus* follows the same developmental process with maximum gonad size attained in the summer. Gonad volume is independent of body size and therefore the gonad index is an accurate measure of the seasonal reproductive effort. Female *P. californicus* have a significantly larger gonad index at maturity than do males indicating a greater reproductive effort for the female than the male.

Spermatogenesis occurs annually, and new spermatozoa are formed within the testis the year they are to be spawned. At the end of the spawning season residual spermatozoa were often observed in some degenerating tubules, but we saw no indication that this sperm was stored for future use. The entire process of oögenesis was not directly observed, but Smiley (1984) has concluded that oögenesis in *P. californicus* occurs in small primary and secondary ovarian tubules located to the anterior of the fecund (or mature) tubules on the gonad basis (see also Mitsukuri 1903). The secondary tubules become the fecund tubules of the next years reproductive season.

The 1:1 ratio of sexes within the adult population of *P. californicus* is similar to that reported for other epibenthic holothurians (Jespersen and Lutzen 1971; Conand 1982; Engstrom 1982; Mosher 1982). Additionally, da Silva *et al*. (in press) have found that this same population of *P. californicus* wanders randomly throughout its habitat without any directional preference or intraspecific influence. A 1:1 sex ratio within a randomly diffusing population should disperse the sexes in such a manner that a relatively even mixture of sexes would occur. This behavior should enhance the probability for successful mixing of the spawned gametes, especially in a species where there is no aggregation of animals during spawning.

Barnes (1975) has stated that the timing of spawning by invertebrates is independent of prior rhythmic reproductive physiology, and that some type of "clamping mechanism" (independent stimulus) is required to induce spawning. Temperature, undoubtedly, is an important factor in the early development of the larvae and therefore significant in the timing of the reproductive cycle, but not necessarily to spawning in particular (Giese 1959; Giese and Pearse 1974; Barnes 1975).

Sea water temperature increases to a peak during the spawning season within Indian Arm fjord where the surface waters are significantly warmer than those at the depths where *P. californicus* commonly occurs (3 - 30m) (Gilmartin 1962). But as indicated above, there is probably no direct relationship between temperature and spawning in *P. californicus*. The relatively gradual seasonal change in sea water temperature at depths greater than 3 m (Gilmartin 1962) would seem unlikely to trigger the pattern of partial spawning observed within the populations of this sea cucumber that were studied.

Spawning in *P. californicus* occurred in concert with an increase in the duration of daily bright sunshine (Fig. A.11 and Table A.1), and Binyon (1972) has stated that many holothurians spawn in probable response to an increase in the intensity of the ambient light regime. The one off-season *in situ* spawning event we observed was also preceded by a significant period of bright sunshine and a localized phytoplankton bloom. McDaniel (1982) in a popular Canadian diving magazine reported a similar occurrence that involved large numbers of *P. californicus* spawning in the early spring coincident with a period of bright sunshine and in the presence of an obvious phytoplankton bloom (see Thorson 1950 p. 2).

Himmelman (1975) has shown that the presence of phytoplankton will initiate spawning in the echinoid *Strongylocentrotus droebachiensis*, and he suggests that a pheromone-like substance released by the phytoplankton is the factor that initiated spawning. If phytoplankton production is the mechanism that initiates spawning in *P. californicus*, localized periodic blooms could trigger the pattern of spawning observed in this sea cucumber. In support of this Mann (1982) has indicated that near-shore phytoplankton production may become stratified in areas where there is a significant warming of the surface waters. In such areas localized nutrient depletion occurs which limits phytoplankton production. He also states that factors such as wind stress and fresh water runoff, which generally fluctuate in intensity and duration, can initiate localized upwelling events that replenish nutrients within these areas. These factors, combined with fluctuations in sunlight, could produce sporadic, small scale phytoplankton blooms following nutrient replenishment during periods of reduced sunlight.

Gilmartin (1962) has reported that there is significant thermal and haline stratification of the top 1.5 - 2.5 m of water in Indian Arm. He also indicates that this is typical (at least thermally) of most mainland B.C. coastal waters. Gilmartin (1964) further reports that the factors controlling primary production within Indian Arm are physical, affecting the stability of the water column which affects the replenishment of nutrients within the euphotic zone. Light intensity could therefore be an indirect factor in stimulating the release of gametes by some echinoderms as phytoplankton productivity would undoubtedly increase during periods of bright sunshine following nutrient replenishment. It seems evident that such may be the case for the initiation of spawning in *P. californicus*.

Reports on the viability of gametes collected by dissection over the spring and summer as well as other reports of spawning by *P. californicus* (Table A.2) would seem to indicate a

Table A.2. Reports of spawning or the collection of ripe sexual products from *Parastichopus californicus*.

Observation	Source
Ripe gametes were noted in June.	Mortensen, 1921.
Spawning reported from June and July.	Courtney, 1927.
Ripe gametes noted in May.	Johnson and Johnson, 1950.
Spawning was observed in April, but stated that spawning occurs in the Spring and early Summer.	McDaniel, 1980.
Spawning was reported in the early summer between May and the middle of July.	Smiley, 1984.
Spawning noted in late June.	Fernald, (In Smiley)

reproductive season somewhat longer than herein suggested. Development of oöcytes collected from immature or nearly mature gonads may occur, but does not necessarily delineate the natural length of the spawning season. On the other hand, spawning, as we have observed, can occur over a broad time span in apparent response to specific environmental triggers.

Where the onset of spawning is dependent upon some exogenous environmental event for stimulation, one might expect the animals to be mature over an extended period of time. This would ensure that the reproductive effort of the particular population has the greatest probability for success (Barnes 1975). In this regard, Strathmann (1974) has suggested that if the spread of sibling larvae by an organism is beneficial for the survival of the species, asynchronous spawning occurring over some extended period of time would be one mechanism for insuring such dispersal. We suggest that this may be the case for the commercial sea cucumber *P. californicus*.

B. EMBRYONIC DEVELOPMENT AND PROCESSES OF METAMORPHOSIS

I. Introduction

Due to the relative ease of their collection, fertilization and rearing, gametes and larvae of echinoderms have traditionally served as the primary organisms for observing and studying the processes of fertilization and early embryonic development in deuterostome eucoelomates (Tyler and Tyler, 1966; Brachet, 1975; Czihak, 1975). The holothuroidea, however depart from the echinodermata as a whole in that those species with planktotrophic larvae have always proved difficult to study. Methods for the stimulation of spawning in these animals are generally unknown, and fertilization of oöcytes collected by dissection is also difficult and often unreliable (MacBride, 1914; Mortensen, 1921; Courtney, 1927; Johnson and Johnson, 1950; Chia and Buchanan, 1969). These problems have caused developmental biologists and embryologists to generally avoid holothurians concentrating on the more easily studied echinoids, asteroids, and to a lesser extent ophiuroids (Kumé and Dan, 1968; Holland, 1981).

Recent expanding interest in the fishery potential of sea cucumbers, and the potential for the mariculture of commercially exploitable species, demands reliable information on the processes of development regardless of the degree of difficulty in collecting such information. In this regard, reported herein is the development and some processes of organogenesis within the embryos, auricularia, doliolaria, and pentactula larvae of *P. californicus* as observed from *in vitro* studies conducted at Simon Fraser University, Burnaby, British Columbia, Canada. The life historical consequences of the observed developmental process are also discussed.

II. Materials and methods

Collection and Fertilization of the Oöcytes

Adult *P. californicus* were collected by SCUBA from Woodlands, Indian Arm Fjord, British Columbia (depth 5 - 15 m), and were transported to the laboratory at Simon Fraser University for dissection and removal of the ovaries and testes. Portions of the excised ovaries of numerous female animals, containing free oöcytes, were placed into beakers containing 1200 ml of Millipore filtered (0.45 µm) sea water then stirred at 60 rpm for 2 hours. Following this, five ml of dilute sperm solution (M. Strathmann, 1978) were added to each beaker and left for approximately four hours. Ovarian fragments were removed by filtering the cultures through 500 µm mesh Nitex screen. The oöcytes were then washed and placed into fresh Millipore filtered sea water. Some cultures were treated with either echinoderm (*Pycnopodia helianthoides*) radial nerve factor (RNF) (Strathmann and Sato, 1969) or with 10^{-3} M dithiothreitol (DTT) (Maruyama, 1980) to induce germinal vesicle breakdown and increase the fertility of the oöcytes. Naturally spawned oöcytes were collected on one occasion *in situ* by pipette and bulb as they exited the gonopore of a spawning female. This animal was then placed, while under water, into a large plastic bag and transported to the laboratory at SFU.

Culturing Techniques

Developing embryos and larvae were maintained at ≃ 10 - 12° C and aerated by slow stirring. Culture salinity was maintained at *in situ* levels of 27 to $28^{\circ}/_{oo}$. Developing larvae were fed from cultures of the marine flagellates *Dunaliella salina*, *Monochrysis lutheri*, and *Isochrysis galbana* (see Strathmann, 1971) aquired from Carolina Biological Supply, Co.

Microscopy

Live embryos and larvae were examined and photographed using Nomarski, differential interference contrast microscopy, while preserved embryos and larvae were examined via scanning electron microscopy (SEM). Larvae for SEM examination were fixed for 15 minutes in 2% glutaraldehyde buffered with 0.2 M sodium cacodylate (pH 7.4) followed by post-fixation for 15 minutes in 2% osmium tetroxide buffered with modified Dorey's solution B (Fankboner, 1978). Larvae were freeze dried, then stub-mounted, gold coated and examined and photographed on a Joel JSM-35 scanning electron microscope.

III. Results

Description of the Oöcytes

The oöcytes of *P. californicus* are translucent spheres measuring ≃ 185 μm in diameter which are surrounded by a jelly coat (JE) of about 30 μm in thickness (Fig. B.1). Oöcytes collected from the excised ovaries of sea cucumbers are predominantly enclosed within an ovarian capsule. Shortly after exposure to sea water these oöcytes exit the capsule by squeezing through a small pore in the capsule wall distorting considerably during the process (Fig. B.2). Oöcytes at collection possess a distinct germinal vesicle (GV) and nucleolus (NU) (Figs. B.1 and B.2). Very few (< 0.01%) of the hundreds of thousands of oöcytes, collected as above, had undergone germinal vesicle breakdown within the ovarian tubules prior to collection.

Examination of naturally spawned oöcytes two hours after *in situ* collection showed the germinal vesicle to be intact and the nucleolus still present. None of these oöcytes were enclosed within capsules, and visually did not appear to be different from oöcytes observed two hours after collection by dissection.

Fertilization

Treatment of oöcytes collected by dissection with RNF or with DTT did not appear to facilitate fertilization in any of the cultures so treated. *In vitro* fertilization levels were consistently quite low (< 5%), but a sufficient number of apparently healthy embryos were obtained to complete this study.

Approximately two hours after the *in situ* collection of naturally spawned oöcytes an attempt to inseminate them in the laboratory was made but proved unsuccessful. Five hours after collection of these oöcytes the sea water contained within the bag used to transport the spawning female to SFU was found to contain numerous 2 cell embryos. These developed normally and ultimately metamorphosed into pentactula larvae.

Development

First cleavage which is holoblastic complete, and meridional is completed four hours after insemination. Second cleavage, which occurs at a right angle to first cleavage, is completed at six hours after fertilization. Subsequent divisions follow at about 2 to 3 hour intervals (Table B.1) to 16 hours post-fertilization, ≃ 64 cell blastulae (Fig. B.3). After 16 hours development becomes asynchronous in both cleavage pattern and time elapsed to the various developmental stages.

Table B.1. Composite developmental schedule derived from five separate *in vitro* cultures of the embryos and larvae of *Parastichopus californicus* reared at 10 - 12° C.

Time After Fertilization	Developmental Stage
3- 4 hours	First cleavage completed.
5- 6 hours	Second cleavage completed.
13-14 hours	Fifth cleavage completed, (32 cells).
16-17 hours	Most embryos† ≃ 64 cells‡
19-20 hours	Most embryos† ≃ 128 cells‡
24 hours	Pre-hatched blastula.
40 hours	Gastrulation beginning, blastopore commonly visible and invagination of the archenteron started.
64 hours	Hatched gastrula.
≃ 4 days (90 hours)	Gastrula with well developed archenteron, the hydrocoel and esophagus are beginning to differentiate.

†Asynchronization of developmental time in simultaneously fertilized oöcytes was common.

‡Asynchronization of cleavage common after 32 cell stage (fifth cleavage).

Table B.1. Continued.

Time after Fertilization	Developmental Stage
6 days	Early auricularia larva with an approximately lateral ciliated band. Invagination of the mid-ventral epithelium to form the buccal cavity has begun.
13 days	Fully developed feeding auricularia.
65-125 days	Metamorphosis to doliolaria larvae may occur at any time.
65-125 days	Very short-lived doliolaria (24 - 48 hours) gives rise to a pentactula larvae.

Twenty hours post-fertilization the majority of the embryos in culture were ≃ 128 cells, and 24 hours post-fertilization pre-hatched blastulae were the predominant stage observed (Fig. B.4).

After forty hours the blastopore forms on the pre-hatched embryo, and invagination of the archenteron begins (Fig. B.5). Twenty four hours later (64 hours post-fertilization), an entirely ciliated hatched gastrula dominates the cultures, and at ≃ 90 hours the larvae assume a characteristic cylindrical shape reaching ≃ 270 µm in length along the animal/vegetal pole axis by 200 µm in diameter (Fig. B.6). Coincident with the invagination of the archenteron (AR) a ball of cells forms within the blastocoel at the tip of the archenteron (Fig. B.7). These cells eventually form the esophagus of the auricularia larvae, and even though they appear to form a distinct vesicle, they are presumed to be part of the developing archenteron and not a separate structure. The archenteron is approximately one quarter the length of the blastocoel at this time.

When the hatched gastrula is 5 to 7 hours old the invaginating archenteron (AR) may reach approximately one half the length of the blastocoel. The ball of cells at the tip of the archenteron forms a hollow anterior vesicle (AV) ≃ 75 µm in diameter (Fig. B.8), which eventually forms the esophagus of the auricularia larva.

While the archenteron lengthens, an evagination at the junction of the archenteron and the anterior vesicle outpockets to form a small lateral vesicle (LV) on the left of the larval visceral mass. This lateral vesicle is the primordium of the hydrocoel and the left somatocoel. Presumedly, the left somatocoel gives rise to the right somatocoel, but this was not directly observed.

Six day old larvae measure ≃ 330 μm long by 195 μm in approximate diameter and are bilaterally configured. A distinct ciliated band (CB) encircles this stage from the anterior to the posterior in a roughly lateral position. The area initially enclosed by the ciliated band appears equivalent to the echinoid oral field as described by Czihak (1971). In some of the larvae the ciliated band has initiated its lobed development characteristic of older larvae (Fig. B.9). As development progresses the mid-ventral epithelium of the larva invaginates and by so doing fuses with the anterior vesicle to form the buccal cavity. This process appears to occur simultaneously with migration of the anterior and posterior ciliated margins of the larva to their mid-ventral postion. With further differentation the stomodaeum opens, the stomach (ST) enlarges, and the entire visceral organ complex looks much as it will in older larvae. A muscular cardiac sphincter (CS) forms between the esophagus and the stomach at the junction of the anterior vesicle (esophagus) (EO) and archenteron (stomach/intestine) (ST). The blastopore/anus (AN) is still located at the posterior of the larva (Fig. B.9).

At thirteen days of age the larvae have developed into well-differentiated feeding auricularia (Figs. B.10 and B.11). This maturation involves the growth of the ciliated band via migration of the anterior ciliated margin of the six day larvae to an approximately mid-ventral position forming the pre-oral transverse ciliated band (PRE). While the posterior ciliated margin of the six day larvae moves to a nearly mid-ventral position as well, forming the post-oral transverse ciliated band (PST). This process appears to involve the folding over of both the anterior and posterior ciliated margins of the larvae to their nearly mid-ventral positions forming both the buccal cavity (BC) and the suboral pocket (SP) (Fig. B.12). The anterior deflection of the intestine and anus to its mid-ventral position just to the posterior of the post-oral transverse ciliated band appears to be one result of this process (Figs. B.10 and B.12). Additionally, the lateral margins of the larvae at the ciliated band fold and curl forming lateral processes adding additional complexity to the ciliated band (Figs. B.10 and B.11). Body growth maintains an overall box shape to the larvae and provides for some increase in size as the 13 day larvae are now ≃ 430 μm long by 270 μm wide.

The larval digestive system is complete and functional in the early auricularia. The anus (AN) opens in a small recession of the ventral epithelium near the post-oral transverse ciliated band (Fig. B.12). The hydrocoel has elongated along the anterior/posterior axis on the left side of the esophagus and

Figure B.1. Mature oöcyte with jelly coat (JE), note germinal vesicle (GV) and nucleolus (NU). Scale bar, 50 µm.

Figure B.2. Mature oöcyte squeezing through pore in capsule. Scale bar, 55 µm.

Figure B.3. 64 and 128 cell embryos. Scale bar, 85 µm.

Figure B.4. Pre-hatched blastula. Scale bar, 90 µm.

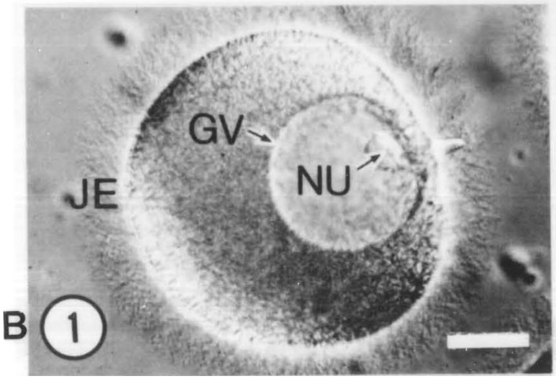
Figure B.5. Early invagination of the archenteron in a pre-hatched embryo. Scale bar, 75 µm.

Figure B.6. Elongate embryo with ball of cells (anterior vesicle) at tip of invaginating archenteron (arrow). Scale bar, ≃ 75 µm.

Figure B.7. Close up of early archenteron and differentiating anterior vesicle (arrow). Scale bar, 140 µm.

Figure B.8. Elongate embryo with well developed hollow "anterior vesicle" (AV) and archenteron (AR). Scale bar, 75 µm.

Figure B.9. Early beginnings of bilateral appearance, note generally lateral ciliated band (CB), esophagus (EO), and Stomach (ST). Also faintly visible is the hydrocoel/somatocoel primordium (HS). the blastopore is still located to the posterior (large arrow). Scale bar, 55 µm.



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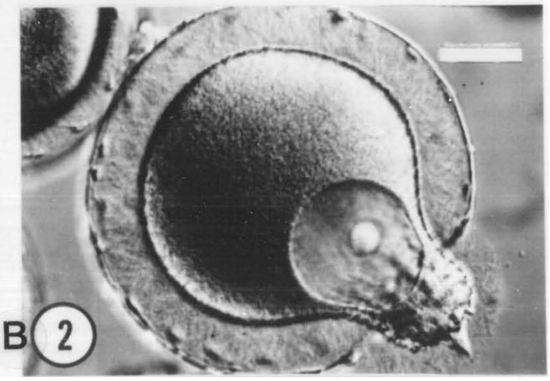

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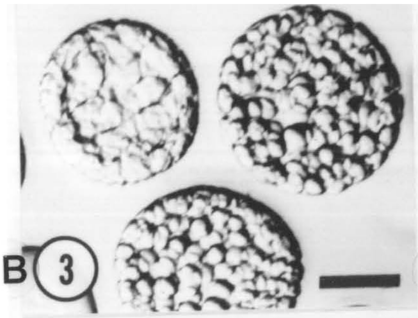

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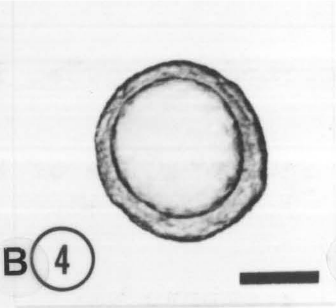

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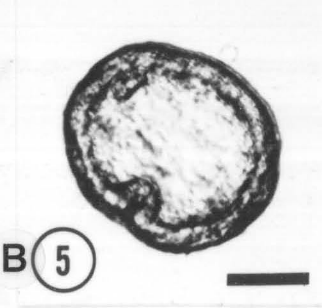

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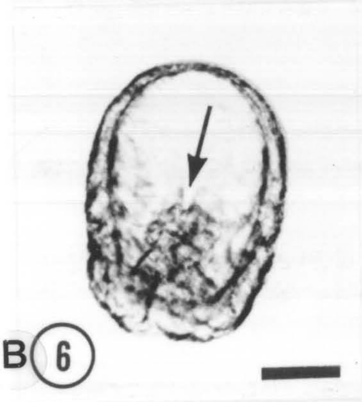

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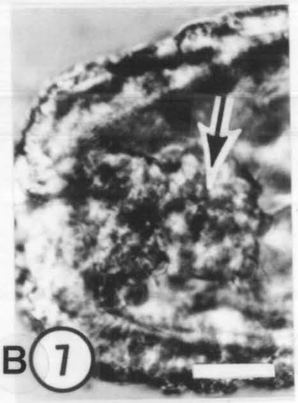

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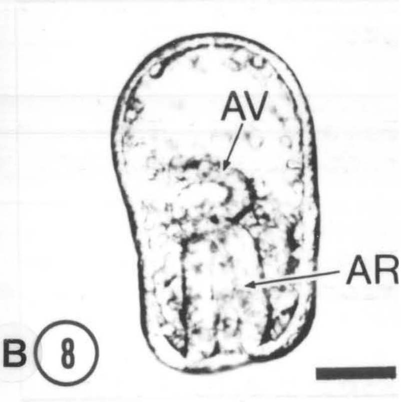

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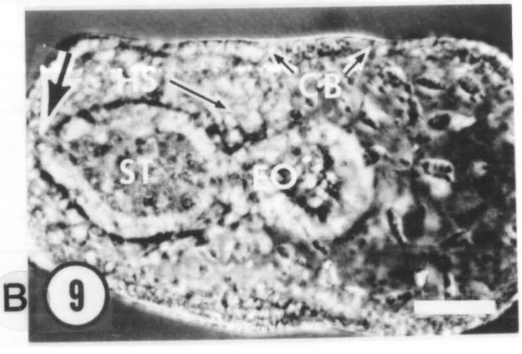
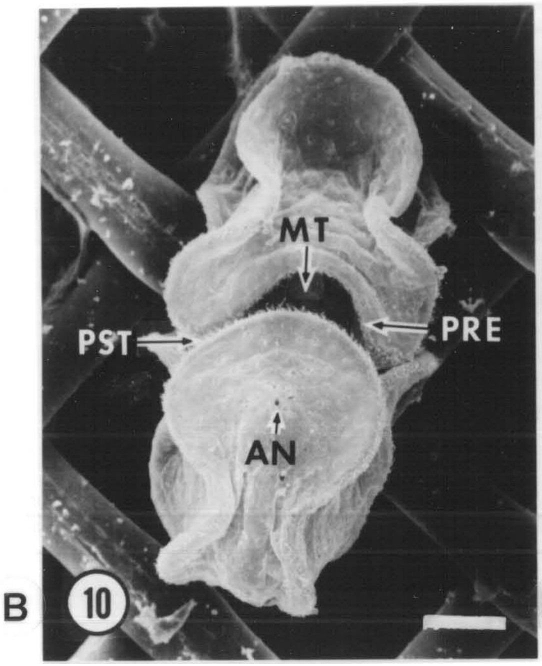


B 9

Figure B.10. SEM ventral view of an auricularia larva of *Parastichopus californicus*. Note region of the mouth (MT), anus (AN), pre-oral (PRE) and post-oral (PST) tranverse ciliated bands. The anterior of the animal is toward the top of the micrograph. Scale bar, 60 μm.

Figure B.11. SEM lateral view of an auricularia larva of *Parastichopus californicus*. Note region of the mouth (MT) and extensive folding and curling of the ciliated band (arrows). The anterior of the animal is to the top of the micrograph. Scale bar, 80 μm.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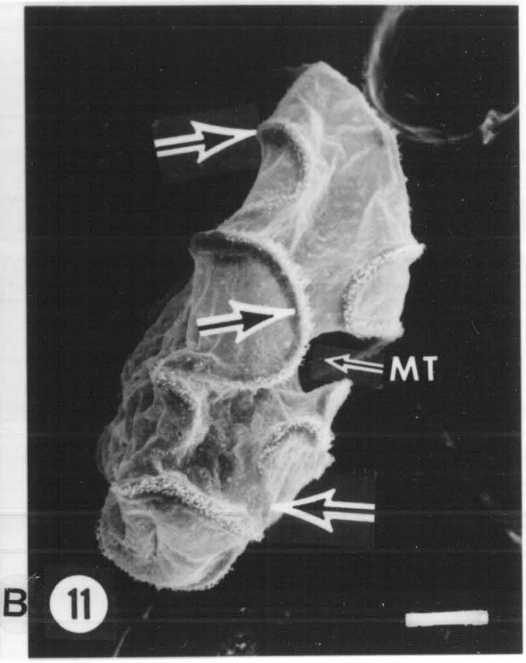
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stomach (ST) (Fig. B.13). The hydrocoelic canal forms to the left of the esophagus, and the hydropore (HP) (dorsal pore) opens to the exterior in an approximately mid-dorsal position. A posteriorly directed finger-like projection of the hydrocoel (HC) pinches off to form the left somatocoel (Fig. B.13). The left and right somatocoels form by the 25th day of development. These larvae now measure ≃ 720 μm long by 500 μm wide.

Growth continues as the larvae age, but there is also some reapportionment of tissue. For instance 60 day larvae are ≃ 750 μm long by about 460 μm wide. During this time little external change is noted, but internally, the hydrocoel and left somatocoel have reached ≃ 170 μm in length. The right somatocoel is about half this long and often difficult to detect. Some ossification of the auricularia may begin about this time as small irregular star-shaped ossicles without fenestrations can often be observed in the extreme posterior of the larvae at the base of the stomach (Fig. B.14). Rod-shaped ossicles can also be seen forming both axially and circumferentially about the hydroporic canal (HP) (Fig. B.15). The auricularia nears metamorphosis as the hydrocoel begins to expand and encircle the esophagus forming lobes that will eventually become the canals of the water vascular system in the primary buccal tentacles of the pentactula (Fig. B.16).

Metamorphosis

Differentation from the auricularia to the doliolaria larva is quite rapid and short lived (24 - 48 hours). The barrel shaped doliolaria which is ≃ 370 μm tall by 210 μm maximum diameter represents a nearly 90% decrease in body volume from the 60 day auricularia, and involves the rearrangement of the ciliated band into five separate ciliated bands that encircle the larva (Fig. B.17). The ventral mouth of the auricularia larva withdraws forming the atrium (AT) into which the primary podia first emerge (Fig. B.18). The larvae have ceased feeding at this time, and the anus has closed. The five primordium of the buccal podia that will soon emerge as the tentacles of the pentactula are visible within the atrium (Fig. B.18).

Metamorphosis from the auricularia through the doliolaria to the pentactula takes only 24 - 48 hours and begins at about 65 days post-fertilization. This process, however, may be delayed at the auricularia stage for upwards of another 60 days, these older auricularia change very little excepting in overall size several of which (120 days old) were nearly 1 mm long. It was noted that metamorphosis occurred throughout this period with new pentactulae settling out continually.

In metamorphosis of the doliolaria to the pentactula, the major changes include proliferation of the water vascular system and loss of the ciliated bands. First, the five primary buccal podia (tentacles) (TN) emerge anteriorly surrounding the mouth.

Next a single tube foot (TF) emerges mid-ventrally at the posterior of the larva (Fig. B.19). The ciliated bands persist for a short while (Fig. B.19), and larvae at this stage may be found swimming within or crawling across the bottoms of their culture vessels. The fully metamorphosed pentactula that results is very similar in size to the pre-metamorphosis doliolaria from which it originated (Fig. B.20).

Behavior and Differentiation of the Pentactula

Newly settled pentactulae actively move via their five primary buccal podia. During feeding, however, they remain anchored to the substratum by a single posterior tube foot (TF) (Fig. B.23) sweeping the substratum with their newly developed tentacles. At metamorphosis few calcareous ossicles are visible within the animal, but 3 - 5 days after metamorphosis fenestrated button ossicles form extensively in the epithelium obliterating all internal structures (Fig. B.21). These primordial larval ossicles later form the table ossicles characteristic of aspidochirote holothurians. The spires of these ossicles protrude out from the body wall but are contained within the epithelium (Fig. B.22).

Figure B.12. Lateral view of an auricularia larva of *Parastichopus californicus*; note the esophagus (EO), stomach (ST), hydropore and hydrocoelic canal (HP), buccal cavity (BC), sub oral pocket (SP) and anus (AN). Scale bar, 70 µm.

Figure B.13. Ventral view of an auricularia larva of *Parastichopus californicus*; note the hydrocoel (HC) with posterior finger-like projection that will form the left somatocoel (arrow). Scale bar, 70 µm.

Figure B.14. Irregular star-shaped ossicle (arrow) within the posterior of an auricularia larvae of *Parastichopus californicus*. Scale bar, 55 µm.

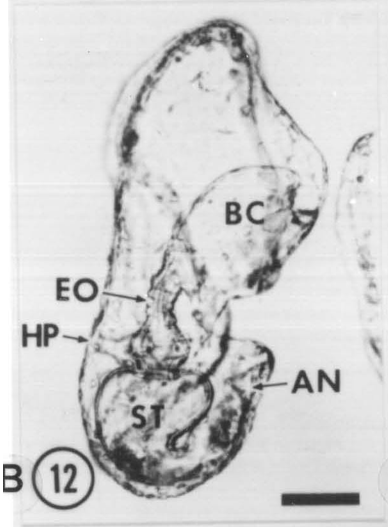
Figure B.15. Rod-shaped ossicles (arrows) around hydroporic canal of the auricularia larvae of *Parastichopus californicus*, note the hydropore (HP). Scale bar, 55 µm.

Figure B.16. Proliferation of the hydrocoel with lobes (arrows) in a nearly competent auricularia larvae dorsal view of *Parastichopus californicus*, HP, hydropore. Scale bar, 115 µm.

Figure B.17. SEM of the doliolaria larva of *Parastichopus californicus*, note the five ciliated bands. Scale bar, 55 µm.

Figure B.18. Some internal structures of the doliolaria larva of *Parastichopus californicus*. Note the atrium (AT) which encompasses the primary tentacles surrounding the mouth of the pentactula larvae (arrow). Scale bar, 60 µm.

Figure B.19. Metamorphosis of doliolaria larva note the presence of the ciliated bands (small arrows), skeletal elements (OS), posterio-ventral tube foot (TF), and tentacles (TN). Scale bar, 60 µm.



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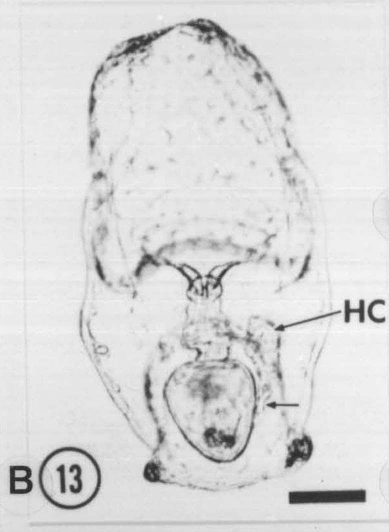

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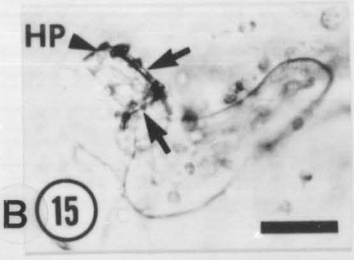

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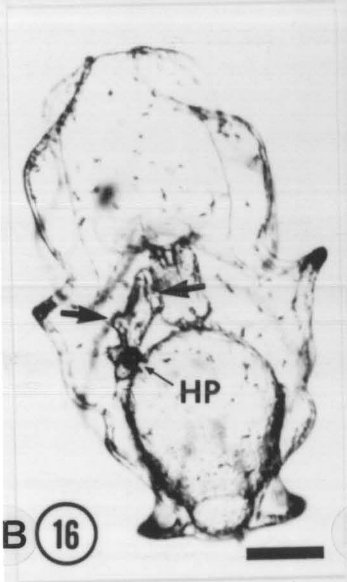

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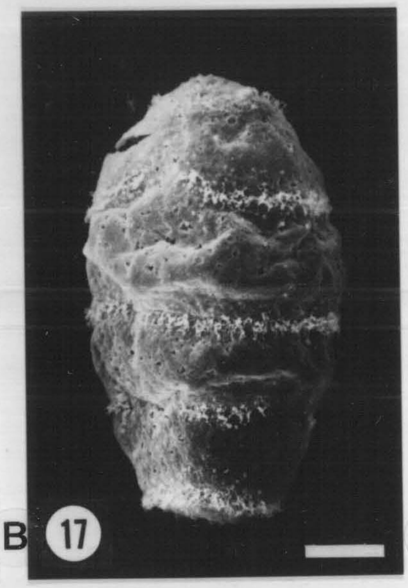

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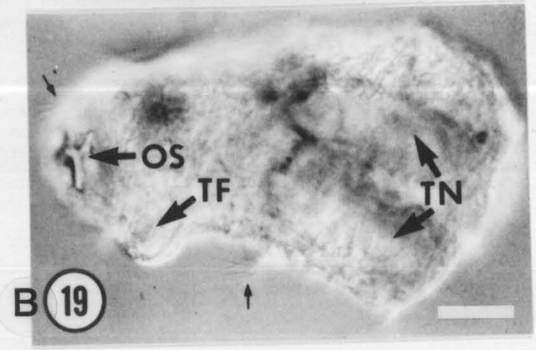


B 19

Alternative Developmental Sequence

While development as described above follows a regular course from auricularia to doliolaria to pentactula one cohort reared in the summer of 1982 deviated significantly from this pattern. Development to the auricularia was normal and consistent with that described above, except that metamorphosis from the auricularia to the pentactula was without the intervening doliolaria stage.

Fifty-five days after insemination some individuals in this cohort displayed an unusual arrangement wherein the hydrocoel completely encircled the esophagus of a diminutive auricularia, and the five primary tentacles characteristic of the pentactula had formed and erupted on the ventral surface surrounding the mouth (Figs. B.24 and B.25). A single tube foot also appeared on the ventral surface of the auricularia, and in some instances preceeds the eruption of the tentacles. The tube foot emerges to the anterior of the anus and by so doing pushes the anus back to a more posterior position. The pentactulae so derived appeared normal and thrived *in vitro*. No incidents of metamorphosis from the auricularia through the doliolaria to the pentactula was observed in this cohort.

Figure B.20. Newly metamorphosed pentactula larva of *Parastichopus californicus* ciliated bands absent and ossification of the body wall beginning (arrows). Scale bar, 65 µm.

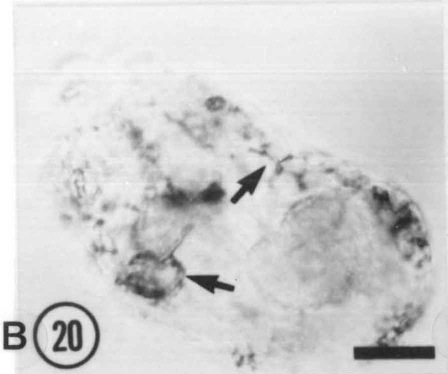
Figure B.21. Fenestrated button ossicles in the epithelium of the pentactula larva of *Parastichopus californicus*. Scale bar, 95 µm.

Figure B.22. Table ossicles with spires in the epithelium of the pentactula larva of *Parastichopus californicus*. Scale bar, 88 µm.

Figure B.23. SEM of the pentactula larva of *Parastichopus californicus* showing the ventral tube foot (TF) and the five primary tentacles (TN). Scale bar, 45 µm.

Figure B.24. Diminutive auricularia of *Parastichopus californicus* with oral podia erupting on the ventral surface of the larva surrounding the mouth. Scale bar, 65 µm.

Figure B.25. Lateral view of a diminutive auricularia larva with primary tentacles erupting on ventral surface of the larva surrounding the mouth. Scale bar, 80 µm.



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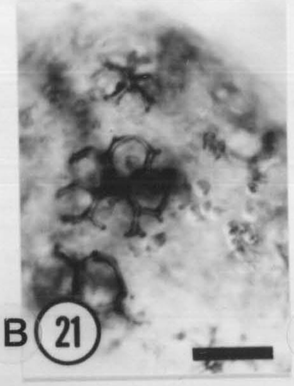

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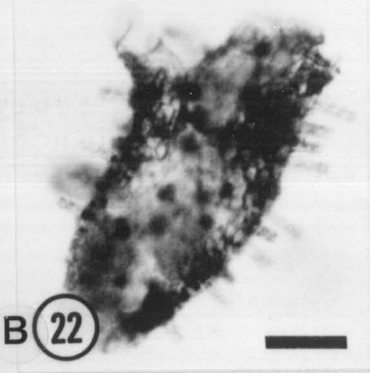

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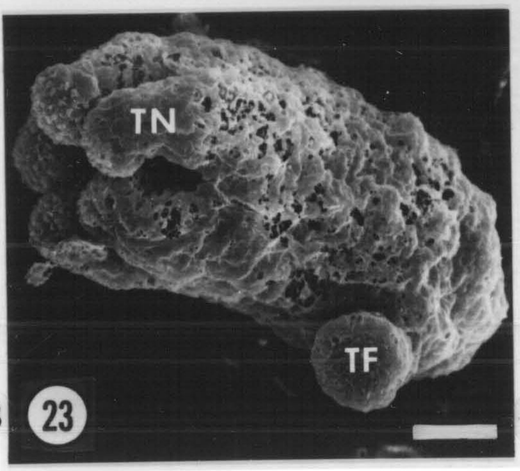


B 23

B 24

B 25

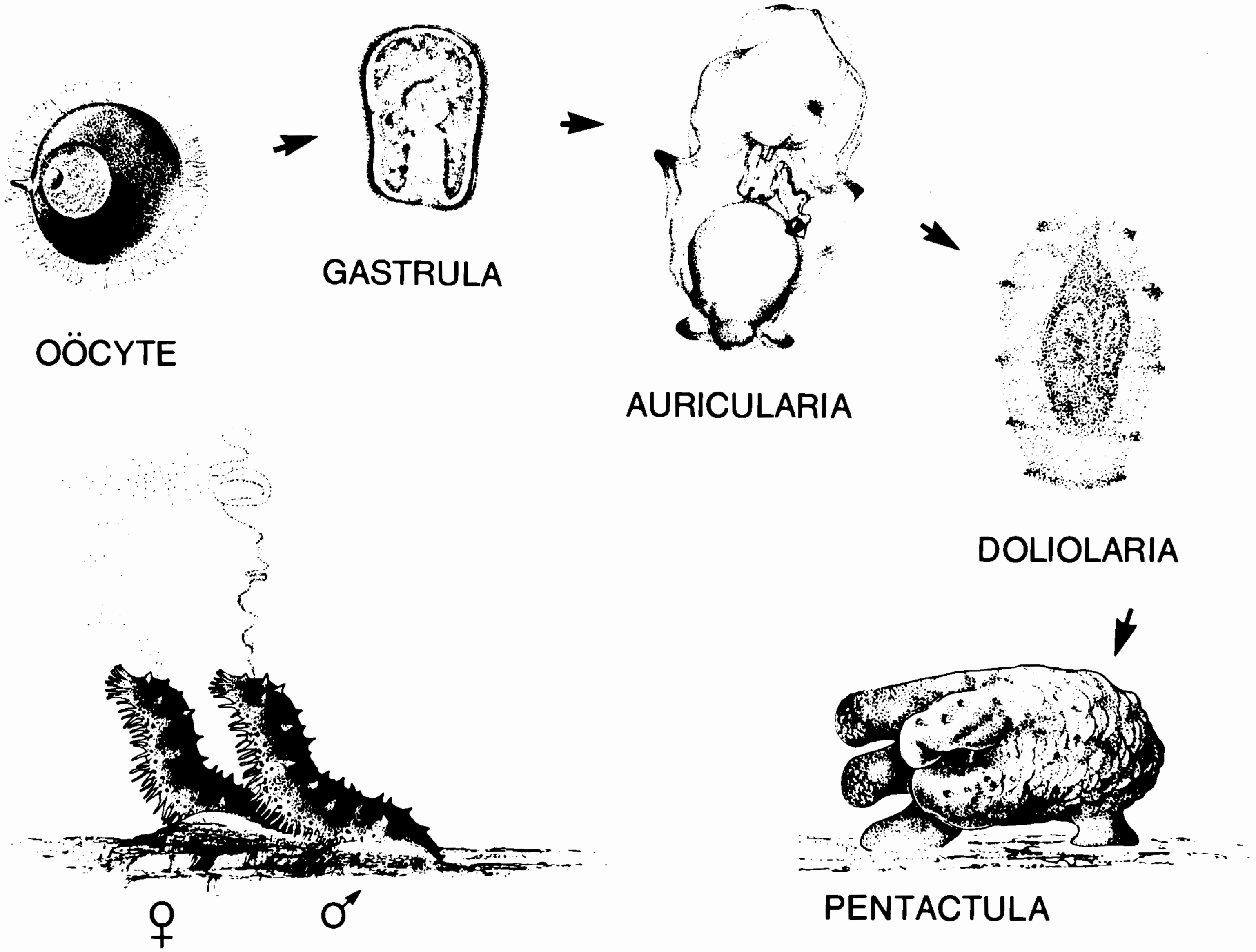
IV. Discussion

The California sea cucumber *P. californicus* of the Pacific coast of North America is commonly found in rocky low-intertidal and subtidal areas away from strong wave action or in quiet bays and on pilings from Baja California to British Columbia (Ricketts and Calvin, 1968; Brumbaugh, 1980; Kozloff, 1983). This sea cucumber is a relatively large (60 - 100 cm long) epibenthic detritivore that may reach densities as high as 0.5 m^{-2}, and which recently has become the focus of an intensifying, albeit limited, commercial fishery within the inland waters of British Columbia, Canada and Puget Sound, Washington (McDaniel, 1984; Sloan, in press). Because of this a significant effort to gather baseline biological and life historical information that can assist fisheries agencies in managing this expanding fishing effort is currently in progress.

Development in *P. californicus* does not deviate significantly from the generalized pattern of embryonic and larval development expected for aspidochirote holothurians possessing a planktotrophic larval phase (Kumé and Dan, 1968) (Fig. B.26) excepting in formation of the coelomic cavities and esophagus.

Coelom formation in holothurians has been described as a bending of the growing tip of the archenteron towards the primordial dorsal surface of the larva forming a distinct

Figure B.26. Life cycle of *Parastichopus californicus* from spawning through settlement of the pentactula larva. Individual developmental stages are not to scale, but display major developmental benchmarks for this holothurian particularly and planktotrophic holothurians in general.


OÖCYTE
GASTRULA
AURICULARIA
DOLIOLARIA
PENTACTULA
♀
♂

vesicle at the growing tip. This vesicle then fuses with the larval epithelium opening the dorsal pore which then pinches off from the archenteron to form the coelom (MacBride, 1914). Hörstadius (1973) has suggested that a coelomic vesicle which originates at the tip of the archenteron migrates to the left side of the larva to form the coelomic complex of the auricularia. Both of these processes reportedly result in the observed asymmetrical coelomic system typical of holothurians (Ubaghs, 1967), with the left complex being much larger and more extensively developed than the right. Neither of these descriptions, however, fits precisely the process of coelom formation that was observed in *P. californicus*.

The anterior vesicle noted during the development of *P. californicus* is synonymous with the earlier described coelomic vesicle, but its ontogenetic fate is much different than that reported by Hörstadius (1973) or suggested by MacBride (1914). In *P. californicus*, the anterior vesicle forms the esophagus of the auricularia larva. While the lateral vesicle forms the hydrocoel and the somatocoels of the auricularia. This process is very similar to coelom formation reported by Czihak (1971) for the sea urchin *Paracentrotus lividus*, except that a single left-handed vesicle is formed in *P. californicus* whereas two vesicles, left and right hand are formed in *P. lividus*.

Metamorphosis and settling in *P. californicus* were first observed 65 days after fertilization. When correlated with *in situ* spawning observations, and taking into account overlapping cohorts that result from multiple spawnings within a population over a three month long spawning season (see Part I.), settlement from the first cohort of the season could begin in early August and continue for at least two months. Settlement from the final cohort of the season would not begin until early November and potentially would not terminate until the end of the year. This would result in a recruitment period of approximately five months duration with pentactulae settling out continuously over this period.

In other laboratory studies, R. Strathmann (1978) observed initial and terminal settlement times for *P. californicus* somewhat different than those reported herein. His observations would result in a settlement period slightly shorter than suggested herein, but still of sufficient duration that a significant temporal expansion of the recruitment period would occur. On the other hand, Rutherford (1973) has noted a very short spawning, developmental, and hatching period for the brooding sea cucumber *Cucumaria pseudocurata*. He further suggests that this pattern of a short spawning and developmental period is common among holothurians generally (see also Boolootian, 1966).

Traditionally two major strategies of larval development have been described for marine invertebrates (MacBride, 1936). These are "embryonic" (direct) development wherein a parent either broods or provides protective encapsulation for the embryo until it hatches having the same general morphology as the adult, and "larval" (indirect) development wherein the embryos develop free from any parental influence and spend some time feeding as distinct larvae that are morphologically different from the adult.

Variations of these basic strategies have been proposed, such as lecithotrophic forms that may occur in the plankton for varying lengths of time depending upon the species (Todd and Doyle, 1981) but do not feed, or demersal forms that exist near the bottom never swimming up into the water column (Thorson, 1950; Mileikovsky, 1971). However, direct development of either brooded or encapsulated embryos, and indirect development as free swimming and feeding larvae constitute the two extremes of the developmental strategy continuum.

Indirect developing forms, as exemplified herein by *P. californicus*, generally have the longest pelagic period of all larval types, and asynchronization of the developmental process in *P. californicus* embryos and larvae extends, at least for some larvae, the pelagic period before settlement. Larvae are then available to settle out of the plankton over a considerable portion of a given year. This extended settlement period may be further increased if the release of the larvae into the plankton is somehow temporally expanded. Such is also the case with *P. californicus* as spawning within the population occurs throughout the late spring and early summer (see Part I.).

Strathmann (1974) suggests that dispersal of sibling larvae by benthic invertebrates may increase the parental cumulative rate of increase if by such dispersal variation in the survival and reproduction of siblings from year to year is reduced. He also notes that increasing the length of the larval pelagic period, extending larval release time (spawning period), or varying the length of the larval pelagic period are possible methods of increasing the spread of sibling larvae. If Strathmann's hypothesis is correct, the particular developmental strategy employed by *P. californicus* in reproduction is very well tuned to insure the spread of siblings.

Additional benefits from this type of spawning and developmental pattrern undoubtedly occur as well. For instance Pennington *et al.* (in press) have observed stage-specific predation upon the developing larvae of the Pacific sand dollar, *Dendraster excentricus*, by eleven common planktonic predators. Earlier stages (ie. gastrula, blastula and prism) are preferentially selected over later stages. In temperate or boreal marine environments where physical parameters vary significantly and are presumed to affect the timing of reproduction patterns (Thorson, 1950; Barnes, 1975) a species such as *P. californicus*, with a developmental strategy aligned toward the indirect end of the developmental continuum, could ameliorate the risks to its developing young from stochastic environmental perturbations, such as severe storms or unusual temperature fluctuations, and predation by varying in time and space the number of embryos, larvae, and newly settled young exposed to the environment at any given time.

In this regard, the single incident of precocious metamorphosis from a reduced auricularia directly to the pentactula may have been an innate response by the larvae of *P. califoricus* to some undetermined stimulus (possibly due to *in vitro* culture). The resulting apparently healthy pentactulae indicates that the auricualria of *P. californicus* may be able to compensate *in situ* for unexpected environmental conditions by settling quite rapidly. This response could insure the survival of a few members of a particular cohort that might not have survived otherwise.

From these observations it is apparent that development in *P. californicus* is highly variable, and that this sea cucumber is quite flexible in its processes of embryonic development. The most obvious reward of such action being an increased potential, to at least some degree, for a successful reproductive effort from season to season.

C. TENTACLE STRUCTURE AND FEEDING PROCESSES

I. Introduction

Aspidochirote holothurians are common deposit-feeding organisms characteristic of coral reef communities (Trefz, 1958; Bakus, 1973; Roberts, 1979). These sea cucumbers have peltate oral tentacles that have been variously described as cauliflower-like structures (Bouland *et al*., 1982), or as "a nasturtium leaf with a central short stalk giving off horizontal branches" (Hyman, 1955; cf. Roberts, 1982). Feeding with this type of tentacle has been described as a shovel or broom-like activity (MacGinitie and MacGinitie, 1949; Mottet, 1976) whereby deposits are scooped or shoved into the sea cucumber's mouth (Trefz, 1958; Bouland *et al*., 1982; Massin, 1982).

This traditional view of tentacular function has been challenged by several recent studies. For instance, Roberts (1979) has proposed that expansion of the peltate tentacle via increasing hydrostatic pressure within the water vascular system opens inter-nodular spaces on the tentacles in which food, etc. can become trapped (cf. Trefz, 1958). Hammond (1982), on the other hand, has discounted this purely mechanical model of feeding as he observed mucus coating the surface of aspidochirote tentacles, and therefore suggested that adhesion is the significant factor in aspidochirote food collection.

More recently, Roberts and Bryce (1982) examined the peltate tentacles of several tropical holothurians at the light microscope level and observed secretory cells in their epithelia. They further stated that these cells produce an adhesive material functional in the collection of food particles, and Levin (1982) has stated that in *Stichopus japonicus* he is "most tempted" to describe tentacle function as an adhesive process.

Bouland *et al*. (1982) have described the ultrastructure of secretory cells in the nodules of the tentacles of *Holothuria forskali*. Interestingly, these secretory cells produce granular vesicles similar to those observed by Fankboner (1978) from the tentacle papillae of *Psolus chitinoides*, to which an adhesive function was attributed (cf. Smith, 1983). Bouland *et al*. (1982), however, believed that mechanical processes alone could account for successful tentacular feeding by *H. forskali*, and thus supported Robert's original mechanical model for tentacle function.

Studies on the structure of holothurian tentacles have, on the whole, emphasized the functioning of the oral tentacles in adult specimens. However, Chia and Buchanan (1969) have reported the presence of papillae on the tips of the tentacles from the pentactula larvae of *Cucumaria elongata*, stating that these are outgrowths of acid mucopolysaccharide cells that produce mucous secretions important in both feeding and locomotion; more recently, Engstrom (1980) has reported that "sediment and detritus adhere to the ... knobby surface of the digits" on the feeding tentacles of young *Chirodota rotifera*. These exceptions not withstanding, the structure and function of the tentacles of pentactula larvae and growing juveniles, as well as a comparison of tentacle function between young and adult forms has gone unreported.

The need for a more comprehensive study of aspidochirote feeding mechanisms comparing and contrasting the life stages within a single species is clearly evident. Thus, reported herein is the form and function of tentacular feeding in adults, juveniles, and pentactula larvae of *Parastichopus californicus* (Stimpson), an aspidochirote species which forms a significant sea cucumber fishery on the Pacific coast of North America.

II. Materials and Methods

Specimen Collection

Adult *P. californicus* were collected subtidally by SCUBA from numerous locations adjacent to the Friday Harbor Laboratories, San Juan Island, Washington State, U.S.A., and at Woodlands, Indian Arm fjord, British Columbia, Canada. Juvenile animals (0.5 cm to 2 cm long and approximately one year old) were collected by SCUBA from subtidal bedrock overhangs and crevices near Lions Bay, Howe Sound, B.C.; and pentactula larvae were cultured *in vitro* at Simon Fraser University, Burnaby, B.C.

Feeding Observations and Tentacle Function

Tentacle function during feeding by adult *P. californicus* was determined from numerous visual observations *in situ* using SCUBA and recorded via macrophotography. Tentacle function was also noted on feeding animals maintained in laboratory aquaria. Observations on the feeding behavior of the buccal tentacles of pentactula larvae and juveniles of *P. californicus* were carried out using Nomarski differential interference contrast optics and/or stereo microscopy.

Tentacles were removed from adult *P. californicus* and lightly pressed against 320 mesh Carborundum particles spread across the bottom of a petri dish filled with sea water. The tentacles were then examined with a stereo microscope to observe the sites and method of attachment of the particles to the tentacles.

Skeletal Elements of the Tentacular Dermis

Morphology of the dermal ossicles from the tentacles of *P. californicus* was determined from preparations of the tentacular skeletal elements which were obtained by dissolving away the tentacle's soft tissue with 6% sodium hypochlorite for approximately 2 hours. The free ossicles were then washed numerous times in distilled water and allowed to air dry. The dry ossicles were mounted on specimen stubs, gold coated, and photographed on an ETEC Autoscan Electron Microscope. Orientation and distribution of the ossicles (as related to function) in the tentacles of adult *P. californicus*, was studied in whole mounts of tentacle stalks and branches. Tentacle ossicles in pentactula and juvenile animals were easily visualized *in vivo*.

Light Microscope Histology and Histochemistry

To determine the nature of the secretory products of the tentacle epithelium, tentacle tissues for histological and histochemical examination were fixed and embedded either in 4% glutaraldehyde buffered with 0.2 M cacodylate (pH 7.4) for 15 minutes, dehydrated to 70% ethyl alcohol, and embedded in JB4 embedding medium (Polysciences, Inc.); or in Bouin's fixative, dehydrated in ETOH, and embedded in paraffin. Paraffin sections were cut at 7 μm while JB4 sections were cut at 1 - 4 μm. Histological staining of paraffin sections was by standard hematoxylin and eosin methods, while JB4 sections for histological examination were stained according to the method of Richardson, *et al*. (1960). Paraffin sections for histochemical determination of muco-polysaccharides were stained by the Periodic Acid Schiff (PAS) method and with Alcian Blue (pH 2.5). While, JB4 sections for histochemical determination of muco-polysaccharides were stained with PAS, Alcian Blue (pH 1.0 and 2.5), Toluidine Blue, and Aldehyde Fuschin (Pearse, 1968).

Scanning Electron Microscopy

Pentactula larvae, the tentacle crowns of juvenile *P. californicus*, and tentacles from adult animals (that had been relaxed in menthol saturated sea water for 24 h) were fixed in glutaraldehyde as above followed by post-fixation in 2% Osmium

Tetroxide buffered with Dorey's solution B, as modified by Fankboner (1978). The various specimens were then washed in distilled water and dehydrated by either freezing the tissues in liquid Nitrogen followed by vacuum evaporation or by critical point drying (CO_2). Specimen preparations were stub-mounted, gold coated and then photographed on a scanning electron microscope as above.

Transmission Electron Microscopy

Tissues for TEM were fixed in glutaraldehyde and osmium as above, dehydrated in a graded series of ethyl alcohol and embedded in Spurr's Low Viscosity Embedding Medium (Polysciences, Inc.). Sections 70 - 100 nm were stained with lead citrate and uranyl acetate then viewed and photographed on a Philips 300 Electron Microscope.

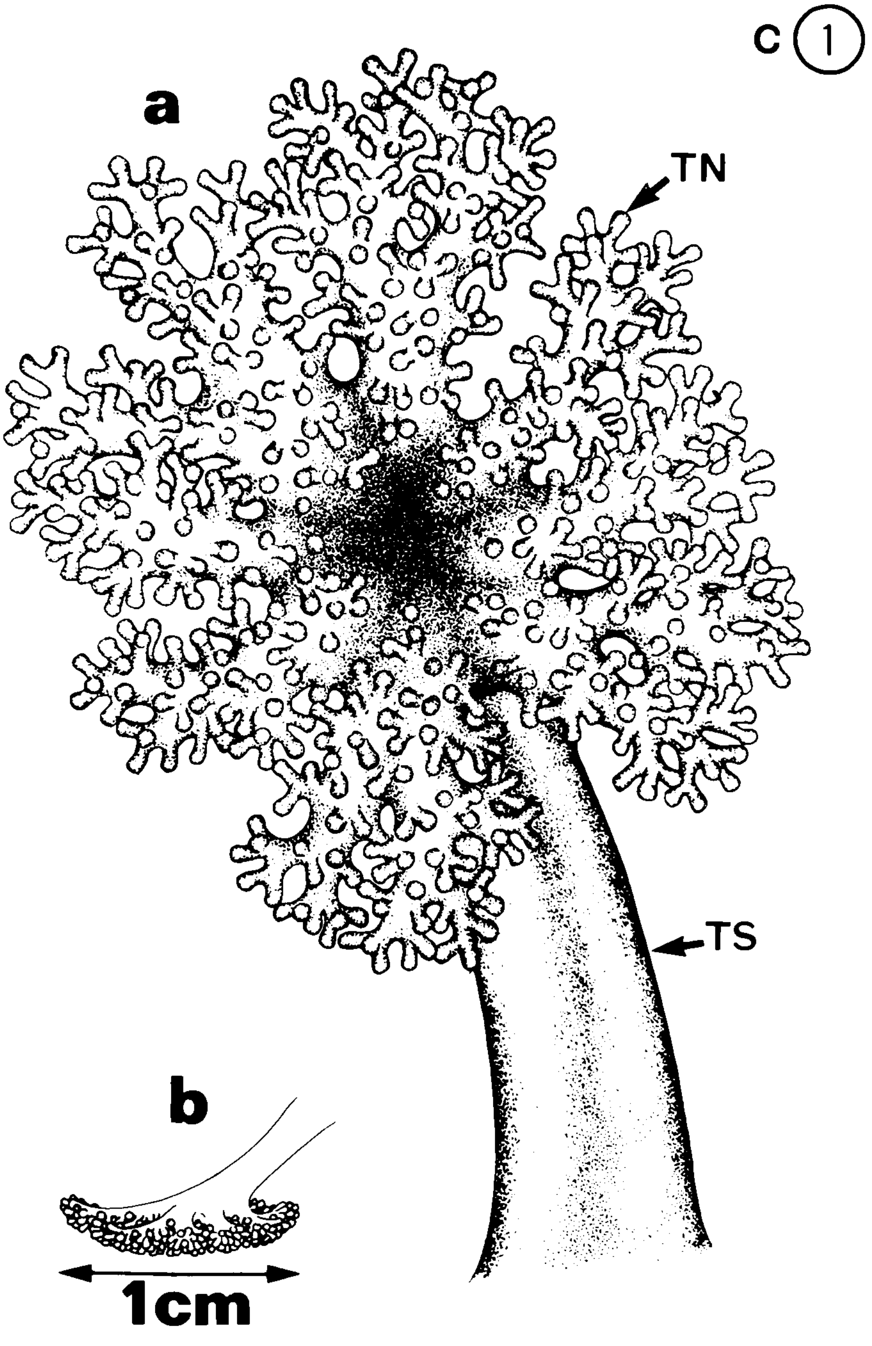
III. Results

Morphology and Function of the Adult Tentacle

The mouth of an adult *P. californicus* is encircled by twenty tentacles, each of which consists of a tentacle stalk with 4 primary branches radiating outward from the tentacle stalk. These primary branches bifurcate several times forming secondary, tertiary, and sometimes quaternary branches (Fig. C.1a). The most distal tentacle branches terminate in capitate nodules which are splayed against the substratum during feeding (Fig. C.1b). The minimum distance between the nodules of fully expanded tentacles was approximately 50 μm. Each tentacle has an associated tentacular ampulla of the water vascular system located at the base of the tentacle and within the perivisceral coelom.

Scanning electron micrographs of an expanded adult tentacle reveal a pad of capitate nodules forming the functional surface of the tentacle (Fig. C.2). The surface of these nodules, which are nearly 100 μm in diameter, is generally microvillous and interspersed with choanocyte-like cells consisting of a central cilium with a circlet of microvilli (Norvang and Wingstrand, 1970) (Fig. C.3). Mucous traces were frequently observed on the tentacle surface (Fig. C.4). Maximally contracted tentacles lose

Figure C.1. Line drawing of the expanded tentacle of an adult *Parastichopus californicus*. **a.** TN, tentacle nodules forming the adhesive surface of the tentacle; TS, tentacle stalk; what appears to be eight primary tentacle branches are actually the first bifurcations of the four primary tentacle branches which in adult tentacles are nearly indistinguishable from the original tentacle branches. **b.** Side view of an expanded tentacle as it appears when being placed against the substratum during feeding.


C ①
a
TN
TS
b
1cm

all semblance of branching, and the adhesive surface of the tentacle appears furrowed, much like the surface of the vertebrate brain. (Fig. C.5).

During feeding the expanded tentacles of *P. californicus* are splayed against the substratum individually and without fixed sequence. Deposited particles adhere to the surface of the tentacle as it is pulled from the substratum. Collapse of the tentacle begins as it is withdrawn from the substratum with the tips of the tentacle branches curling inwards to form a cup which aids in the mechanical grasping of larger sediment particles (Fankboner, 1978; cf. Levin, 1982). Large inorganic particles (ie. bits of shell etc.) can be easily jostled free of the tentacle surface, before the tentacle is placed into the mouth, with water movement created by the observing diver. This process was occasionally observed to occur when no apparent disturbance of a tentacle was noted.

As a tentacle is lifted from the substratum the interstices of the tentacle branches and nodules are free of trapped material; collected sediment occurs only on the surface of the tentacle. Stereo microscopic examination of tentacles that had been pressed against carborundum particles showed that the particles adhere to the exposed surfaces of the tentacle nodules. This attachment is somewhat transitory though as minor movements of the tentacles would detach some particles. This was especially true as the time after attachment increased. Observations on sea cucumbers sweeping the glass fronts of

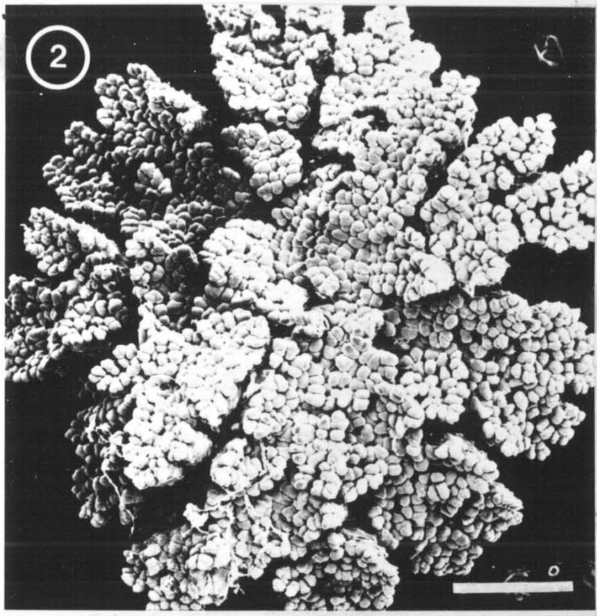
Figure C.2. SEM preparation of a tentacle from an adult *Parastichopus californicus*. Scale bar, 1 mm.

Figure C.3. Micrograph of the microvilliated surface of a tentacle nodule. Note the cilium (C) of a uniciliated cell with its associated circlet of microvilli (MV). Scale bar, 1 μm.

Figure C.4. Mucus strands (arrows) occurring on the adhesive surface of the tentacle. Scale bar, 250 μm.

Figure C.5. Micrograph of a maximally collapsed feeding tentacle of an adult *Parastichopus californicus*. Note the absence of branching and the furrowed appearance of the surface of the tentacle. Scale bar, 1 mm.

C ②



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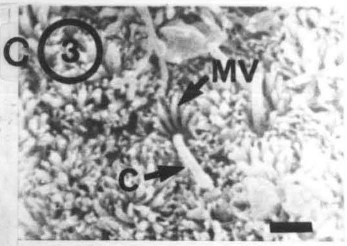

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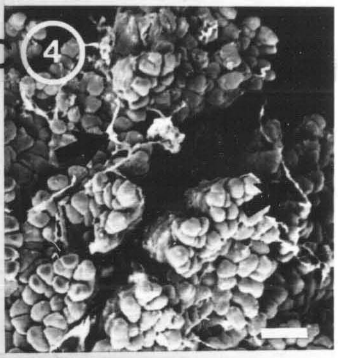

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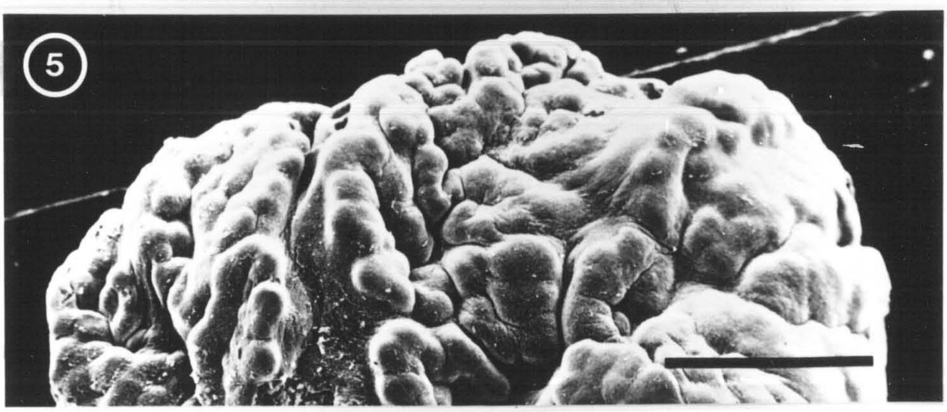


aquaria with their tentacles show the tentacles to stick to the glass by the tentacle nodules, and the tentacles must be forcedly withdrawn from the glass as the animal moves along.

When a tentacle is inserted into the pharynx the tentacle cup is inverted and the functional surface of the tentacle is wiped clean of collected sediments against the wall of the pharynx. An apparent increase in coelomic pressure within the peripharyngeal sinus of the buccal bulb (as indicated by active swelling) causes a balloon-like distension of the pharyngeal wall against which the tentacle is wiped. Transfer of collected sediments from the tentacles to the pharynx is quite efficient as no bits of food remain on the tentacle when it is removed from the mouth.

Larval Tentacles

The tentacular crown of the pentactula larvae consists of five capitate tentacles (Fig. C.6) with papillae protruding from the surface (Figs. C.6 and C.7). The larval tentacles appear notably sticky and pentactulae hold tenaciously to the bottoms of culture dishes by their tentacles against the flow of pipetted sea water. During feeding the tentacles of the pentactula are placed into the pharynx in no apparent sequence as with the adults.

Juvenile Tentacles

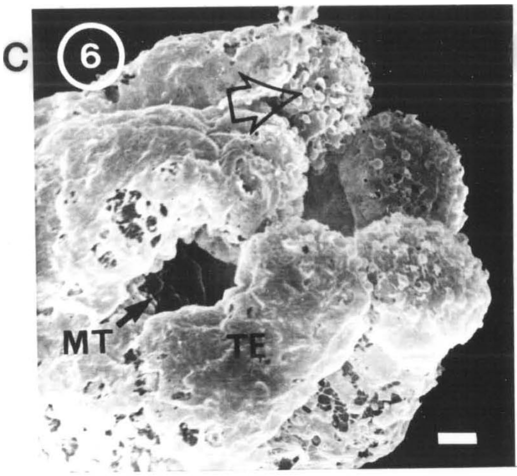
There is great variation in the size and degree of development in yearling specimens of *P. californicus*. This differential degree of maturation is most obvious in the development of the tentacle crown of the juveniles. For instance, juvenile tentacles show the same basic structure as adult tentacles (ie. bifurcation of the tentacles into primary and secondary branches, etc. that terminate in capitate nodules), but the branching of these tentacles is not yet as extensive as in the adults (Fig. C.8). The smallest one year animal examined had 12 tentacles, 11 equal in size and one much smaller with very simple branching. On the 11 tentacles of equal size the four primary tentacle branches were visible and the beginings of the secondary branches could be seen. Larger juveniles (older by possibly as much as 6 months) showed more extensive branching of the tentacles as well as an increase in the number of tentacles (Fig. C.9). The largest one year animal examined had a full compliment of 20 uniformly developed tentacles. New tentacles appear as bud-like protrusions between the stalks of existing tentacles, and soon grow to the same size and branching configuration of existing tentacles. Feeding juveniles insert their tentacles into the pharynx in no observable order or sequence as with the adults and larvae.

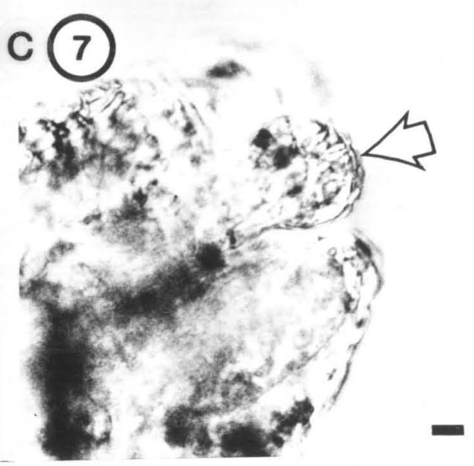
Figure C.6. Tentacle crown of a pentactula larva. Note the papillae (arrow) protruding from the surface of one of the five tentacles (TE) surrounding the larval mouth (MT). Scale bar, 10 μm.

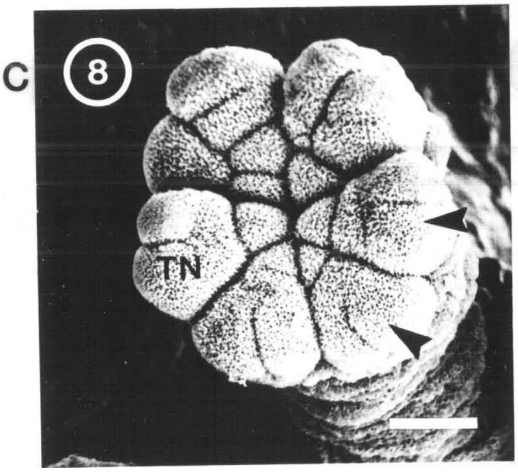
Figure C.7. Light micrograph of the tentacle tip of a living pentactula larva, note the protruding papillae on the tentacle surface (arrow). Scale bar, 10 μm.

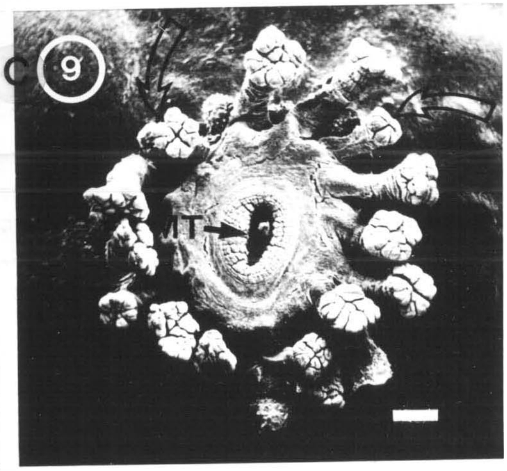
Figure C.8. A juvenile tentacle showing the microvilli on the surface of the tentacle nodules (TN). Note the initial bifurcations of a primary tentacle branch (arrows). Scale bar, 100 μm.

Figure C.9. Tentacular crown surrounding the mouth (MT) of a yearling *Parastichopus californicus*. Arrows indicate tentacles not yet as extensively branched as the other tentacles present. Scale bar, 400 μm.


C ⑥
MT
TE


C ⑦


C ⑧
TN


C ⑨
MT

Structure of the Tentacle Nodules

Transmission electron micrographs show the nodule epithelium to consist predominately of columnar cells which contain electron-dense membrane-bound granular vesicles (GV) 0.6 - 0.7 μm in diameter which originate above a basally-located nucleus and aggregate in the apex of the cell. The free surface of the epithelium is covered with tufts of microvilli that often contain stacks of the granular vesicles (Figs. C.10 and C.12). Uniciliated cells (UC) with their circlet of microvilli were also noted (Fig. C.10). The tips of the microvilli are embedded in a 0.2 - 0.3 um thick cuticle (Fig. C.11). The cuticle was not observed in all micrographs of the epithelium. The cells at the distal tips of the tentacles nodules are packed very tightly together, but branches of collagenous fibers (CF) occur interstitially and extend to the surface of the nodules. The granular vesicles described above could be faintly visualized with the light microscope and were shown histochemically to contain weakly acidic sulphated mucosubstances (purple "β" reaction with Toluidin Blue, very dark purple staining with Aldehyde Fuschin, a strong PAS reaction, and positive for weakly acidic sulphated mucosubstances with Alcian Blue; Pearse, 1968).

Figure C.10. TEM-section of the nodule epithelium. CT, interstitial branches of connective tissue; GV, aggregations of electron dense granular vesicles; MV, microvilli; UC, uniciliated cell. Scale bar, 8 μm.

Figure C.11. TEM-section of the nodule epithelium showing the microvilli imbedded in the cuticle (CU). Scale bar, 8 μm.

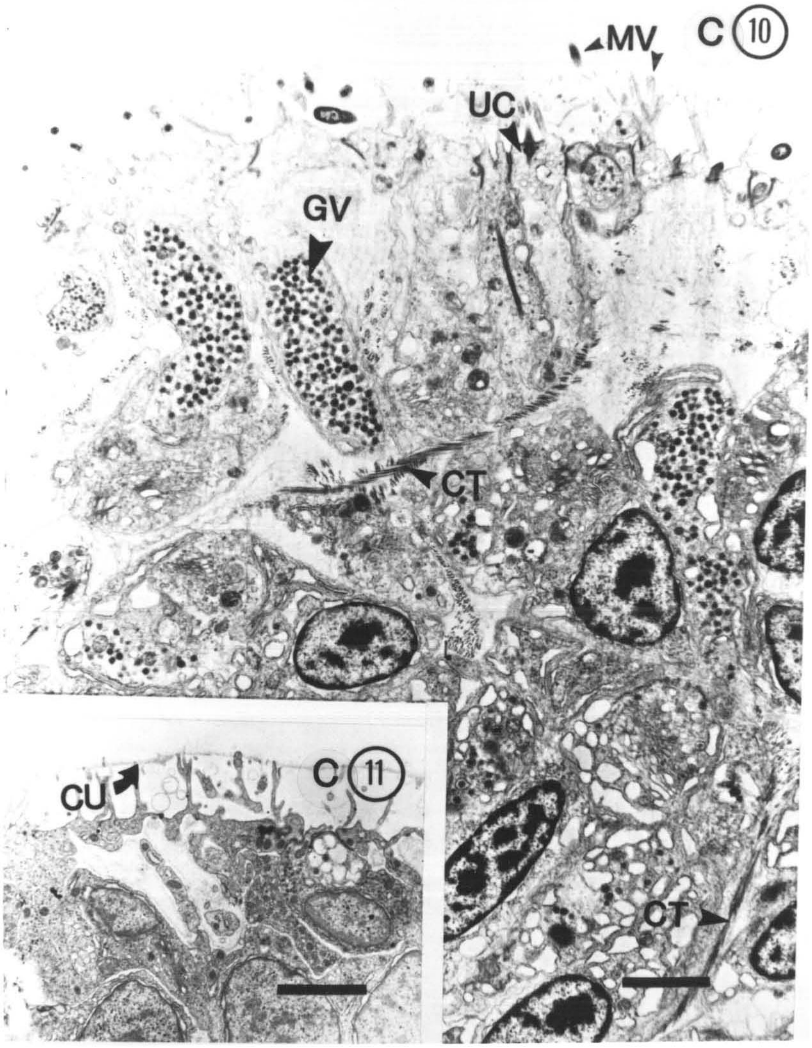
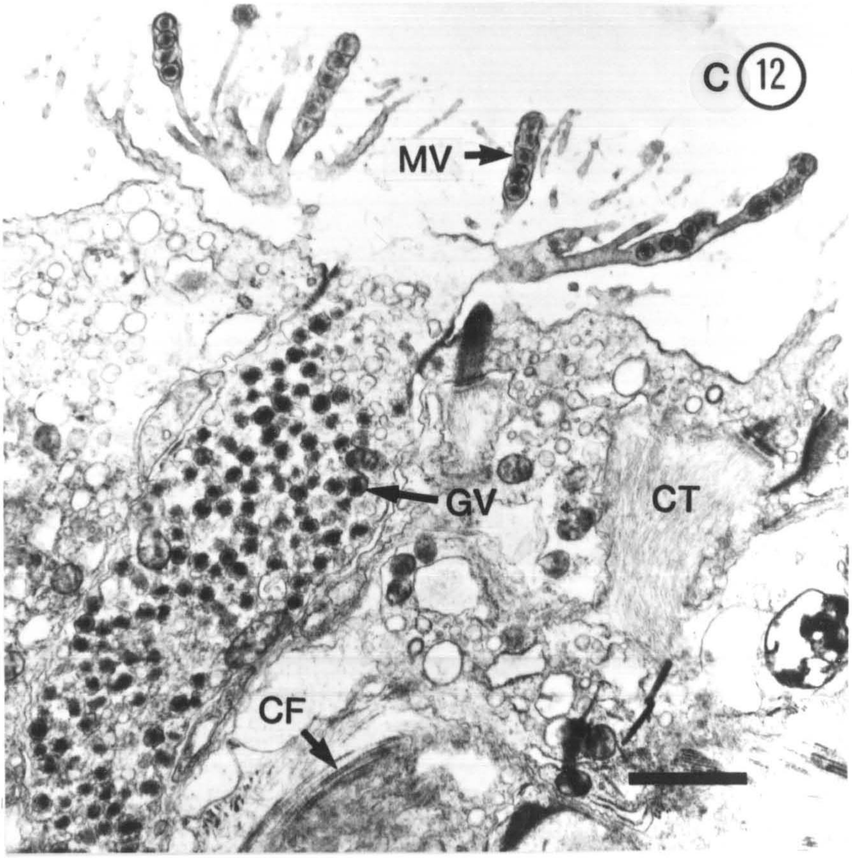

MV
C ⑩
UC
GV
CT
CT
CU
C ⑪

Figure C.12. TEM-section of the nodule epithelium. CF, collagenous fibers; CT, connective tissue; GV, granular vesicles; MV, microvilli (some containing electron dense granular vesicles). Scale bar, 5 μm.


C
12
MV
GV
CT
CF

Fine Structure of the Water Vascular System Epithelium

The epithelium of the water vascular system (WVS) is composed mainly of myoepithelial cells (ME) which contain an inner bundle of myofibrils (MF) associated with numerous mitochondria (MI) (Fig. C.13). The myofibril bundles rest against a (B) basement membrane of densely-packed collagen fibers (CF) that is common to both the water vascular system and the nodule/tentacle branch epithelium (Figs. C.13 and C.14). The myofibril bundles and the myofibrils themselves are oriented longitudinally (Fig. C.14), and form an extensive longitudinal muscle layer that lines all the tentacular branches of the water vascular system.

The collagenous basement membrane (or dense collagenous layer) is formed of regularly arranged fibers approximately 0.02 μm thick with a cross-striating pattern that repeats every 0.05 μm (Fig. C.15). This collagenous layer is itself 0.3 - 0.4 μm thick and individual fibers extend from the collagenous layer (Figs. C.10 and C.12). into the nodule/tentacle epithelium and to the surface of the tentacle nodules (Figs. C.10 and C.12).

Skeletal Elements of the Tentacles

The ossicles of the tentacles of *P. californicus* are predominately arc-shaped rods that branch on each end. Occasionally, unbranched or paired rods were observed (Fig.

Figure C.13. TEM-section of the epithelium of the tentacular branch of the water vascular system. AM, amoebocyte: B, basement membrane; C, cilium; CT, connective tissue; ME, myoepithelial cell; MI, mitochondria; MF, muscle fibers; WVS, tentacular branch of the water vascular system; Scale bar, 5 μm.

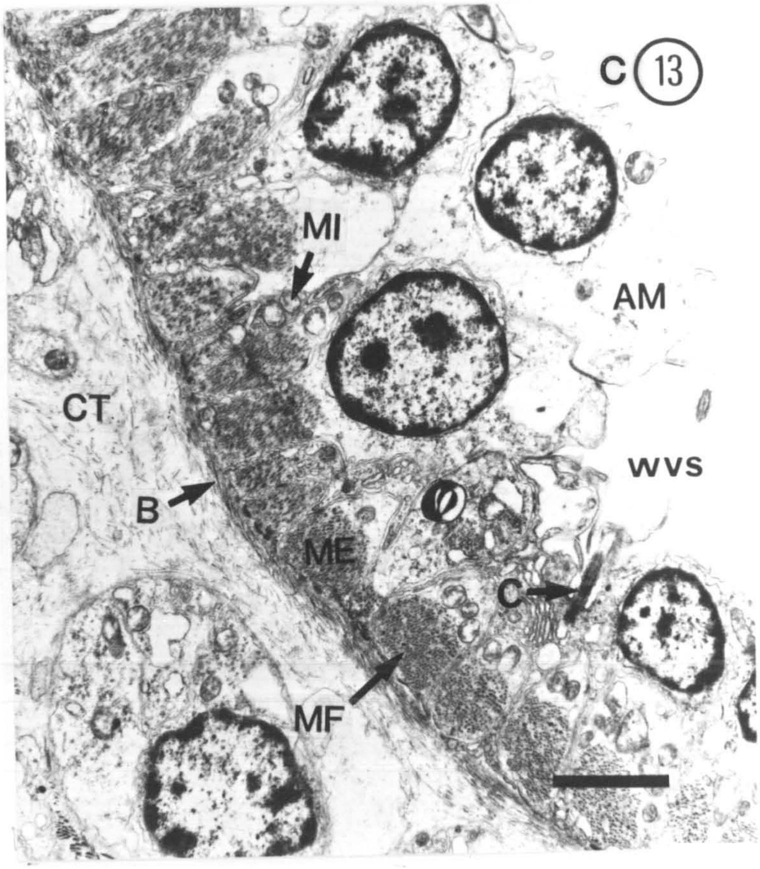
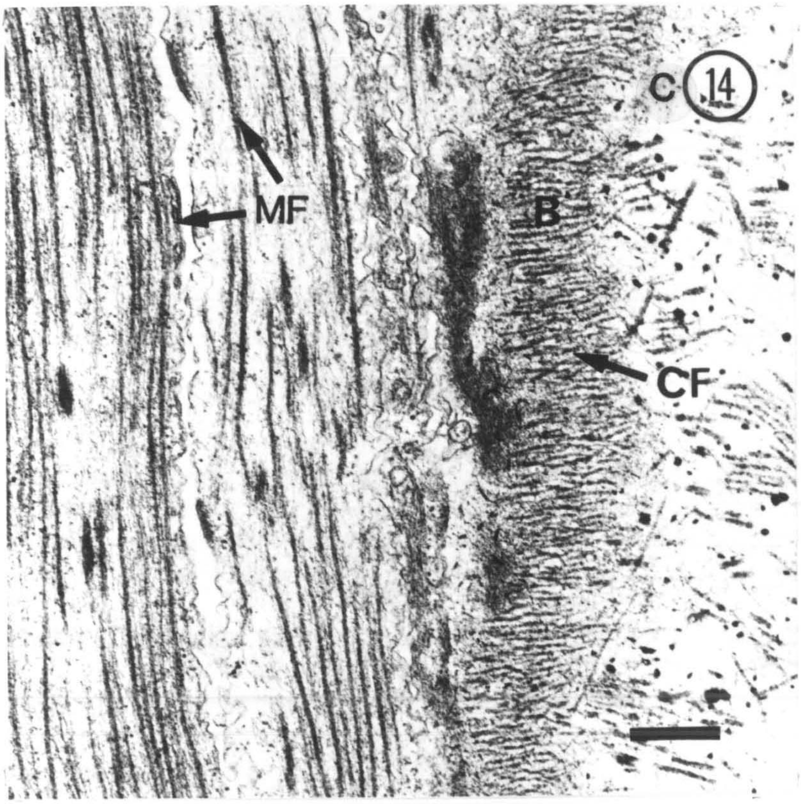
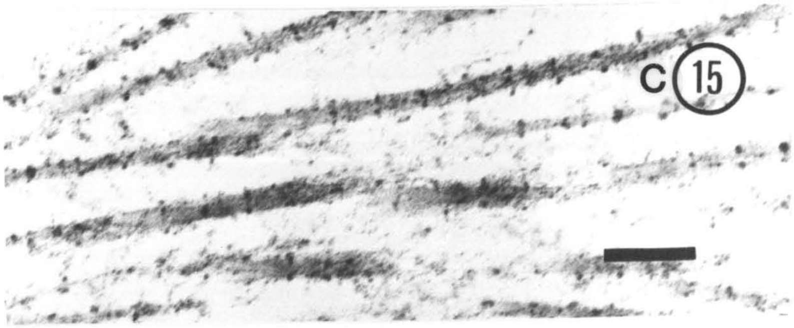

C 13
MI
AM
CT
WVS
B
ME
C
MF

Figure C.14. A glancing longitudinal section through the myoepithelium and basement membrane of a tentacular branch of the water vascular system. B, basement membrane; CF, collagenous fibers; MF, myofibrils. Scale bar, 0.4 μm.

Figure C.15. TEM of the collagenous fibers. Note the repeating cross striations. Scale bar, 0.1 μm.

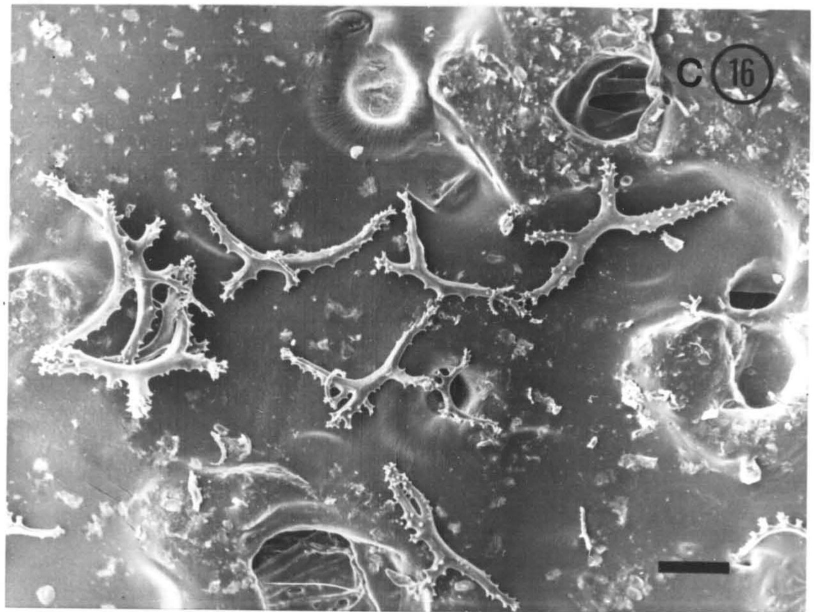

C 14
MF
B
CF

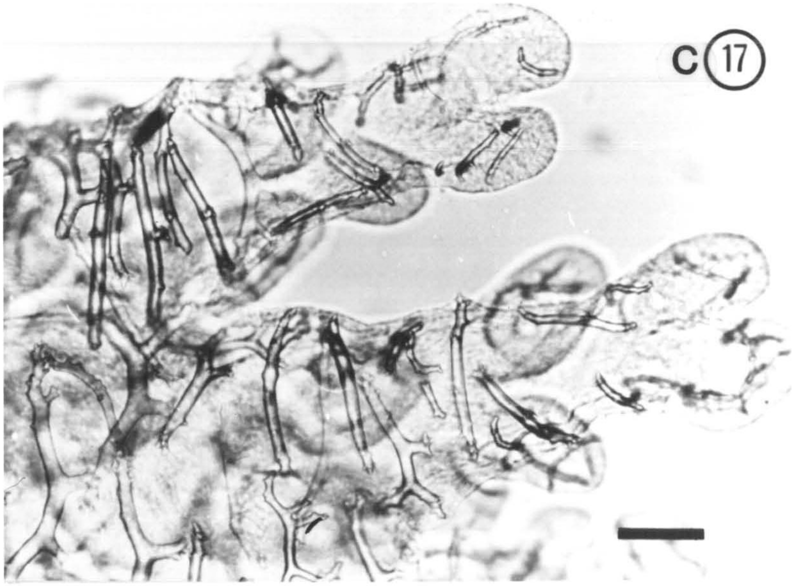

C 15

C.16). Ossicles are found only in the tentacle branches and do not occur in the main tentacle stalk of adult animals. Ossicles were observed, however, in the tentacle stalks of pentactula larvae and juveniles. The ossicles of terminal tentacle branches and bases of the tentacle nodules are approximately 100 μm long while, the ossicles in all other tentacle branches are approximately 300 μm long. These ossicles lie, like supportive ribs, in numerous planes with the main axis of an ossicle situated at about 90° to the axis of the tentacle branch (Fig. C.17). The arc of each ossicle runs parallel to the circumference of the tentacle branch.

Figure C.16. Rod-shaped dermal ossicles from the tentacles of *Parastichopus californicus*. Scale bar, 100 µm.

Figure C.17. Rod ossicles imbedded in a cleared tentacle branch of an adult *Parastichopus californicus* showing their supportive function in maintaining the circular shape of the tentacle branches. Scale bar, 100 µm.


C
16


C
17

IV. Discussion

The majority of holothurians feed, via oral tentacles, on deposited particulate material or ingest organic rich sediments through which they burrow (see references in Fankboner, 1978). The most complete investigations into the structure and function of sea cucumber tentacles have centered on adult dendrochirote holothurians that collect suspended particles from the water column surrounding the tentacles (Fankboner, 1978; Smith, 1983) or sweep the substratum adjacent to the animal for deposited material (Brumbaugh, 1965; Nordhausen, 1972).

The mechanisms by which dendrochirote holothurians feed has been a matter of some issue in recent years. Fish (1967) claimed on the basis of *in vitro* observations that trapping of food by the dendritic tentacles of *Cucumaria elongata* resulted from a sticky coating of pharyngeal mucus on the surface of the tentacles. To the contrary, however, Fankboner (1978 and 1981) has shown that food particles adhere to adhesive papillae which occur on the distal tips (buds) of the tentacles of *Leptopentacta* (= *Cucumaria*) *elongata*, *Psolus chitinoides*, and several other dendrochirote genera. Pinching or grasping of food particles by these holothurians as the tentacles contract was considered to be of secondary importance (Fankboner, 1978). Furthermore, Smith (1983) has stated that, "By far the most important method of seston capture is by adhesion on the tentacle nodes," of *Neopentadactyla mixta*.

Feeding in *P. californicus* consists of the repeated extension, collapse and withdrawal of the tentacles placed against the substratum. The food-laden tentacle is lifted from the substratum and inserted into the mouth. Expansion of the tentacles is apparently accomplished by the relaxing of the longitudinal muscle fibers associated with the tentacular branches of the water vascular system, and increasing the water pressure within these branches. The canals of the tentacular branches of the water vascular system extend to the tips of the tentacle branches and facilitate the extensive expansibility of the tentacles. Tentacle contraction occurs through the constriction of the longitudinal muscle fibers that occur in all branches of the tentacles and the tentacle stalk.

The ossicles of the tentacles provide circular support to the tentacle branches (and in younger animals the tentacle stalks as well)(cf. Engstrom, 1980b) in the absence of a layer of circular muscles. These ossicles function much as the folds in the bellows of a concertina in that their right angle orientation to the tentacle branch axes and parallel relation to each other allows a great deal of tentacle contraction while not diminishing the ability of the ossicles to provide skeletal support to the tentacle branches.

Adhesion is known to play an important role in the function of echinoderm tube feet (Hermans, 1983), and approximately 44% of the total adhesive capacity of the tube feet of the sea star

Asterias vulgaris has been suggested to result from the stickiness of mucous secretions on the "suckers" of the tube feet (Binyon, 1972). Hermans further suggested that the presence of two types of secretory cells in echinoderm tube feet may indicate that a "duo-gland" adhesive/de-adhesive system is operating in echinoderm tube feet, and since only a single gland system has been described in holothurian tentacles, an adhesive only system facilitates food capture by the tentacles of these echinoderms.

The ultrastructure of the tentacle nodules of *P. californicus* is very similar to that of *Holothuria forskali* (Bouland *et al.*, 1982), and generally similar to that of *Psolus chitinoides* (Fankboner, 1978) and *Neopentadactyla mixta* (Smith, 1983). Important morphologically homologous structures, which appear to be functionally analogous as well, include the electron-dense secretory vesicles, epithelial microvilli containing these vesicles, and uniciliated cells with a circlet of microvilli.

The electron-dense granular vesicles of holothurian tentacles are both morphologically and histochemically homologous to secretory vesicles produced in the adhesive glands of many invertebrate species (Hermans, 1983), and Fankboner (1978) has suggested an adhesive role for the granular vesicles from the adhesive papillae of the tentacles of *P. chitinoides* (cf. Smith, 1983). Bouland *et al.* (1982), though, state that the function of these vesicles in the tentacle nodules of *H.*

forscali is unknown. In each of these reports the vesicles were observed within the microvilli of the cells in which they were produced, and these authors felt that the granular vesicles were secreted through the microvilli. This seems a reasonable route for secretion of these vesicles, in view of their presence in the microvilli, but to date, no direct evidence for secretion in any of these fine structure preparations has been observed.

The uniciliated cells of the nodule epithelium have generally been considered to be sensory in function (Fankboner, 1978; cf. Burke, 1980; Bouland *et al*., 1982). Indeed Bouland *et al*. (1982) believe that the tentacle nodules are sensory sites homologous to the sensory hillocks of echinoid globiferous pedicellaria. Fankboner (1978) has suggested both a mechanoreceptive and a chemoreceptive function for these uniciliated cells, while a strictly chemoreceptive function has been suggested, but not determined, for similar cells on echinoderm tube feet (Burke, 1980). Millott and Coleman (1969) describe "specialized" sensory cells of the echinoid podial pit as having irregularly branched microvilli on the free surface sometimes associated with a single cilium or flagellum; Pentreath and Cobb (1972), however, state that "there is no physiological evidence to show conclusively that these 'specialized' cells transduce any particular stimulus into signals within the nervous system." They also suggest that the epithelial cells of echinoderms are generally sensory in function and often lack specialization.

Ensnarement of food particles by the tentacles of *P. californicus* occurs through two processes. Particles may become trapped by the cupping formation of the tentacle at the onset of contraction, but we believe this mechanical action of the tentacles is of relatively little importance in feeding, as there is no reverse process of tentacle expansion or relaxation while the tentacle is in the pharyngeal cavity. Adherance of food particles to the tentacle nodules by the secretion of an adhesive substance at the surface of the nodules is the most important method of food collection by *P. californicus*. The extended tentacles effectively become adhesive discs that are placed against the substratum being grazed, and thus detritus brought in contact with the tentacle adheres to it and is drawn into the mouth.

The tentacles of the pentactula larvae function in a similar manner, except that adhesion is the sole mechanism by which food material can be collected. The tentacle morphology and histology of juvenile *P. californicus* includes the same structural components as the adult tentacles. Juvenile tentacles, however, do not show the extensive proliferation of branching and concomitant growth of the adult tentacles.

D. BIOLOGY, ECOLOGY AND BEHAVIOR OF THE JUVENILE LIFE STAGE

I. Introduction

Aspidochirote holothurians are common epibenthic detritivores with a worldwide distribution (Mitsukuri, 1903; Trefz, 1958; Ricketts and Calvin, 1968; Jespersen and Lützen, 1971; Bakus, 1973; Lawrence, 1979; Roberts, 1979; Sloan, 1979; Conand, 1981; Roberts and Bryce, 1982), and while much of the adult life history of these animals has been studied little is known of the biology and ecology of the juvenile life stage from settlement to sexual maturity (Bakus, 1973; Conand, 1981).

The California sea cucumber *Parastichopus californicus* (Stimpson), an aspidochirote commonly found in the rocky subtidal along the Pacific coast of North America from Baja California to British Columbia (Ricketts and Calvin, 1968; Brumbaugh, 1980; Kozloff, 1983), has over the years been the focus of a considerable amount of scientific study. Johnson and Johnson (1950) have noted the incidents of mature gametes collected by dissection from adult animals. Margolin (1976) has reported that adult *P. californicus* respond by swimming to escape the touch of many predatory sea stars commonly encountered within its preferred habitat. Cameron and Fankboner (1984) report on tentacle structure and processes of feeding in adult and juvenile animals. Additionally, Swan (1961) has reported that adult *P. californicus* expel their visceral organs on a seasonal basis in the fall and early winter of each year, but Fankboner and Cameron (in press) have shown that this process is actually one of visceral atrophy and not evisceration.

Interestingly enough none of the sea stars reported by Margolin (1976) to initiate swimming behavior in *P. californicus* are considered to regularly prey upon this sea cucumber (Greer, 1961; Mauzey *et al.*, 1968; Shivji, 1983), but the prey potential of juvenile *P. californicus* to these stars and their ability to respond to the touch of these predatory sea stars is unknown. Additionally, the extent to which visceral atrophy may occur in juvenile animals has not been reported. With this in mind, and with the discovery of apparent nursery areas where newly settled and juvenile *P. californicus* were regularly observed, a study of various aspects of the biology and ecology of the juvenile life stage in this sea cucumber was undertaken.

II. Materials and Methods

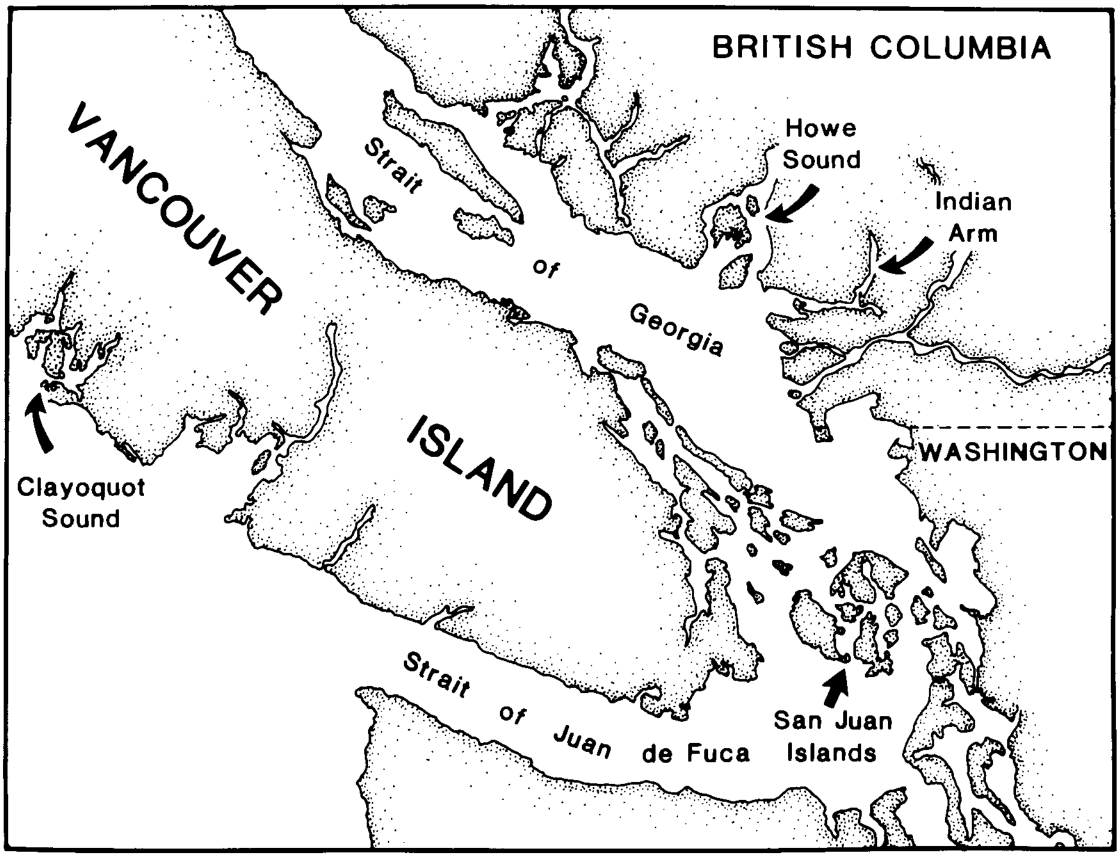
Occurrence and Distribution

Two hundred and twenty-eight SCUBA dives to observe or collect *P. californicus* were made in various locations surrounding the San Juan, Islands, Washington; and Indian Arm Fjord, Howe Sound, and Clayoquot Sound, British Columbia (Fig. D.1). These dives included systematic and repeated sampling of distinct habitats and populations of *P. californicus*. The presence or absence of juvenile animals was noted, and some general characteristics of each habitat observed.

Size and Growth

Traditionally the juvenile sea cucumber that results from the metamorphosis of the doliolaria or the vitellaria larva, or is released by the parent after brooding has been termed a pentactula because it possesses five primary buccal podia (MacBride, 1914, p. 539; Hyman, 1955, p. 188). Even though this life stage is morphologically similar to, and generally found in the same habitat as the parent, it has been called a larva (Barnes, 1980, p. 995). For the purposes of this paper these newly settled animals are termed larvae as long as they possess

Figure D.1. Geographic overview of the regions surrounding the southern portion of Vancouver Island showing all major study areas: Clayoquot Sound, Howe Sound, Indian Arm fjord, and the San Juan Islands.


BRITISH COLUMBIA
VANCOUVER
Strait
of
Georgia
Howe Sound
Indian Arm
Clayoquot Sound
ISLAND
WASHINGTON
Strait of Juan de Fuca
San Juan Islands

no more than their five original buccal podia. With the appearance of one additional buccal podium the animal is then called a juvenile of the 0+ year class.

Holothurians are quite plastic in their body dimensions rendering single measurements of linear size, such as length, very unreliable. Additionally, because of the nature of their respiratory process (repeated filling and emptying of their internal fluid filled respiratory trees), day to day measurements of mass in adult *P. californicus* can vary by as much as 100 gm (Cameron, personal observation). Therefore a bi-dimensional index of size was determined for individual animals.

This size index is determined from the relationship of contracted length (in cm) X contracted width (in cm) X a scaling factor of 0.1 (see Yingst, 1982). Measurements were taken from animals that were teased to stimulate contraction until the body wall became rigid from internal turgor-like pressure of the coelomic fluid. The very smallest animals were measured to the nearest 0.1 cm while larger animals were measured to the nearest 0.5 cm.

The accuracy of the size index as a measure of body dimension was determined by correlation analysis of the size index to damp weight of the body wall. This weight included the body wall, buccal bulb and longitudinal muscles. The gut, gonad (if present), respiratory trees, polian vesicle, and coelomic fluid were all removed prior to weighing the body wall.

Individual growth in newly settled pentactula larvae reared *in vitro* at Simon Fraser University was measured with an ocular micrometer through a Wild M5A stereo microscope (≃ five months after settlement). Individual growth within two populations of newly settled juvenile sea cucumbers discovered in the spring of 1982 and 1984 within the rocky subtidal at Kelvin Grove near Lions Bay, Howe Sound, B.C. was observed by periodic collection (≃ every three months) and sizing. Individual size index determinations from two samples of juvenile *P. californicus* collected from Croker Island were pooled and separated into size/age classes by probability paper analysis (Cassie, 1954; Birkeland *et al.*, 1982).

Seasonal Dynamics of the Viscera

The processes of visceral atrophy in juvenile *P. californicus* were noted from dissections of juvenile animals collected from Croker Island, Indian Arm fjord.

Behavior

The behavior of newly settled pentactula larvae that had been reared *in vitro* at SFU and 0+ year class animals collected at Kelvin Grove was observed via stereo microscopy as described above. Behavioral observations were also made on 0+ year class animals *in situ* at Kelvin Grove.

The response of juvenile animals (0+ year class) to the touch of the predatory sea star *Pycnopodia helianthoides* was observed in three trials on 10 different animals. The tip of a single ray from a small *P. helianthoides* was placed mid-dorsally on the experimental animal for a maximum stimulation period of 20 seconds. The ray was removed at this time or before when first response was observed. Time to first response and duration of the response was noted for each application in each trial. The intensity of the response was subjectively assigned to one of four categories: no response (NR), within 20 seconds of stimulation; local contraction of the body wall at the site of stimulation (CONT); arching of the body (ARCH); and undulating contractions of the body or "swimming" (SWIM) (Margolin, 1976).

Prey Potential

The prey potential of *P. californicus*, specifically juveniles, to a predatory sea star was observed in running sea water aquaria at the University of Washington's Friday Harbor Laboratories. Adult specimens of the sea star *Solaster endeca* were starved for two to six weeks then allowed to forage after *P. californicus* of various sizes (ages). Three or five sea cucumbers of uniform size per trial were placed into an aquarium in which a sea star was already present.

Morphological Observations

Juvenile *P. californicus* ≤ one year old and < 1 cm long were prepared for histological examination of their internal organ systems by first relaxing the organism in magnesium chloride then fixing entire animals for 15 minutes in 4% glutaraldehyde buffered with 0.2M sodium cacodylate (pH 7.4). Specimens were then dehydrated in a graded series of ethyl alcohol to 70% and embedded in JB4 embedding medium (Polysciences, Inc.). Eight to ten sequential sections 1 - 2 µm thick were cut at approximately 15 - 20 µm intervals along the length of whole animals. These sections were stained by the method of Richardson *et al.*, (1966), and examined and photographed on a Nikon Optiphot light microscope. Whole, fixed and dried, or living 0+ year class juveniles were dissected open and the viscera examined via stereo microscopy.

Newly settled pentactula larvae and juvenile animals (0+ year class) for scanning electron microscopy (SEM) were fixed as above then post-fixed in 2% osmium tetroxide buffered with modified Dorey's solution B for 15 minutes (Fankboner, 1978). Specimens were dehydrated either by critical point drying (CO_2), or by freezing in liquid nitrogen followed by vacuum evaporation. Specimens were stub mounted, gold coated, and examined and photographed on a Joel JSM-35 scanning electron microscope.

The general external morphology of pentactula larvae and juvenile animals was observed by stereo microscopic examinations of living specimens. Micrographs of these animals were taken through a Wild M5A stereo microscope equipped with a Nikon AFM photo-microscope attachment.

Dermal ossicles from the body wall of pentactula larvae, juvenile (0+ year class), and adult *P. californicus* were prepared for SEM by corrosion of the soft tissues with 6% sodium hypochlorite. The free ossicles were then washed numerous times in distilled water and allowed to air dry. The dry ossicles were gold coated, examined, and photographed as described above.

Symbionts

Notations on the occurrence of *Arctonoë pulchra*, an ectozoic polynoid, worm were made on all 0+ year class animals collected at all sites, while the presence of *Enteroxenous parastichopoli*, a worm-like endoparasitic gastropod that is commonly found within the coelomic cavity of adult *P. californicus* attached to the anterior gut, was observed only from animals dissected for visceral condition observations. The occurrence of an unidentified sporozoan parasitic on the gut of this holothurian was also noted from dissected animals.

III. Results

Occurrence and Distribution

Juvenile *P. californicus* of the 0+ year class (previous Fall settlement) were either regularly or never observed at specific study locations (Table D.1). Juvenile animals at Woodlands Bay and Croker Island were commonly found within dense mats of the filamentous red alga *Sarcodiotheca gaudichaudii* or attached to the thalli and stipes of *S. furcata* (Croker Island only), while at Ritchie Bay (Clayoquot Sound) 0+ year animals were found on the parchment tubes of the sedentary polychaete *Phyllochaetopterus* sp. which also support a significant growth of the red alga *Callophylis flabellulata*.

Juvenile *P. californicus* (0+ year class) collected at Kelvin Grove were most often found in fissures or crevices that afforded overhang protection on a nearly vertical rock wall extending from ≃ 2 to 20 m in depth. Most of these young were observed in ≃ 10 m of water. Divers were able to collect numerous 0+ year class individuals (11 - 42 per 25 minutes of diving time) by searching only in areas as described above.

Newly settled sea stars (*Pisaster brevispinus, P. ochraceus, Pycnopodia helianthoides, Evasterias troschelii* and others) and sea urchins (*Strongylocentrotus franciscanus* and *S.*

Table D.1. Study locations within the San Juan Islands; Indian Arm, Fjord; Howe Sound; and Clayoquot Sound that were repeatedly visited over an extended period of time (except Clayoqout Sound) from which the presence or absence of 0+ year class *Parastichopus californicus* was noted.

Location	Juveniles Present†	# of Dives at Site	Time Span of Dives
Cantilever Pier, Friday Harbor Labs	-	39	May 79 - Mar 80
Pt. George‡, Shaw Island	-	45	May 79 - Dec 84
Shady Cove, San Juan Island	-	6	May 79 - Mar 80
Woodlands Bay, Indian Arm	+	77	Sep 80 - Oct 83
Ritchie Bay, Clayoquot Sound	+	7	Jan 81
Croker Island, Indian Arm	+	32	Jun 82 - Oct 83
Kelvin Grove, Howe Sound	+	22	Jun 82 - Dec 84

†(+) = presence of numerous size/age classes within the population. 0+ year class most definitely present. (-) = animals within these areas are all adults and are of approximately the same size.

‡One juvenile animal, probably early 1st year class, was collected at Pt. George in November of 1979. No other juvenile animals were discovered at this location.

dröebachiensis) were also regularly observed in the summer and early fall of each sampling year at Kelvin Grove and Indian Arm (both Woodlands Bay and the northern tip near Croker Island). *Pisaster brevispinus*, *P. ochraceus*, *Pycnopodia helianthoides* and *E. troschelii* were all observed at Woodlands Bay, while *Pisaster ochraceus* and others were observed at Kelvin Grove. Adults of these sea stars, as well as others, commonly occured within the epibenthic community where these young echinoderms were observed.

Recently settled *S. franciscanus*, ≃ 25 - 50 m^{-2}, (test diameter ≤ 1 cm) were observed in great numbers each year we were sampling at Kelvin Grove. A first or possibly second year juvenile specimen (test diameter ≃ 5 cm) was seen on one occasion, but no adult *S. franciscanus* were ever observed in this area. Massive settlements (estimated at > 300 m^{-2}) *S. dröebachiensis* were noted in the shallow subtidal at the northern tip of Indian Arm opposite the west side of Croker Island in the summers of 1982 and 1983, but few if any of these young survived. Rainwater runoff during the very wet winter season turns the top 1.5 to 2.5 m of water in Indian Arm virtually to fresh water (compare Gilmartin, 1962), and massive mortality, to almost equal recruitment, among these young echinoids was observed at this time (see Himmelman *et al.*, 1984). Occasionally older *S. dröebachiensis* ≃ 3 - 6 cm test diameter were observed under rocks and in crevices within this area.

Size and Individual Growth

From probability paper analysis of size frequency data, ages of juvenile *P. californicus* are reported as 0+ year class through 4th year class. 0+ year class animals are < 12 months old; 1st year animals are ≃ 12 to 23 months old; 2nd year animals are ≃ 24 to 35 months old; 3rd year animals are ≃ 36 to 47 months; and 4th year animals are ≃ 48 to 55 months old. Mature animals are thought to be ≥ 56 months old (Table D.2).

Contracted pentactula larvae with no internal ossification measured within a few days of metamorphosis are ≃ 150 μm long (inclusive of their tentacles). *In vitro* growth of the pentactula proceded slowly (Table D.3), and only one instance of the development of secondary podia, either locomotory or feeding, was noted. Approximately 50 days after first settlement within one cohort a single juvenile was observed with seven tentacles while all the remaining animals in this cohort still had only five tentacles. The tentacles in aging pentactulae soon lengthen considerably and by ≃ 75 days after first settlement the largest pentactulae have oral tentacles that when fully extended may reach one half the length of the body.

Individual growth in juvenile animals (as observed from field collected specimens) appears to be a lengthening of the body in an anterior direction. As the young sea cucumbers grow the primary posterior tube foot retains its terminal position near the anus. Additional tube feet appear anterior to this first pedicle alternating irregularly across the ventral surface among the radii of the trivium (Figs. D.2 and D.3). Near the end of the first year post-settlement a small cluster of tube feet appears terminally around the primary tube foot (Fig. D.4). In no instance was the emergence of additional tube feet or dorsal papillae noted on pentactulae reared *in vitro* before an electrical failure within the culture system resulted in the deaths of all laboratory reared young. The smallest 0+ year class animals collected from Kelvin Grove (≃ 0.5 cm in contracted length) had distinctly proliferated water vascular systems with numerous tube feet, dorsal papillae, and buccal tentacles (Table D.4).

Individual growth within two populations of newly settled juvenile *P. californicus* from Kelvin Grove was observed periodically over the months following the settlement of larvae in 1981 and 1983. Juvenile animals were first noted in the early spring of 1982 (≃ 8 months post-settlement) when they were ≃ 0.3 - 1.0 cm in contracted length (Fig. D.5), and were first collected in the summer of 1982 when they were ≃ 0.5 - 1.9 cm in contracted length. Juveniles of this population were sampled nearly quarterly until June 1983 over which time there was an ≃ 750% increase in the average size index of this population (Table D.5). A second population of juveniles was discovered at this same location in the summer of 1984 and collected ≃ every other month until Dec. 1984. Analysis of variance for unequal

Table D.2 Probability paper analysis of pooled size frequence data from two samples of juvenile *Parastichopus californicus* collected at Croker Island, Indian Arm Fjord, B.C.

Year Class	Size Index $\bar{x}$(SD)	Range	*n*	Percentage of Total
1	1.01(0.83)	0.00- 2.50	34	34.3
2	4.45(1.69)	2.51- 7.50	52	52.5
3	8.97(1.38)	7.51-11.50	11	11.1
4	13.40(0.61)	11.51-14.50	4	4.0

Table D.3. Length/width measurements of the pentactula larvae of *Parastichopus californicus* reared *in vitro* at Simon Fraser University as measured on January 3, 1983 from pentaculae that had settled by mid-August of 1982 or animals ≃ 4.5 - 5 months old.

Animal #	Length (mm)	Width (mm)	Size Index†
1	1.16	0.42	4.87×10^{-4}
2	2.91	1.25	3.62×10^{-3}
3	0.83	0.42	3.48×10^{-4}
4	1.08	0.83	8.96×10^{-4}
5	0.66	0.42	2.77×10^{-4}
6	0.58	0.33	1.91×10^{-4}
7	0.58	0.33	1.91×10^{-4}

†The size index here is calculated as elsewhere in the paper and is based on cm units.

Table D.4. Observations on the development of the external structures associated with the water vascular system from individual 0+ year class *Parastichopus californicus*.

Size Index	#Tube Feet	#Papillae	#Buccal Tentacles†
0.018	13	22	--
0.020	14	24	13
0.024	8	15	--
0.024	16	19	--
0.033	18	25	--
0.045	22	29	15
0.049	19	22	--
0.060	24	44	--
0.075	25	31	--
0.092	48	37	17

†It was not possible to count the buccal podia in the majority of the animals examined.

Table D.5. Average size index and approximate age of juvenile *Parastichopus californicus* collected at Kelvin Grove, Howe Sound, B.C. from Jul 1982 through Dec 1984.

Date Collected	*n*	Size index $\bar{x}$(SD)	Age†
Jul 28, 1982	25	0.039(0.026)	≃ 10 mo
Oct 19, 1982	42	0.118(0.090)	≃ 12 mo
Jan 3, 1983	11	0.155(0.105)	≃ 14 mo
Apr 7, 1983	19	0.179(0.128)	≃ 18 mo
Jun 9, 1983	21	0.299(0.197)	≃ 20 mo
Jul 3, 1984	27	0.030(0.022)	≃ 8 mo
Jul 17, 1984	12	0.022(0.022)	≃ 9 mo
Sep 26, 1984	17	0.084(0.063)	≃ 11 mo
Dec 3, 1984	16	0.050(0.028)	≃ 13 mo

†This is an approximation assuming a mid-October 1981 recruitment.

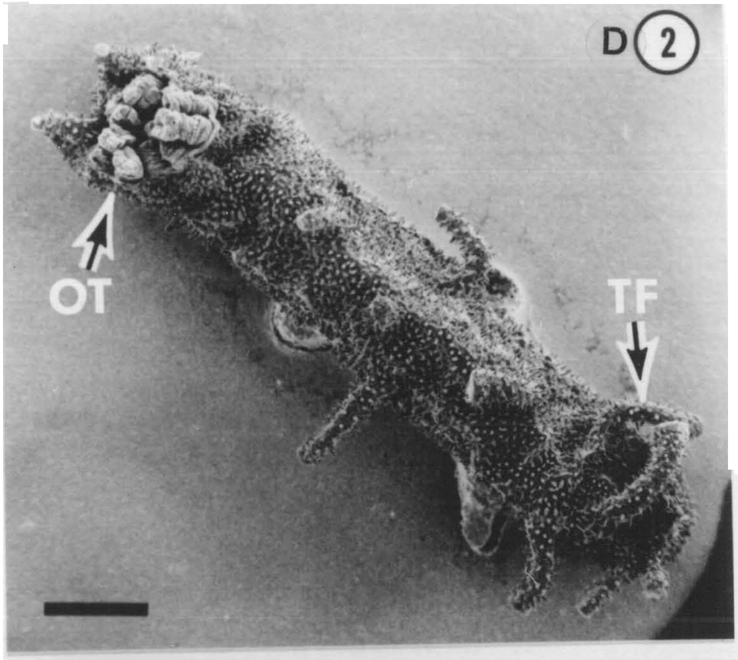
sample size was performed separately on the individual size index observations from these two populations of newely settled *P. californicus*, and in both instances the null hypothesis that all sample means were equal was rejected $P < 0.05$ in both cases, for the first sampling $F = 13.819$, $\nu_1 = 4$, $\nu_2 = 108$, and for the second sampling $F = 8.835$, $\nu_1 = 3$, $\nu_2 = 67$. Determination of which means were significantly different was accomplished by Newman-Keul pairwise comparison (Table D.6).

Individual growth in the first population was greatest in the summer and remained static in the winter increasing again in the spring (Fig. D.6). While a different growth pattern was observed within the second population, wherein, there was an obvious decrease in body size during the winter among this group of animals (see Table D.5 and Fig. D.6).

Correlation analysis of the relationship of size index to stripped body weight of juvenile animals collected at Croker Island showed that there is a significant positive relationship ($r = 0.96$, $n = 61$) between the two values. The prediction equation is $\hat{y} = 7.796x - 7.339$, x = size index, $\hat{y}$ = predicted body weight (Fig. D.7).

Figure D.2. SEM (ventral view) of an entire juvenile *Parastichopus californicus*. Note eight oral tentacles (OT) and ventral tube feet (TF). The largest and oldest tube feet are located toward the posterior of the animal. Scale bar, 600 µm.

Figure D.3. Light micrograph (lateral view) of an entire juvenile *Parastichopus californicus*. Note the oral tentacles (OT), ventral tube feet (TF), and dorsal papillae (DP). The "fuzzy" appearance of the papillae and tube feet is caused by the spires of the table ossicles within the body wall epithelium. Scale bar, 1.0 mm.


D 2
OT
TF

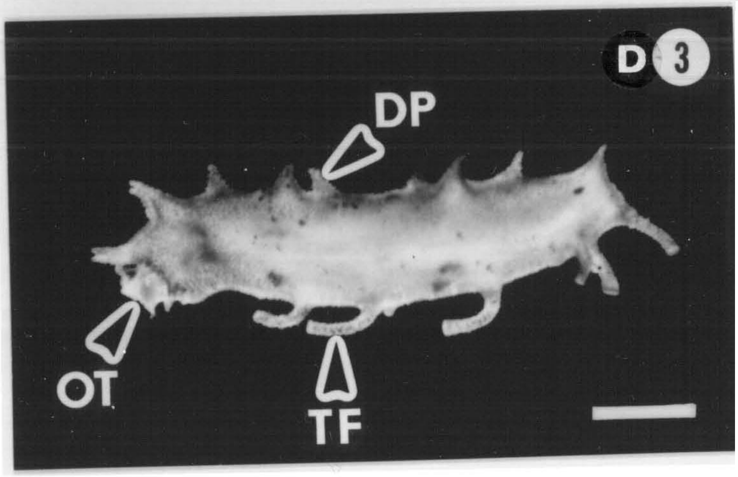

D 3
DP
OT
TF

Figure D.4. SEM of the cluster of tube feet at the posterior of a juvenile *Parastichopus californicus*. Note the large primary tube foot of the pentactula (arrow) and table ossicles within the epithelium. Scale bar, 435 μm.

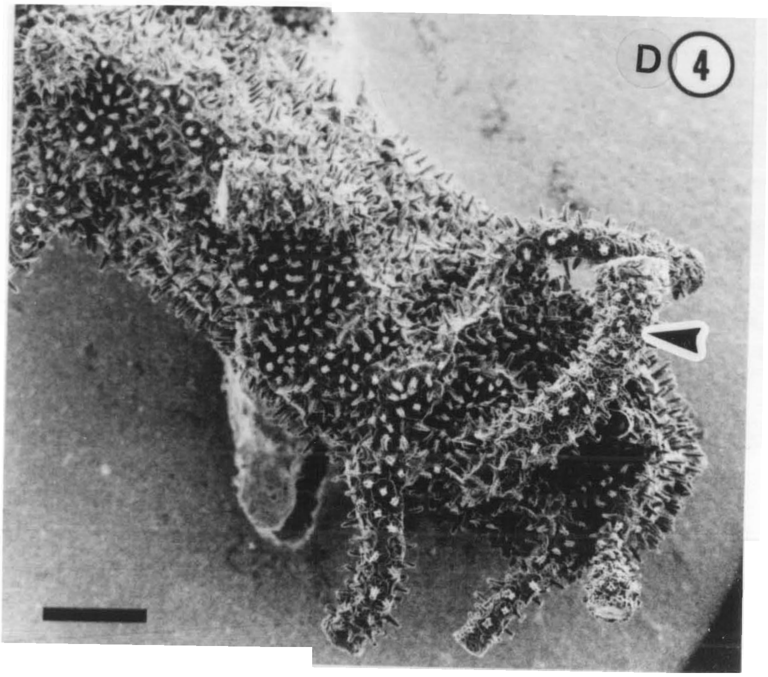

D
4

Figure D.5. Nine juvenile *Parastichopus californicus* of the 0+ year class on natural substratum. Scale bar, 0.5 cm.

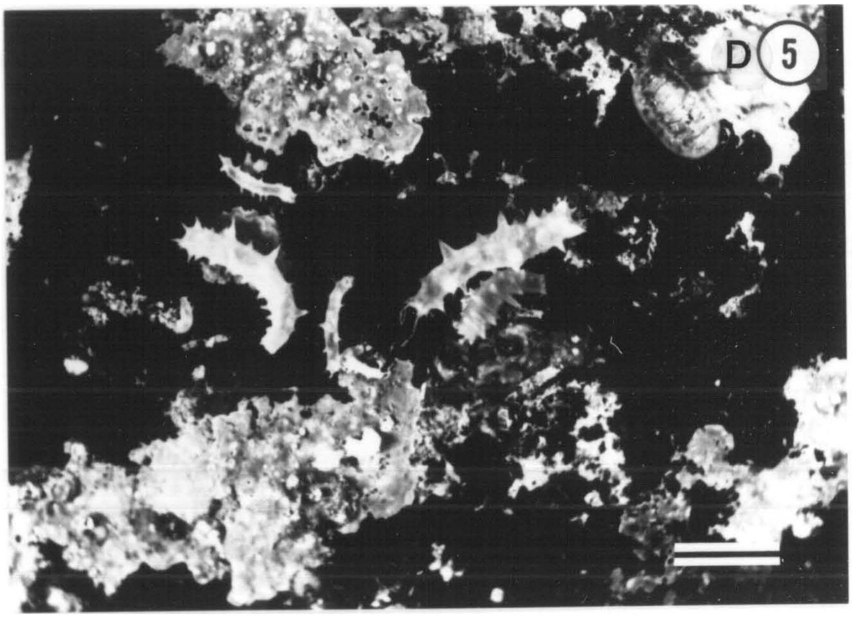

D
5

Figure D.6. Individual *in situ* growth within two groups of newly settled juvenile *Parastichopus californicus* from Kelvin Grove, Howe Sound, British Columbia.

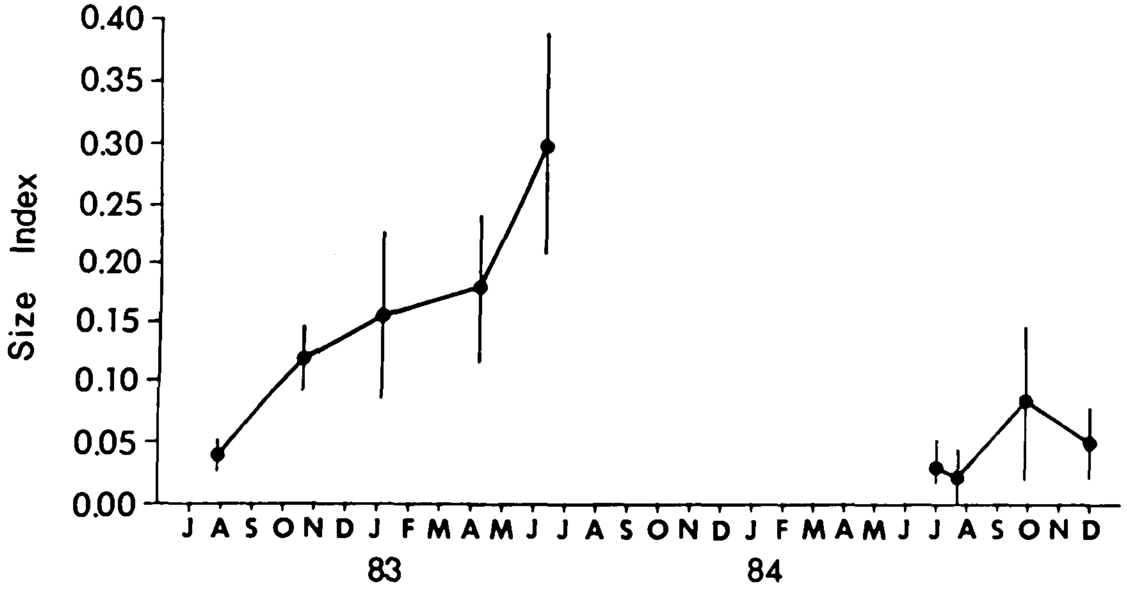
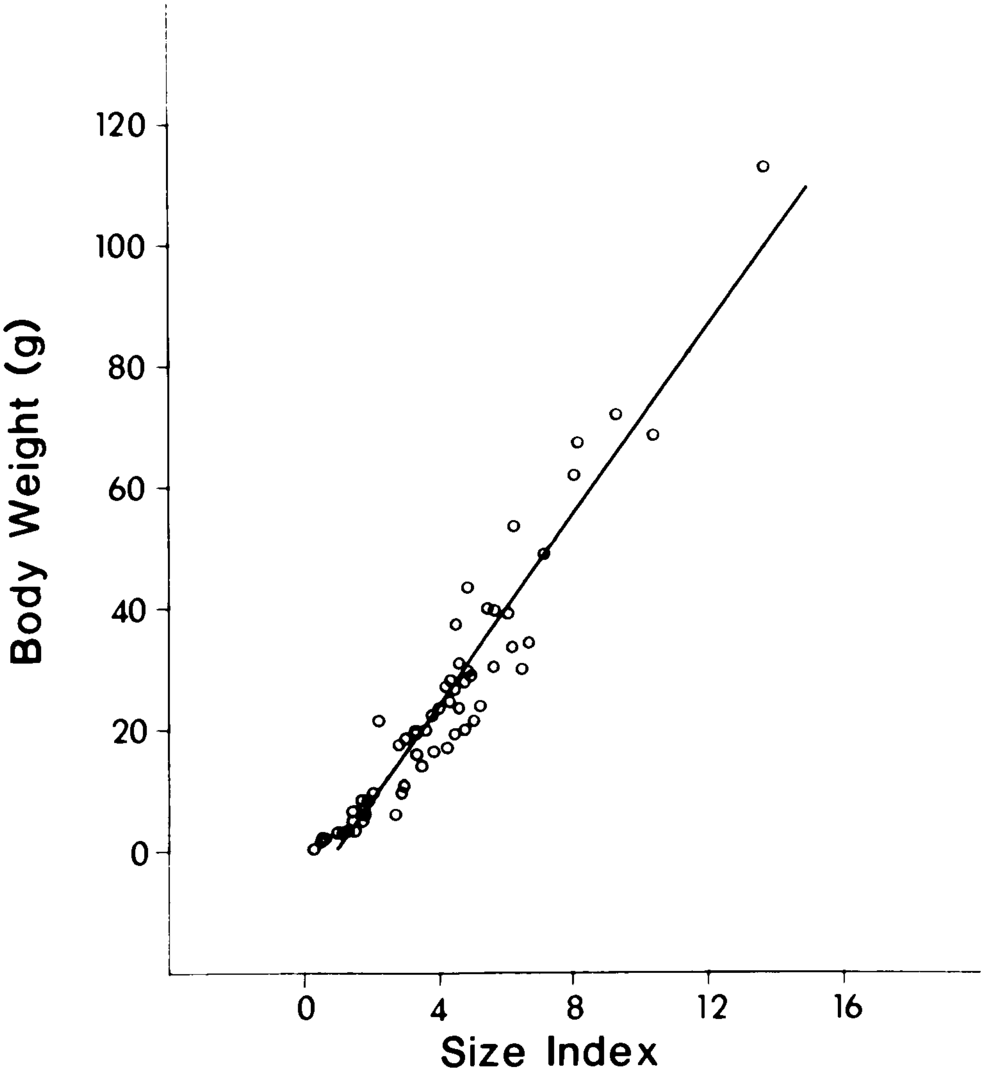

Size Index
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
83
84

Figure D.7. Regression of size index to damp weight of the body wall, $\hat{y} = 7.796x - 7.339$, $\hat{y}$ = predicted body weight, x = size index, r = 0.96 of *Parastichopus californicus* ≤ 4 years of age.


120
100
80
60
40
20
0
Body Weight (g)
0
4
8
12
16
Size Index

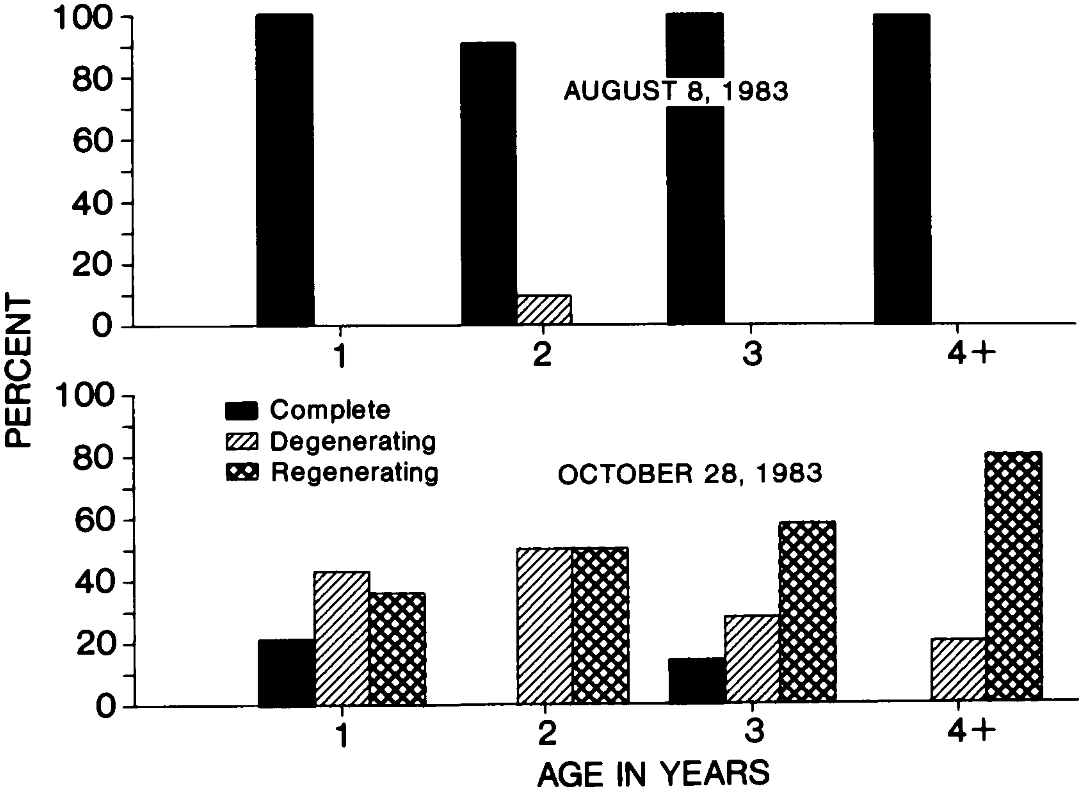
Seasonal Dynamics of the Viscera

Of 63 animals collected in August of 1983 at Croker Island all except three had complete viscera with their guts full of ingested material (Fig. D.8). In a similar sample collected at the same location in October of the same year (*n* = 36) the viscera were in a state of flux (atrophy and regeneration) as is common for the adults at this same time of the year (Fankboner and Cameron, in press) (Fig. D.8).

Behavior

The newly settled and growing pentactulae are quite active and locomote and feed via their buccal podia. When feeding the larvae sweep the substratum surrounding them collecting micro-organisms etc. Strings of fecal material were often noted in the culture vessels and/or being evacuated from the cloaca of the larvae indicating that successful feeding was occurring *in vitro*. Approximately 90 days after settlement cloacal pumping was observed suggesting the presence of functional respiratory apparatus. Pentactulae were often observed standing up-right on their single tube foot waving their tentacles above them hydra-like (compare Edwards, 1909) or by releasing the grip of the tube foot they may walk across the substratum on their tentacles (see Cameron and Fankboner, 1984 [Part C]).

Figure D.8. Condition of the viscera in juvenile *Parastichopus californicus* ≤ 4 years of age collected from Croker Island, Indian Arm.



JUVENILE VISCERAL CONDITION

Juvenile *P. californicus* observed *in situ* at Kelvin Grove feed by firmly attaching themselves to the substratum with their tube feet sweeping the rock surfaces surrounding them with their tentacles. Attempts to dislodge these animals with a suction pipette was often difficult as the terminal cluster of tube feet maintain such a grip on the substratum that many animals could not be collected without prying them free with a dissecting probe.

Juvenile *P. californicus* experimentally stimulated with a ray from the predatory sea star *Pycnopodia helianthoides* responded with varying levels of intensity and duration (Table D.7). Numerical values from 0 - 3 were assigned to the various response categories: NR = 0, CONT = 1, ARCH = 2, and SWIM = 3. A total response value for each animal tested was summed. Margolin (1976) observed 100% swimming in 100% of the contacts of *P. helianthoides* on adult *Parastichopus californicus* which results in an expected response value of nine for three trials on each of the 10 trial animals. No single juvenile's total response value equaled nine, and many were considerably less than this expected value (Fig. D.9).

Prey Potential

Juvenile *P. californicus* exposed to the predatory sea star *Solaster endeca* were variously preyed upon. The smallest animals were most readily consumed while older and larger animals

Table D.6. Newman-Keul pairwise comparison of the mean size indices from two populations of juvenile *Parastichopus californicus* collected from Kelvin Grove, Howe Sound, B.C. Means are ranked from smallest to largest values, and nonsignificant differences are underlined.

Population I.

07/28/82	10/19/82	01/03/83	04/07/83	06/09/83
0.39	0.118	0.155	0.179	0.299

Population II.

07/17/84	07/03/84	12/03/84	09/26/84
0.022	0.030	0.050	0.084

Table D.7. Response of juvenile *Parastichopus californicus* to contact of a ray from *Pycnopodia helianthoides*. Each animal was stimulated three times for a maximum period of 20 seconds. Data are reported as length of stimulation (in seconds) - length of response (in seconds) - intensity of response†.

Animal#	Trial #1	Trial #2	Trial #3‡
1	20-045-CONT	20-000-NR	20-000-NR
2	8-189-CONT	8-172-ARCH	20-112-SWIM
3	3-062-CONT	16-079-CONT	20-040-CONT
4	7-088-SWIM	6-062-SWIM	2-043-CONT
5	18-037-CONT	17-055-ARCH	8-030-CONT
6	11-068-SWIM	8-092-SWIM	5-030-CONT
7	5-050-ARCH	10-050-ARCH	10-025-CONT
8	5-079-SWIM	7-103-SWIM	20-000-NR
9	20-000-NR	18-092-ARCH	20-000-NR
10	20-000-NR	3-030-CONT	15-030-CONT

†NR = no response, CONT = local contraction of the body wall at the site of stimulation, ARCH = arching of the body, and SWIM = sinusoidal undulations of the body that result in "swimming" behavior.

‡Trials 1 and 2 were done the same day separated by a 30 minute rest period. Trial 3 was run the following day.

Table D.8. Observations of predation on *Parastichopus californicus* by *Solaster endeca* in laboratory aquaria.

Size Index $\bar{x}$(SD)	*n*	Results
0.91(0.12)	5	All 5 consumed in 24 hours.
1.67(0.05)	3	All 3 consumed in 48 hours.
3.68(0.48)	5	3 consumed in 43 hours. Star terminated all foraging activity at that time.
5.26(0.73)	3	Star actively foraged and made numerous and repeated unsuccessful attacks in the first 12 hours then terminated all foraging activity. 0 sea cucumbers consumed.

escaped predation more easily. The largest of the trial animals ($\bar{x}$ size index = 5.26, $\hat{y}$ = 33.7 gm, age ≃ 1 - 2 years post-settlement) were never captured by a foraging sea star even after 12 hours of continuous and repeated attacks (Table D.8). Adult *P. californicus* maintained in laboratory aquaria for 2 - 6 mo containing 1 - 2 specimens of either *S. endeca* or *S. stimpsoni* were occasionally attacked but never consumed by the sea stars.

Morphology

The pentactula larvae at settlement are ≃ 250 - 350 μm long (Fig. D.10). Five capitate primary tentacles (TN) encircle the mouth (Cameron and Fankboner, 1984), and there is a single postero-ventral tube foot (TF) at the extreme ventral terminus of the animal. Under the stereo microscope the body wall at settlement is slightly transparent, but the whole animal is somewhat opaque appearing a uniform white color.

Shortly after metamorphosis the process of skeletal formation intensifies, and the body wall becomes densely packed with fenestrated button ossicles totally masking from view all internal detail (Fig. D.11). Irregular plate ossicles with numerous holes surround the oral aperature forming a hood-like structure that appears to protect the buccal podia. Additionally, three nearly triangular fenestrated plates form around the cloacal aperature producing a somewhat triangular

Figure D.9. Response of juvenile *Parastichopus californicus* to the touch of *Pycnopodia helianthoides* as compared to the expected response of adult animals (from Margolin, 1976). The score for each juvenile animal is a sum of values for three trials on each animal. No response (NR) = 0, contraction of the body wall (CONT) = 1, arching of the body (ARCH) = 2, and swiming (SWIM) = 3.

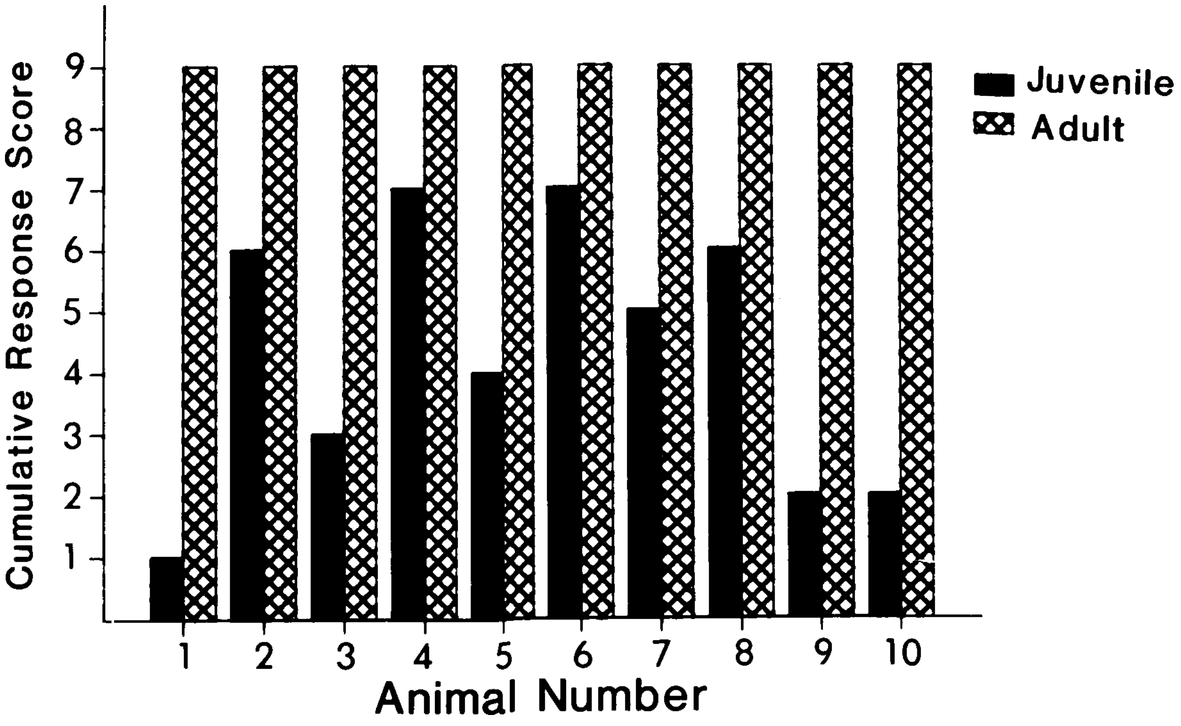
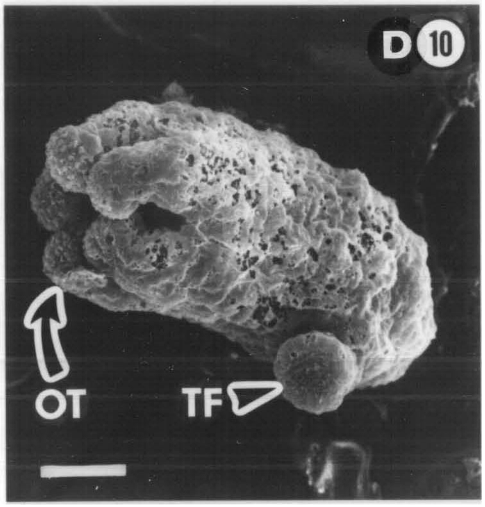
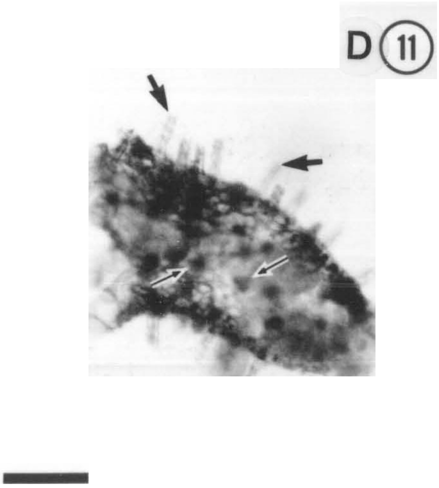

Cumulative Response Score
1
2
3
4
5
6
7
8
9
1
2
3
4
5
6
7
8
9
10
Animal Number
Juvenile
Adult

Figure D.10. SEM of a newly settled pentactula larva of *Parastichopus californicus*. Note the single primary tube foot (TF) and the five primary oral tentacles (OT). Scale bar, 85 µm.

Figure D.11. Pentactula larva of *Parastichopus californicus* with ossicles obliterating all internal structures. Note spires of the table ossicles (large arrows) and pigment spots (small arrows). Scale bar, 130 µm.


D 10


D 11

opening for discharge of fecal material and the in-flow and out-flow of respiratory water. It should be remembered that the echinoderm skeleton is entirely internal, and all skeletal pieces in living pentactulae are completely contained within the epithelium.

Within a few days of metamorphosis the buttons of the body wall begin to form the spires or legs characteristic of the aspidochirotid table ossicle. The diameter of the table surface in these ossicles is ≃ 80 - 85 μm and the spires or legs are 60 - 65 μm tall (see Fig. D.13). Numerous fenestrations occur in the table top of these ossicles, with four large primary openings surrounded by one or two rows of smaller irregular holes. As these tables develop the spires extend above the level of the body surface, but are contained within the epithelium (Fig. D.11). There are numerous cross supports on the legs or spires of juvenile table ossicles, and the tips of the spires bear numerous thorn-like projections. Occasionally brown or red pigment spots (Fig. D.11) were observed on the body walls of some pentactulae. This coloration pattern coupled with the protrusion of the spires of the ossicles gives the pentactulae an appearance superficially like that of adult *P. californicus*.

As the pentactulae grow into juvenile animals the process of ossification continues until the body wall is essentially composed of tightly contiguous table ossicles (Figs. D.12 and D.13). These ossicles are of the same general size and construction as the tables of the pentactulae. Oval buttons and

"C" shaped ossicles were also observed from the body walls of juvenile animals (Figs. D.13 and D.14). The button ossicles underlie the tables within the epithelium, and all the body wall ossicles rest upon a collagenous matrix (CM) (Figs. D.15, D.16, and D.17). This matrix is proportionately very thin, 80 - 120 µm, in juvenile sea cucumbers, but constitutes the bulk of the body wall in adult sea cucumbers (≃ 2 - 3 mm).

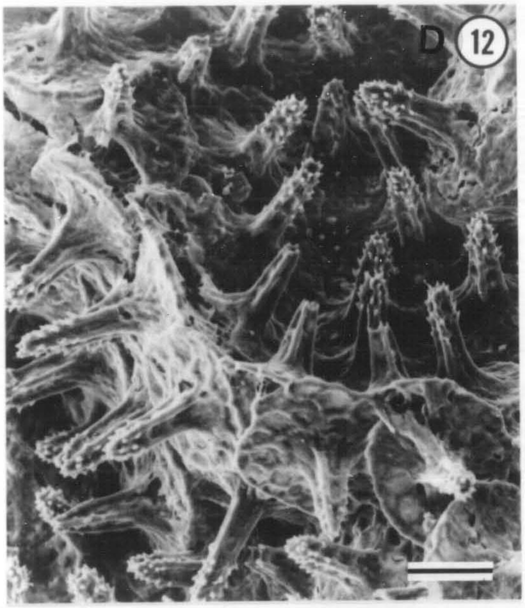
Table ossicles from the body wall of an adult *P. californicus* were also examined and found to be smaller and less complex than those from juvenile animals. The table diameter was only 60 - 65 µm, with spires 60 - 65 µm tall. The table surface in adult *P. californicus* ossicles consists of four large primary holes with four smaller holes interspersed between the larger holes at the margin of the table surface (Fig. D.18). There are fewer cross supports on the spires of adult table ossicles, and the tips of the spires are less complex than those of juvenile animals (Fig. D.18).

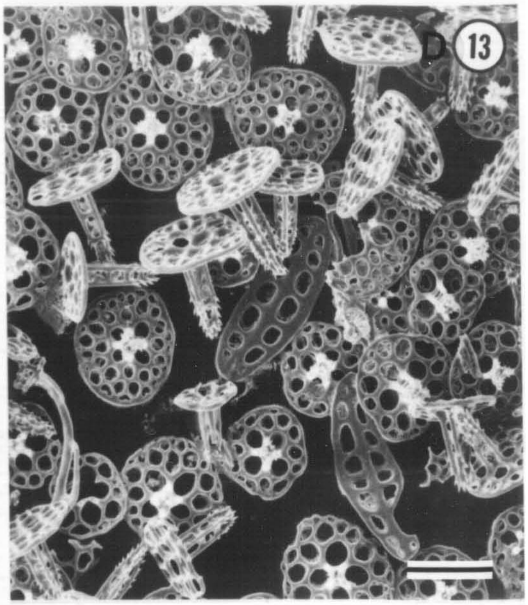
Stereo microscopic observations of the body surface (papillae and tube feet included) also show the extensive nature of the development and proliferation of these ossicles, and the characteristic way in which the spires extend out from the body surface (Fig. D.19). The pattern of ossicle distribution in late 0+ and 1st year animals (size index ≥ 0.825) visually did not appear to be different from that of much younger (smaller) animals.

Figure D.12. SEM of the surface of a juvenile (0+ year class) *Parastichopus californicus* showing the arrangement of the table ossicles within the epithelium. Scale bar, 40 µm.

Figure D.13. SEM of the table ossicles from juvenile *Parastichopus californicus* These ossicles are of the same size and morphology as the table ossicles of newly settled pentactula larvae. Note button ossicles in the center and lower right of the micrograph. Scale bar, 65 µm.

Figure D.14. "C" shaped ossicle (arrow) from the body wall of *Parastichopus californicus*. Scale bar, 30 µm.


D
12


D
13

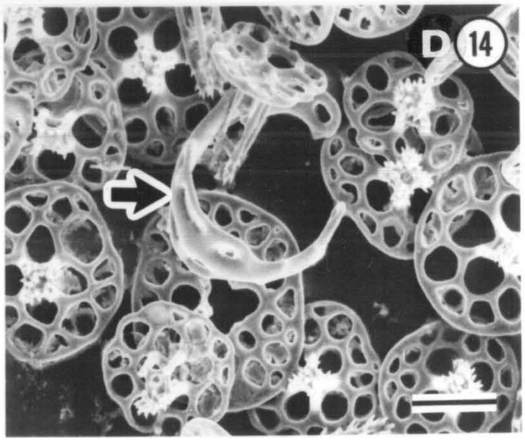
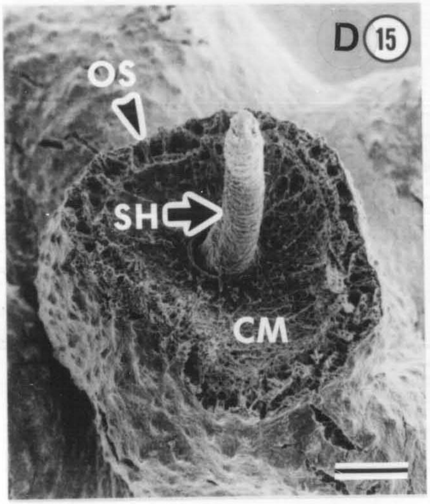
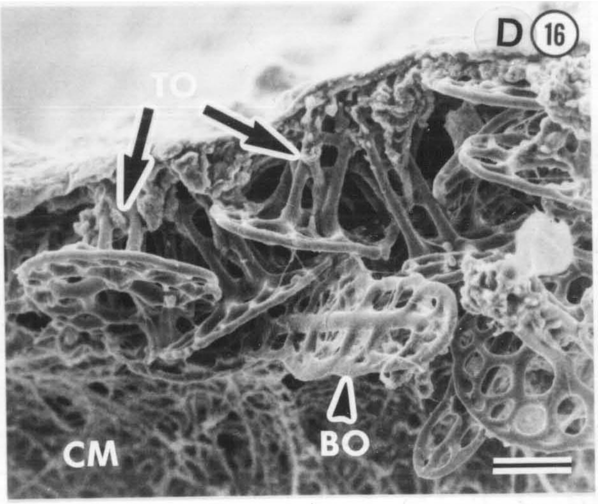

D
14

Figure D.15. SEM of fractured dorsal papilla from a 0+ year class *Parastichopus californicus*. Shaft (SH) is the central supporting canal of the papilla which is part of the water vascular system. Note the location of the ossicles (OS) in the periferal edge of the papilla. Underlying the layer of ossicles is a dense collagenous matrix (CM) that is continuous with the rest of the body wall. The thickness of the collagen here is due to the nature of the construction of the papillae and not representative of the thickness of the collagen of the body wall generally. Scale bar, 160 μm.

Figure D.16. SEM close up of the table ossicles embedded in the epithelium of a dorsal papilla. Note the button ossicle (BO) under the layer of table ossicles and between it and the collagenous matrix (CM). Scale bar, 40 μm.

Figure D.17 Light micrograph of the body wall of a 0+ year class *Parastichopus californicus* showing the location of the ossicles (OS) and the collagenous matrix (CM) of the body wall. Note also the radial nerve (RN) and longitudinal muscle (LM) of a single ambulacrum. The open space between the nerve and muscle is a radial branch of the water vascular system. PV, perivisceral body cavity. Scale bar, 70 μm.


D ⑮
OS
SH
CM


D ⑯
TO
CM
BO

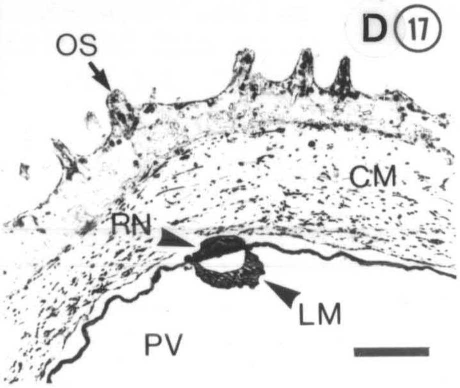

D ⑰
OS
CM
RN
LM
PV

Figure D.18. SEM of the table ossicles from an adult *Parastichopus californicus.* Scale bar, 30 µm.

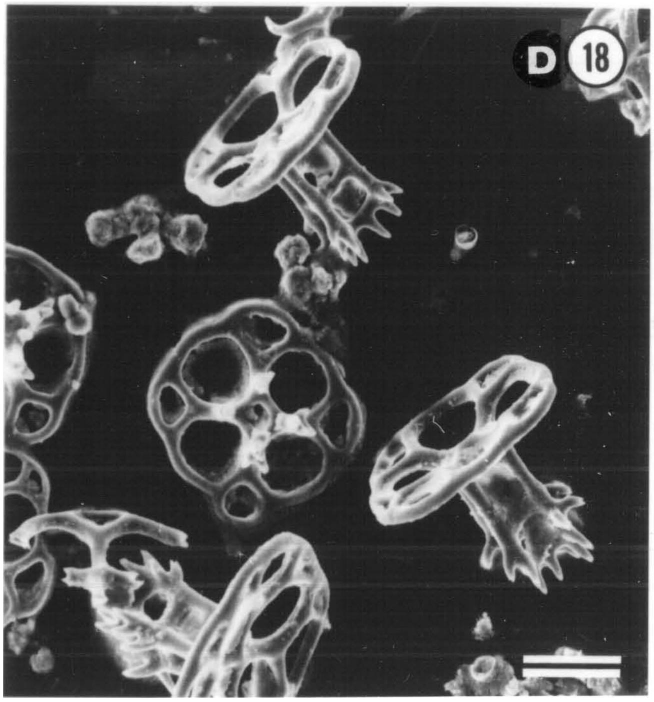
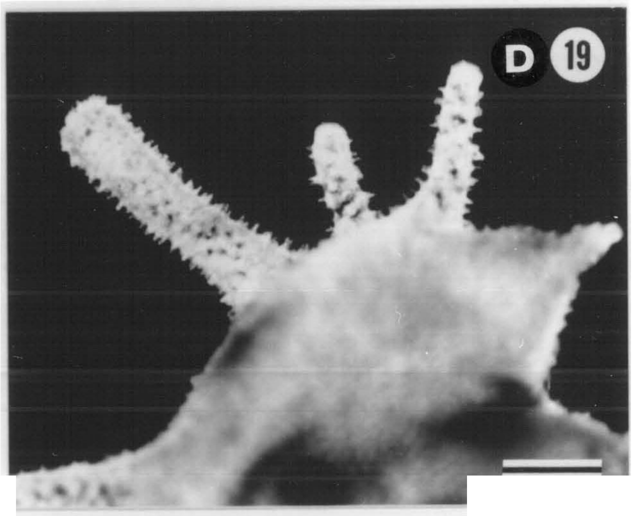

D
18

Figure D.19. Light micrograph of the tube feet from a living 0+ year class *Parastichopus californicus* showing the characteristic protrusion of the spires of the table ossicles. Scale bar, 435 µm.


D
19

Visceral organ morphology in 0+ year class *P. californicus* is identical to that of the adults. The gut consists of an anterior descending, middle ascending, and posterior descending sections forming a single loop. There is a single polian vesicle, two simple, balloon-like, respiratory trees attached to the cloaca, and a rete mirabile associated with the gut.

Juvenile sea cucumbers in the first year post-settlement are quite variable in color. The smallest animals are uniformly white while the dorsal coloration of the majority of late 0+ age class animals is a uniform pink or an intense red-maroon. Intermediate forms are often mottled with various shades of red and pink. This same coloration pattern carries through to at least the 2nd year age class (3 years post-settlement) when body coloration turns the dull brown-maroon characteristic of most adult *P. californicus*.

The red-maroon coloration of the younger animals is very similar to the coloration of the red algae with which they were often associated. This was especially so *in situ* where ambient light conditions rendered the juveniles almost invisible. Often very young (< 2 cm in length) *P. californicus* were missed on first examination of a particular habitat and until the habitat was closely examined.

Symbionts

Of the 14 different samples of animals collected at numerous locations which included 0+ year class to early 1st year class animals, only one contained animals with the scale worm *Arctonoë pulchra* present. On October 19, 1982 forty-two ≃ 1st year animals (assumed August - December 1981 settlement) were collected from Kelvin Grove three of which were host to a single specimen of *A. pulchra* (Table D.9). Two samples of juvenile sea cucumbers (0+ through 4th year class) collected at Croker Island were dissected for visceral examination of which six of the 63 animals collected on August 8, 1983 had single specimens of *E. parastichopoli* attached to their guts. One of these sea cucumbers was lacking its viscera. The parasite was attached to the anterior region of the esophagus (Lützen, 1978) which is never lost during atrophy or evisceration (Fankboner and Cameron, in press) (Table D.10). Pale white blisters were also noted on the gut of one of the animals that had been dissected. These have been shown to be the encysted stage of an unidentified sporozoan occasionally found on the gut of adult *P. californicus* prior to the onset of/or during early visceral atrophy (Fankboner and Cameron, in press).

Table D.9. Size of juvenile (0+ year class) *Parastichopus californicus* and the symbiotic polynoid *Arctonoë pulchra* found associated with them.

Parastichopus calilfornicus			*Arctonoë pulchra*
Length(cm)	Width(cm)	Size Index	Length(cm)
2.2	0.6	0.132	0.5
3.2	0.7	0.224	0.6
2.8	0.4	0.112	0.8

Table D.10. Size and age of juvenile *Parastichopus californicus* infested with the endoparastic gastropod *Enteroxenous parastichopoli*.

Size Index	Year Class	# *E. parastichopoli* Present in Coelom
4.20	2nd	1
6.75	3rd	1
9.36	4th	1
4.20	2nd	1
4.62	2nd	1
5.00	2nd	1†

†This specimen of *E. parastichopoli* was gravid.

IV. Discussion

The discovery of juvenile (0+ year class) *P. californicus* at Kelvin Grove allowed us to follow growth in newly settled sea cucumbers, and to examine the biology and ecology of these young animals to evaluate the significance of the juvenile life stage in the life history of this commercially important holothurian. This study was somewhat opportunistic as juvenile *P. californicus* < one year old and < 1 cm in length are difficult to detect. The numbers of animals collected on each sampling trip was dependent upon the accessibility of sites where juveniles were known to occur, the length of time a diver could stay in the water, and on a desire to sample a sufficient number of animals to experiment with yet without depleting the population. With this in mind divers swam predetermined routes collecting all juvenile sea cucumbers < 5 cm in length that were observed during 25 minutes of diving time. It was felt that the 5 cm length constraint might bias the mean individual growth downard slightly, but seemed a natural cutoff point from subjective notation of very few animals 5 - 10 cm in length within the habitats examined.

Individual growth, as observed over nearly the first two years after recruitment, is most rapid in the spring and early summer slowing down in late summer and stopping or regressing in the winter months. This pattern of growth is similar to body weight changes observed in the adults of the species (Fankboner and Cameron, in press).

Growth to the adult size is determinate with often all the sea cucumbers in any one habitat being generally the same size (Bakus, 1973; Conand, 1981; Cameron, personal observation). Adult body weight in *P. californicus* is not constant over the year though, as ≃ 25 % of the summer maximum body weight is lost during the winter when feeding has stopped and visceral atrophy and subsequent regeneration are occurring (Fankboner and Cameron, in press).

The size structure of the populations of sea cucumbers at the Kelvin Grove, Croker Island and to a lesser extent Woodlands Bay regularly consisted of numerous small and intermediate sized animals suggesting that successful settlement of juveniles into these areas occurred quite regularly. Recruitment at Kelvin Grove during 1981 and 1983 was noted with the appearance of 0+ year animals in the summers of 1982 and 1984. No juveniles that could have been 0+ animals were observed in the summer of 1983. This may not be the regular pattern of recruitment at Kelvin Grove as subjective observations of this population of sea cucumbers included animals that appeared to be of all year classes, 0+ to adult. Apparently settlement had occurred regularly each season over the previous four or five years at least.

The noteworthy occurrence of numerous echinoderm species settling into those areas where young *P. californicus* were commonly found, and the subsequent high mortality within these areas, coupled with the absence of adults of at least one of the species, seems to indicate that some environmental parameter was appropriate for the settlement or congregation of pelagic larvae at these sites. Tidal current patterns may aggregate the larvae of these animals in these areas until they are sufficiently competent to settle or until so far into their competent period that they settle into habitats that are marginally suitable for the adult animals (Thorson, 1950 and 1966).

The process of visceral atrophy and or seasonal evisceration has been examined previously in adult holothurians (Swan, 1961; Byrne, 1983; Fankboner and Cameron, in press).; Examination of 0+ through 4th year classes of juvenile animals showed the processes of visceral degeneration and subsequent regeneration that occurs in adult animals also occurs in all age classes of sea cucumbers. This process is staggered throughout the population though with < 100 % of the population in either the degeneration or regeneration phases at any one time. Yet all of the animals in a given population undergo visceral atrophy and subsequent regeneration seasonally (Fankboner and Cameron, in press).

Adult *P. californicus* display an interesting "swimming" response to the touch of predatory sea stars (Margolin, 1976) yet none of the stars that stimulate this response are reported to regularly feed upon *P. californicus* (Mauzey *et al.*, 1968). Juvenile sea cucumbers also respond to the touch of a predatory sea star, but not with the same level of intensity as reported for the adults. The large sun star *Pycnopodia helianthoides* which is known to be a voracious predator of numerous benthic invertebrates (Greer, 1961; Mauzey *et al.*, 1968; Paul and Feder, 1975; Shivji *et al.*, 1983) elicits the most intense and regular response in adult *P. californicus* (100% swimming, 100% of times contacted, Margolin, 1976) yet appears seldomly if ever to consume *P. californicus in situ* (Fisher, 1928; Greer, 1961; Mauzey *et al.*, 1968; Paul and Feder, 1975).

At the Woodlands Bay site where the density of *P. helianthoides* reaches ≃ 15 - 20 m^{-2}, quiescent *P. helianthoides* were often seen with one or more rays draped across the dorsal surfaces of *P. californicus* eliciting no unusual behavior of any kind. Mechanical stimulation of the star by a diver (either prodding or handling) initiated active movement in the star, and stars so stimulated invariably elicited typical swimming behavior in sea cucumbers they touched. This suggests that active or excited stars somehow stimulate swimming behavior when quiescent stars do not (cf. Sloan, 1980, p. 110).

Three congeneric species of predatory sea stars, *Solaster dawsoni*, *S. endeca*, and *S. stimpsoni* which sometimes occur in areas inhabited by *P. californicus*, also elicit a significant swimming response in adults of this species (56 - 91% of contacts resulted in swimming; Margolin, 1976). A considerable portion of the diets of these three species of *Solaster* is known to consist of holothurians from 1 - 3 cm long (*Cucumaria* *lubrica*) up to 10 - 15 cm long (*Eupentacta* spp.) though not *P. californicus* (Mauzey *et al*., 1968; Birkeland *et al*., 1982).

Juvenile *P. californicus* were readily consumed by *S. endeca* in laboratory predation experiments up to a specific size threshold above which the stars appeared unable to capture the sea cucumbers offered to it (Fisher, 1928). Size refuges from predation are common among marine invertebrates wherein older animals reach a size sufficiently large enough to avoid being consumed by predators that commonly feed upon smaller individuals of the species (Paine, 1976). It appears that juvenile *P. californicus* ≥ 2 years of age are immune to predation by *S. endeca*, and probably other predatory sea stars as well.

The behavior reported as swimming by Margolin (1976) consists of rapid undulating or sinusoidal movements of the body which propels the sea cucumber across the bottom in a random and haphazard fashion. Often the sea cucumber is elevated off the bottom for short periods of time. Margolin (1976) reported a forward (anterior) directional component to this movement, but my personal observations suggest that wave surge or tidal currents account for any directional aspect to this movement (Kroop, 1982).

Swimming behavior is displayed by all size classes of sea cucumbers but is most effective in older (larger) animals (Sloan, 1980, p. 110), the distance that an animal moves while swimming is a function of its overall length. The sweep of the sine-type or undulating movement is much more vigorous in longer animals, and results in a greater displacement with increased thrust off the substratum. This same behavior in animals of the 0+ year class results in negligible displacement of the animals, especially in aquaria with virtually no water motion. The efficiency of the swimming escape behavior undoubtedly increases with size (age), and may be a factor in providing the apparent size refuge from predation by predatory sea stars.

Numerous macroscopic symbionts of holothurians are known (see Jespersen and Lützen, 1971). Yet, their presence and or prevalence in juvenile animals has not been reported. The purpose of this study was not to account for the varied and numerous symbiotic relationships that may occur in juvenile *P. californicus*, but some of the more obvious symbionts could not be ignored. The presence of *A. pulchra*, *E. parastichopoli*, and an unidentified sporozoan gut parasite infesting various year classes of this sea cucumber indicates that juvenile animals are not immune to infestation by these symbionts. The presence of presumedly juvenile *A. pulchra* on 0+ year class *P. californicus* would be interesting to follow to determine if growth in this polynoid is arrested by the size of its host or if the worm outgrows its host and seeks another larger host animal.

The microscopic ossicles or sclerites from the body walls of holothurians are the only hard body parts from this class of echinoderms, and are often used as major taxonomic characters for identification (Hyman, 1955, p. 134). Rowe (1969) indicates in his review of the family holothuriidae that his generic and specific identifications are based "almost entirely on the form and combination of the calcareous spicules" from the body wall and podia. This macroscopically amorphous skeleton is poorly represented in the fossil record which has resulted in a dearth of paleontological information on holothurians as a group (Pawson, 1966). Because of this, recognition of taxonomic affinities among fossil sclerites and those of living holothurians while relatively simple for some groups can be very difficult if not impossible for others (Frizzell and Exline, 1966). Changes in the general construction of the ossicles of holothurians with increasing age, as observed in *P. californicus*, is not a newly described process (Mitsukuri, 1897; Clark, 1922; Diechmann, 1937; Feral, 1979), but may confound efforts to systematize extant forms (Liao, 1979) or to characterize affinities between fossil and living holothurians.

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