SOME ASPECTS OF THE TAXONOMY AND BIOLOGY
OF THE GENUS *LEPTOSYNAPTA* (HOLOTHUROIDEA)
IN BRITISH COLUMBIA

by

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B.Sc., University of Victoria, 1965

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biology

We accept this thesis as conforming
to the required standard

UNIVERSITY OF VICTORIA

September, 1973

Supervisor: Dr. D. V. Ellis

ABSTRACT

Two species of *Leptosynapta, L. clarki* and *L. transgressor* (Heding, 1928), have been found in B.C. waters; the latter species has not been fully established as a distinct species. Various biological aspects of these species have been compared. Each species, when alive, showed a distinct external pigment pattern. The pigments of both species were analysed and believed to be naphthaquinone. 98% of adult *L. clarki* examined had 12 tentacles (range 9-13) while 65% of *L. transgressor* examined had 10 tentacles (range 8-14). Ossicles varied according to age and location of specimens and were not reliable characters for distinguishing between the species in adult specimens. Immature *L. clarki* could be distinguished by their primary ossicles which were lacking in immature *L. transgressor*. Small specimens of *L. transgressor* (6-42 mm) tended to have anterior and posterior anchors which were of one type while larger specimens (18-103 mm) had longer posterior anchors resembling those of *L. clarki*. There was some evidence that ossicles were related to environmental factors such as sediment type and exposure. Oocytes of both species developed as sediment temperatures dropped in the fall and winter, and maximum oocyte diameters were reached during minimum temperatures (Dec.-Feb.) Mature oocytes of *L. clarki* were larger (average 404 μ) than those of *L. transgressor* (average 272 μ) and there were slight differences in sperm shape and length. Fertilization occurred after the seasonal rise in temperature in the spring, though *L. transgressor* was a month (April) later in breeding than *L. clarki* (March). *L. clarki* broods its young at least until the pentacula stage. Evidence that *L. transgressor* broods its young is inconclusive; only fertilized eggs and very early embryological stages have been found in the females. The release of juveniles from the adult females of *L. clarki* has been witnessed for the first time. The release takes place through the five anal pores of the adult. *L. clarki* occurs from Queen Charlotte Islands to central California while *L. transgressor* has only been found from southern Vancouver Island to Puget Sound. *L. clarki* is mainly intertidal and often found in *Zostera* (Eel Grass) beds. It inhabits a wide range of sediments from sandy silt to gravel. *L. transgressor* is subtidal only (6-40 meters) and inhabits finer sediments ranging from sandy silt to medium sand. The faunal communities associated with each species are quite distinct. The commensal polychaete, *Malmgrenia lunnulata* has been found on both species of *Leptosynapta* and the commensal crab, *Pinnixa schmitti* has been found in the burrows of *L. clarki* for the first time. These results present sufficient information to establish *L. transgressor* as a distinct species from *L. clarki*.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Derek Ellis for his help in the preparation of this manuscript and for providing the funds and equipment which made this study possible. I am also grateful to Dr. A. R. Fontaine who reviewed parts of the manuscript and gave valuable criticism and advice, particularly on pigmentation.

Special thanks are extended to Elizabeth Deichmann who gave helpful suggestions during the preliminary stages of the work, and Dr. J.F.L. Hart who provided encouragement and contributed to the collections.

Appreciation is also extended to those who identified specimens for the community studies, particularly Rita Gustus; Tom Gore for his help with photographs; to Jim McCallum for his assistance in sediment analysis; to Briony Goddard for typing the manuscript; to Doug Hartley for his help in sampling; and to Norma Morton for general assistance. I am also grateful to Jack Brooks, Fisheries Officer at Sooke, for providing his boat for sampling; and to the Dept. of Transport for making their launch available. Collections of specimens received from Dr. D. Abbott and Tom Quigley of California, and U. Lie of Washington, are gratefully acknowledged.

Finally, I am most indebted to my husband, Allan, for his encouragement and help with the manuscript, and for the many hours he spent looking after our children so that I could finish the work.

INTRODUCTION

A number of new species of marine animals that inhabit sediment bottoms have been identified since investigations of marine macrofauna in British Columbia began at the University of Victoria in 1964. There remains, however, a paucity of taxonomic information on certain of these animals and studies in this direction are badly needed in order to obtain a more comprehensive picture of benthic communities. That group of "sea cucumbers" known as the apodous holothurians are frequently encountered along this coast yet little is known of their life histories or taxonomy, particularly from the west coast of North America. For this reason, it was decided that a preliminary examination of the whole apodous group in B.C. coastal waters be undertaken and, as a result, the examination has led to a detailed study of the genus *Leptosynapta*.

Classification of Apodous Holothurians

The two orders which are collectively known as "apodous holothurians" are the orders Apoda and Molpadonia. Classification based on Fell (1963) is given below and includes those genera which are found in British Columbia coastal waters:

Phylum Echinodermata

Subphylum Echinozoa

Class Holothuroidea

Order Apoda

Family Synaptidae

Subfamily Synaptinae

Group Heterournae

Genus *Leptosynapta*

Family Chiridotae

Genus *Chiridota*

Order Molpadonia

Family Molpadiidae

Genus *Molpadia*

Review of the Biology of Apodous Holothurians

Apodous holothurians lack true tube feet, though their tentacles are considered modified tube feet or "buccal podia." Members of this group are burrowing forms, usually having a vermiform shape and a thin body wall through which the viscera can be seen. The body wall is embedded with either anchor and plate or wheel-shaped ossicles which function as protective, supportive or locomotory devices. The anchor and plate ossicles of *Leptosynapta* aid in locomotion and function in the following manner: circular muscle contractions force the anterior region of the animal forward, at the same time propping the posterior anchors against the wall of the burrow. The animal anchors itself by this action and will not slip backward.

Tentacles of Apoda number roughly 10-20 and are pinnate, peltate or digitate in character. No radial canals are present and the tentacular canals arise from the water ring since there are no tentacular ampullae. The five longitudinal muscle bands are in single strips in the radii and do not interrupt the circular muscle layer at the radii as is the case in other holothurians. The digestive tract is only slightly looped or straight and the anus terminal. Respiratory trees are lacking and respiration is believed to be carried out through the skin and buccal podia. There is a simple haemal system with dorsal and ventral sinuses connected to lacunae in the wall of the digestive tract. The gonad is usually branched and often hermaphroditic (Hyman, 1955).

It is believed (Hyman, 1955) that apodous holothurians compensate for their lack of podia by having more complicated sense organs than other holothurians. The papillae in synaptids are glandulosensory in nature, that is, a sensory bud surrounded by gland cells. The sensory buds are made up of neurosensory cells whose basal filaments converge to form a nerve fiber that enters the ganglion of the dermal nerve plexus. A ganglion lies beneath each papilla. Sensory buds also occur on the inner surface of the tentacle stalks of synaptids and are believed to be used to test the surrounding medium (Hyman, 1955).

Cornucopia-shaped, ciliated urns occur in all members of the apoda and are found along the mesenteries near the body wall. They appear to gather coelomocytes and waste materials and empty these into the coelom as "brown bodies".

Autotomy, that is, fragmentation of the body including the body wall as well as the viscera, occurs in synaptids by constriction of the body into short pieces (Clark, 1899). Only the anterior end can be regenerated in synaptids and all other pieces die. Autotomy takes place under unfavourable environmental conditions such as high temperature, or chemical irritation.

The genus *Leptosynapta* is among the smallest of holothurians. The species in this group are pink to white in color and have 10-13 tentacles which are usually small. The ossicles are in the shape of anchors and plates.

The genus *Chiridota*, slightly larger than *Leptosynapta*, has 12 broad peltate tentacles and wheel-shaped ossicles with six spokes which are aggregated into wheel papillae. Some species have C-shaped rods present in the body wall. Specimens taken from British Columbia are rosy colored with brownish pigment spots. The general coloration is more intense than that found in *Leptosynapta* specimens. Both *Chiridota* and *Leptosynapta* are sometimes taken in the same haul subtidally from soft sediment. Three species of *Chiridota* have been reported from the B.C. coast (Heding, 1928; Edwards, 1907).

Holothurians in the order Molpadonia are of moderate size. They are pear or sausage-shaped burrowing forms whose bodies are narrowed posteriorly to a tail-like region with the anus at the tip. Unlike the Apoda, this order has tentacular ampullae, respiratory trees and double longitudinal muscle bands. The genus *Molpadia* is commonly dredged along the B.C. coast in soft sediment. *Molpadia intermedia* is a rich wine color and about 10-12 cm. long. The 15 tentacles are digitate in form. Anchors are found in the body wall but these differ from those in *Leptosynapta* because they are attached to the swollen, fenestrated (punctured) inner ends of 5 or more radiating rods. Two other genera in the order Molpadonia, *Caudina* and *Paracaudina* have been reported from the north-west Pacific (Ohshima, 1915) but it is not yet known whether these occur along the coast of B.C.

Description of Study

During preliminary studies of the order Apoda in B.C., it became apparent that only one genus, *Leptosynapta*, was available locally in sufficient numbers for satisfactory taxonomic work. Two forms of *Leptosynapta* were found in the collections made and these appeared to comply with Heding's (1928) description of *L. clarki* and *L. transgressor*.

Heding's work has been criticized by some contemporary holothurian authorities on the basis that he did not take into consideration changes in size and shape of ossicles with age or ecological differences. His descriptions were often based on preserved fragments of specimens and there was no mention of live color of the animals. Deichmann (personal communication) suggested that Heding's *L. transgressor*, taken from the same haul as *L. clarki*, was probably the young of the latter species. No work has been done on *L. transgressor* since the original description was made.

In this study the two local forms have been compared to determine if they are indeed separate species. A contemporary approach to taxonomy which includes morphological, reproductive and ecological aspects of the biology of each species has been followed. The following procedure has been taken in this investigation:

1. Time series samples of specimens from local areas on a monthly basis over a twelve month period.
2. Morphological examination of specimens, particularly for ossicle development.
3. Examination of pigment patterns and pigment analysis.
4. Examination of gonads to compare gamete or embryological development and thus obtain a picture of the breeding cycle of each species as related to temperature.
5. Survey of new areas to determine geographical distribution of each species.
6. Study of the ecology of each species involving exposure, depth, sediment type, faunal community, mutualism and predation.

<u>Collecting Stations</u>

Three stations in the Victoria area were chosen for time series collections (Fig.2 & 3).

Station 1.	Sooke Harbour	Lat. 48 21.85 N	0-.5 M depth
	(*L. clarki*)	Long. 123 42.90 W	
Station 2.	Sooke Harbour	Lat. 48 22.02 N	6-7 M "
	(*L. transgressor*)	Long. 123 42.89 W	
Station 3.	Bosun Bank	a.Lat. 48 42.25 N	20-30 M "
	(*L. transgressor*)	Long. 123 32.35 W	
		b.Lat. 48 42.00 N	25-40 M "
		Long. 123 32.39 W	

The positions given indicate the approximate mid-points of dredgings taken. Location "a" was used at Bosun Bank from May - Aug., 1968, but since specimens became scarce here after this period, from September to April location "b" was used.

Only one station was used for *L. clarki* because this species was not found in sufficient numbers elsewhere for regular sampling. For convenience in terminology the working hypothesis that the two forms are separate species will be adopted and they will be called by different specific names throughout this dissertation.

<u>Collecting and Preserving Methods</u>

Intertidal collecting was done by digging up the sediment with a trowel or small shovel and screening with a 10 inch diameter hand screen with 1.5 mm mesh, or simply extracting the animals from turned over sand using forceps (Fig.1).

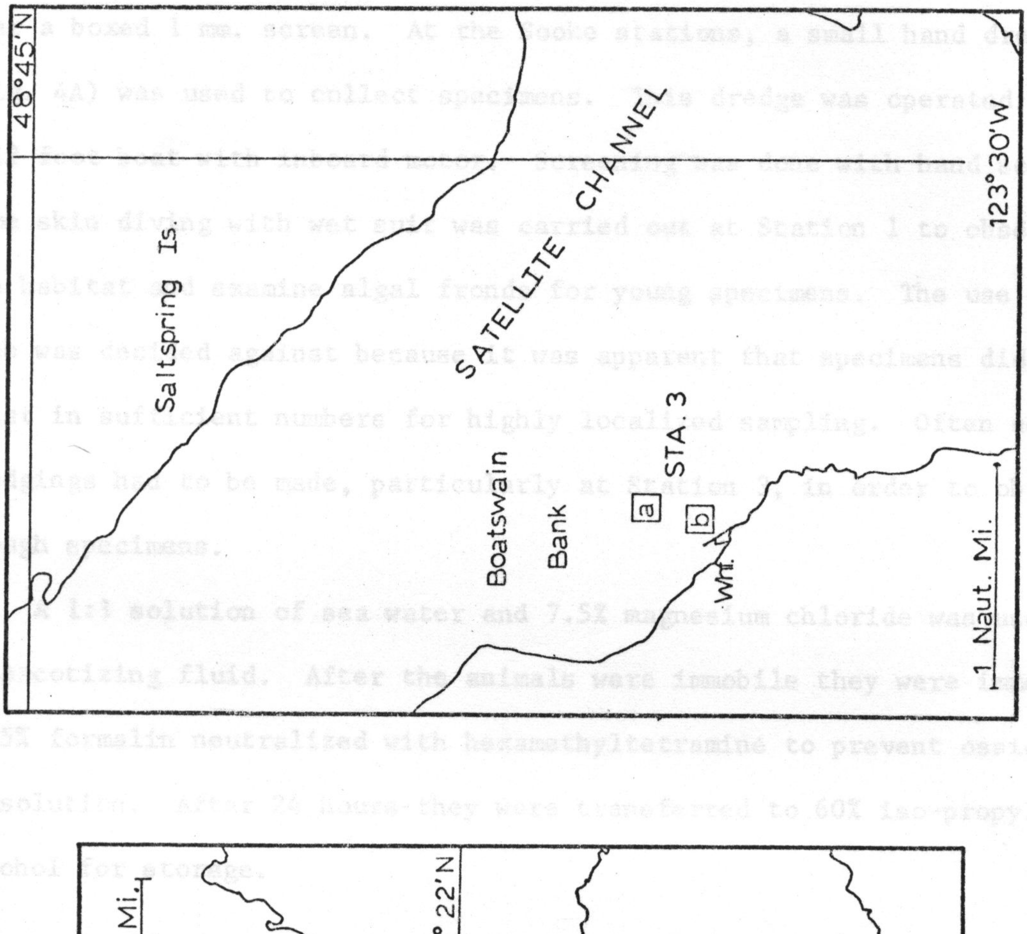
Subtidal collecting at Bosun Bank was carried out from a Department of Transport 30 foot launch using a 3 foot long triangular dredge (Fig.4B)

Figure 1. Collecting *L. clarki* in *Zostera* beds, Masset, Q.C.I.

Figure 2. Bosun Bank: Station 3 (*L. transgressor*).
Location "a" used May to August, location "b" used Sept. to April.

The term Bosun Bank as used throughout the text refers to Boatswain Bank in Figure 2.

Figure 3. Sooke Harbour: Station 1 (*L. clarki*).
Station 2 (*L. transgressor*).


48°45'N
Saltspring Is.
SATELLITE CHANNEL
123° 30'W
Boatswain
Bank
STA 3
a
b
Wh.
1 Naut. Mi.
0.25 Naut. Mi.
48° 22'N
STA 2
STA 1
Whiffin
Spit
123°43'W
SOOKE
HARBOUR
Govt.
Whf.

which was operated by a winch. Screening was done by hosing the sediment over a boxed 1 mm. screen. At the Sooke stations, a small hand dredge (Fig. 4A) was used to collect specimens. This dredge was operated from a 12 foot boat with inboard motor. Screening was done with hand seives. Some skin diving with wet suit was carried out at Station 1 to observe the habitat and examine algal fronds for young specimens. The use of a grab was decided against because it was apparent that specimens did not exist in sufficient numbers for highly localized sampling. Often several dredgings had to be made, particularly at Station 3, in order to obtain enough specimens.

A 1:1 solution of sea water and 7.5% magnesium chloride was used as a narcotizing fluid. After the animals were immobile they were immersed in 5% formalin neutralized with hexamethyltetramine to prevent ossicle dissolution. After 24 hours they were transferred to 60% iso-propyl alcohol for storage.

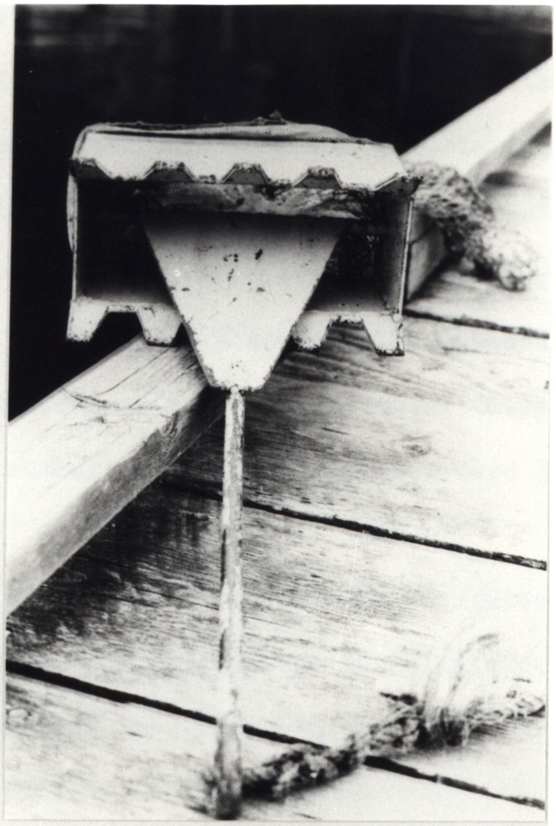
Taxonomic Review of the Genus *Leptosynapta*

Verrill (1867) proposed the genus *Leptosynapta* as separate from typical specimens of the genus *Synapta* (Eschscholtz) which have fifteen tentacles and prominent verrucae. He described *Leptosynapta* as having a more slender form, no prominent verrucae, and fewer, shorter, more digitate tentacles. He regarded *L. tenuis* (NW Atlantic) as the type species, and included the European *L. inhaerens*.

For the next thirty years this genus was regarded as invalid and the genus *Synapta* was used in its place. Östergren (1898) divided *Synapta* into six genera, one of which he kept as *Synapta* with the type specimen *S. inhaerens*.

Figure 4. A: Hand dredge used at Sooke Harbour.

B: Triangle dredge used at Bosun Bank.


4A


4B

Clark (1907) criticized Ostergren for using Eschscholtz's name for the group since Eschscholtz intended large oriental fifteen-tentacled forms to be included in *Synapta*. Clark realized that certain *Synapta* species conformed with Verrill's *Leptosynapta*. He suggested that Verrill's genus be adopted with *L. inhaerens* (which he believed was synonymous with *L. tenuis*) as the type species. Clark gave a key to nine species, eight of which were found in the North temperate zone. The major divisions of the key were based on tentacle number. Additional species were listed in Clark's paper of 1924a.

Heding's (1928) diagnosis of the genus was somewhat different than Clark's (1907). He described eight new species and in all listed twenty-five species: 6 European, 8 Australian and Indo Pacific, 1 West Indian, 5 North American Pacific and 5 North American Atlantic.

Deichmann (1930) diagnosed the genus after Clark (1907) and gave a key, based entirely on ossicles, to 8 species from the western Atlantic. Two of these, *L. inhaerens* and *L. roseola* were found in the north-western Atlantic.

The *L. inhaerens*, *L. tenuis* and *L. clarki* complex

Clark (1907) believed that *L. inhaerens* was probably a circumpolar species ranging south to subtropical waters. He gave the following distribution: "Europe: coasts of Norway, Denmark, Great Britain, France, Italy; American Pacific: Puget Sound, Washington; Mendocino, Pacific Grove and Point Loma, California; American Atlantic: Maine, South Carolina and Bermuda Is.." This authority realized the variability of the ossicles and pointed out the danger of putting undue emphasis on this character because of the "personal factor" which arose in ossicle measurements due to the difficulty of getting the anchors to lie flat.

Östergren (1898) suggested that the American form be regarded as a variety, subspecies or separate species and Deichmann (1930) supported this view. Ohshima (1915) mentioned *L. inhaerens* at four stations in Japan and he believed they corresponded well with the American form. No indication was given of the depth the specimens were found or the number of tentacles they had.

A more comprehensive discussion of *L. inhaerens* was given in a later paper by Clark (1924a). He paid special attention to the size of the anchors and plates in specimens from Europe and North America. His findings revealed that ossicles of specimens from Northern Europe were very large while those from Southern Europe and the western Atlantic were smaller. Those on the Pacific Coast had smaller ossicles than those on the Atlantic Coast and South-west Pacific specimens had smaller ossicles than those in the North-west Pacific.

That same year Clark (1924b) reported a small Synaptid about 10 mm long collected at Alert Bay in 10 Fathoms of water. He stated that "it seems to represent" *L. inhaerens*.

On the American coasts, Heding (1928) replaced *L. inhaerens* by *L. tenuis* on the Atlantic and *L. clarki* on the Pacific. *L. clarki* was described from specimens collected in a lagoon near Nanaimo (Fig. 18). Heding attempted to solve the *L. inhaerens*-*L. tenuis* problem by ordering specimens from Wood's Hole. He found these different from *L. inhaerens* of the Norwegian coast and outlined the differences in a tabular form (Table 1). Studies by Everingham (1961) further established *L. clarki* as a species distinct from *L. inhaerens*.

Table 1.

Character Comparisons in Three Species of *Leptosynapta*

(after Heding (1928))

Character	*L. clarki*	*L. tenuis*	*L. inhaerens*
Tentacle rods	perforated	not perforated	not perforated
Sensory cups	3-4 (7)	15-25	2-8
Digits	8	9-11	13-17
Anchors	2 kinds	2 kinds	1 kind

(The shape of the digit rods and the shape of the ciliated funnels differed in each species.)

Leptosynapta albicans

Selenka's (1867) original description of this North Pacific species contained very little information. It is interesting to note that the illustration by Selenka showed anchors and tentacle rods which are typical of *L. clarki*.

Clark (1901) described two small specimens which were dredged at Scow Bay, Puget Sound. He suggested they were *L. albicans* because they lacked sensory cups on the tentacles, but judging from the small size they were probably immature specimens. Some years later Clark (1907) admitted that *L. albicans* was probably synonymous with *L. inhaerens* but Caso (1962) disagreed. Lie (1968) believes that in the American North Pacific *L. clarki* replaces *L. albicans* and *L. inhaerens*.

L. transgressor

Heding (1928) described this species from specimens dredged at Nanoose Bay and Roxton Passage. He distinguished it from *L. clarki* by the number of tentacles (10), the shape of the rods, and the presence of only one kind of anchor. Two other species were listed from the Pacific coast by Heding: *L. roxtona* and *L. lens*.

L. roxtona

This species was taken in Roxton Passage in 15-20 fathoms. Only 3 fragments were obtained but Heding was convinced this was a new species because of the unique anchors and plates.

L. lens

This form was found at La Jolla, California and was distinguished by the squarish notch in the posterior margin of the calcareous ring sections. Heding believed the species was close to *L. albicans* and Deichmann (personal communication) places it as a synonym of that species.

CHAPTER I

PIGMENTATION

INTRODUCTION

Few other phyla show the diversity of pigment classes that are represented in the integument of echinoderms. Four major pigment classes: carotenoids, melanins, porphyrins and quinones have been isolated from echinoderm skin (Vevers, 1966; Fox & Hopkins, 1966). It is also interesting that the distribution of pigment types does not appear to be correlated in any way with phylogenetic relationship.

While the subject of skin pigments has not been pursued to any great extent, most of the literature (reviewed by Vevers, 1966; Fox & Hopkins, 1966) is concerned with carotenoids, which besides being found in the integument of many species of asteroids, ophiuroids, holothurians, and crinoids, are of metabolic importance, especially in oogenesis. The carotenoids are all of dietary (exogenous) origin but they may undergo a specific molecular rearrangement during metabolism.

Melanic pigments have been reported from the integuments of a few species of echinoids, ophiuroids and holothurians; they are of endogenous origin.

Porphyrins have been detected in the skin of a few asteroids and appear to be of exogenous origin. However, dendrochirote holothurians synthesize the porphyrin haeme, and use haemoglobin as an internal respiratory pigment.

Quinones are known from the skin of most echinoids and crinoids. They were first found in a holothurian by Mukai (1958, 1960) and have only been identified in a single species. In the crinoids, they are anthraquinones, probably of endogenous origin, while in echinoids and holothurians they are naphthaquinones which are synthesized endogenously or may be exogenously obtained from plant foods.

Although pigment biochemistry cannot be used in echinoderm phylogeny, pigmentation in the sense of color patterns and pigment distribution can be used taxonomically and has already been employed usefully in the ophiuroids. For example, pigment patterns have successfully been used in keys to the Caribbean ophiuroid fauna (Clark, 1933; Fontaine, 1953).

According to Clark (1907), a variety of colors occur in the Synaptidae. The usual colors are white, flesh, dull yellow, green, grey, and dull red. The color is seldom uniform but is often scattered over the body on papillae or spots of white, brown or red. Some species are more darkly colored on one side. Subtidal species are nearly colorless while the more brightly colored forms inhabit coral beds. Color is used as camouflage in a West Indian species which lives on seaweeds (Clark, 1898). The colors are due to pigment cells which are found in the connective tissue of the body wall. The cells may be scattered or crowded and are round, globular or radiant in shape.

Some mention was also made by Clark (1907) of the chemical composition of synaptid pigments. The pigments dissolved or modified rapidly in acids or corrosive sublimate ($HgCl_2$). Pure alcohol dissolved the pigments of some species but did not affect others.

Little or no mention is made of color in North Pacific synaptids in original descriptions, probably due to the fact that most of the identifications were made on preserved specimens whose pigments had leached out in the preserving liquid. It is obvious, then, that an examination of the pigment patterns of synaptids as well as more comprehensive biochemical pigment studies, would enhance their taxonomy and alleviate the difficulty of sorting out the species in this little known group.

In this chapter the distribution and pattern of the external pigmentation has been examined and a simple experiment has been carried out as an attempt to identify the type of pigment in both species of *Leptosynapta*.

METHODS

Pigment pattern and distribution was examined *in situ* by observing live narcotized animals under a dissecting microscope. Small pieces of tissue were then excised and mounted for examination under a compound microscope.

The experiment on Leptosynaptid pigments was based on solubility tests and simple microchemical color reactions observed under the microscope. The limited amount of material precluded a detailed chemical and spectrometric analysis. To test for solubility, fresh animals were narcotized and small pieces of pigmented skin were excised and placed in test tubes of solvent for a period of up to 24 hours. As preliminary results showed that the pigment was possibly a quinone, *Strongylocentrotus franciscanus* integument, already known to contain naphthaquinones (Fox & Hopkins, 1966), was tested also to provide a comparison.

To test for primary fluorescence (natural fluoresence, without the addition of reagents), pigmented areas were excised, mounted in sea water, and examined under a Leitz fluorescent microscope (HBO-200 light source, BG-3 or UG-5 exciter filters and appropriate barrier filters). Attempts were then made to excite the pigment to fluoresce by adding various reagents to the prepared slides.

RESULTS

Pattern and Distribution

The patterns and distribution of pigments were different in each species. Pigments of *L. clarki* were distributed over the entire body surface, giving the whole animal a pale pink color. Raised papillae were found over the body surface and pigment cells, which were roundish-oblong in shape, encircled the base of each papilla (Figs.5A &6A). Specimens of *L. clarki* collected from various locations showed similar pigmentation except those from Kye Bay and Denman Island whose pigments were much paler in color.

L. transgressor lacked the overall pink color of *L. clarki* and was basically white with dark red pigment spots which were composed of dense clusters of roundish-shaped pigment cells (Figs.5B & 6B). There was a higher density of pigment spots on the dorsal side than on the ventral and similarly, the anterior end was more pigmented than the posterior end. Pigment spots often extended anteriorly on to the tentacle stalks as far as the digits. This species lacked raised papillae.

Solubility Tests - See Table 2

Microchemical Tests

L. transgressor showed no natural, primary fluoresence; *L. clarki* pigment displayed a faint orange primary fluoresence. The pigment of neither species was excited to fluoresce by adding weak acid (5% acetic acid) or weak base (1N NaOH) to the preparation.

No characteristic color change occurred when concentrated sulfuric acid (H_2SO_4) was used to treat small pieces of pigmented skin. The pigment was destroyed but there were no transient color changes.

Figure 5. Pigment pattern in: A. *L. clarki*

B. *L. transgressor*

5 A

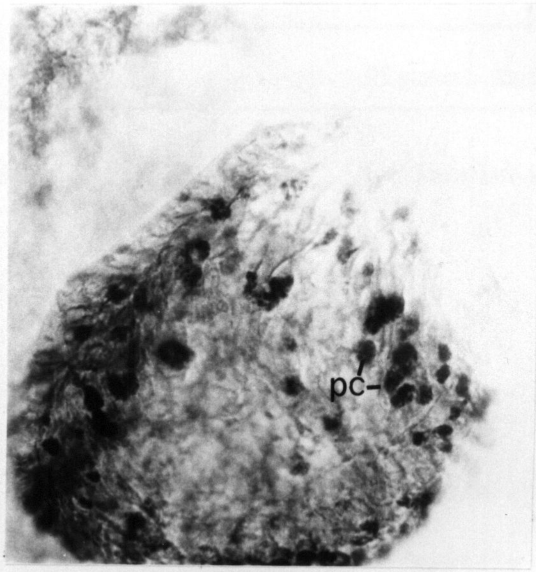
1 mm.

5 B

Figure 6. Arrangement of pigment cells (pc) in pigment spots of:

A. *L. clarki*

B. *L. transgressor*



6 A

20μ

6 B

Table 2.

Solubility Characteristics of the Pigments of the Synaptids, *Leptosynapta clarki* and *Leptosynapta transgressor* and the Urchin, *Strongylocentrotus franciscanus*.

Reagent	Pigment Reaction
Distilled water	Insoluble
Ethanol	"
Benzene	"
Ether	"
Acetone	"
Pyridine	"
0.1% HCl	Rapidly Soluble
Methanol + 2 drops HCl	" "
Formic Acid 90%	" "
NaOH 0.2%	Slowly Soluble
H_2O_2	Bleached within Seconds

The color of pigmented skin was neither diminished or enhanced by addition of the reducing agent, sodium hydrosulfite ($Na_2S_2O_4$), or the oxidizing agent, sodium nitrite ($NaNO_2$). That is, the pigment *in situ* showed no redox properties.

When treated with 5% $FeCl_3$ solution, the pigment *in situ* showed a marked and rapid darkening in color, eventually becoming green-black in color.

The microchemical color reactions noted above were also given by pieces of *S. franciscanus* integument.

DISCUSSION

The solubility and color reaction data are compatible with the tentative diagnosis of the Leptosynaptid pigments as quinones. Particularly diagnostic are the solubilities, the absence of a characteristic fluorescence naturally or in acid or basic media, and by the reaction with $FeCl_2$ (the so-called Jaksch-Pollak test) which indicates the phenolic character of the molecule. The only reaction which does not agree with this diagnosis is the absence of a redox reaction. However, since the test was performed on pigment *in situ* in fresh skin, the pigment may not show redox color changes while complexed to a protein moiety before denaturation. It is noteworthy that *S. franciscanus* skin pigment gives identical reactions under the same test conditions. Evidently the pigment of both species belongs to the same class of quinones. However, the faint primary fluorescence of the *L. clarki* pigment suggests a minor difference in molecular configuration. It must be emphasized that the diagnosis of a quinone structure is only tentative until a more adequate analytical survey can be made.

Nevertheless, there is sufficient evidence now to eliminate some major pigment classes. The insolubility in organic solvents and absence of a blue or green color reaction with H_2SO_4 means that the pigments are not carotenoids. The ready solubility in weak acids, weak bases, and weakly acidic alcohol, plus the instability to H_2O_2 eliminates the possibility of melanin. The absence of a specific brilliant fluorescence, either naturally or when activated by weak acids or bases, eliminates pterins or porphyrins. The solubility data suggest an ommochrome, but ommochromes give a specific, transient violet color reaction with H_2SO_4, and do not react with $FeCl_3$. These negative properties enhance the argument that the Leptosynaptid pigment is quinoid in nature. Resumes of the chemistry and diagnostic features of animal pigments are given by D.L. Fox (1953) and H.M. Fox & Vevers (1960).

The identification of the *Leptosynapta* pigments is interesting, though of little taxonomic value in separating the species, the only difference being the faint primary fluorescence of *L. clarki* which appears to be lacking in *L. transgressor*. The pattern and distribution of pigments, so easily observed and so obviously different in each species, is of greater taxonomic importance.

One may speculate on the function and ecological aspects of these pigments as well. The function of integumentary pigments has not yet been determined but two suggestions have been proposed in the literature, that of photosensitivity (Millot, 1957) and as an aid in the consumption of oxygen (Cannan, 1927).

There is evidence that visible light plays a role in the distribution of naphthaquinones and that these pigments may act as photo-masking mechanisms. Harvey (1956) found that pale specimens of a sea urchin at

Naples darkened when exposed to light, while darker specimens paled after a month in the dark. Darker urchins are generally found in shallow water while paler ones are in deeper, more turbid waters. Light colored urchins in shallow water mask the light by covering their surface with bits of algae held by their spines.

The deeper habitat of *L. transgressor* may explain the lack of pigmentation over the entire body surface as in *L. clarki*. The abundant pigmentation of *L. clarki* could act as a mask against the brighter light of intertidal and shallow water areas. On the other hand one could argue about the unimportance of light in animals which spend a good deal of their life buried in sediment.

Because of the redox properties of naphthaquinones it has been suggested (MacMunn, 1889) that this pigment may be used in respiration, but it has not been proven experimentally that naphthaquinone actually absorbs oxygen. Cannon (1927) found that naphthaquinone had a low redox potential but he believed it might be involved as an intermediary in cellular oxidative processes.

Since Leptosynaptids are primarily skin breathers, the proposal made by Cannon could be of consequence, particularly in areas of low oxygen availability. Digestive and reproductive activities take place in the anterior part of the body in synaptids, therefore they require more oxygen there than in the rest of the body. The aggregation of pigment in the anterior end of the body of *L. transgressor* could be advantageous to this species whose habitat would obviously have less oxygen than that of *L. clarki*. The possibility of photo-masking cannot be ruled out here either since *L. transgressor* appears to have a functionally dorsal side where pigmentation is definitely more abundant than on the ventral side.

It has already been mentioned that *L. clarki* has raised papillae over the body surface and these papillae are pigmented around the base. The habit of tube building in this species could account for such papillae which would allow water circulation into the tube and around the bases of the papillae. In such an arrangement, the pigment could very likely act as an agent in reactions involving the absorption of oxygen.

It is difficult to determine why specimens of *L. clarki* from Kye Bay and Denman Island were so lightly pigmented. A factor common to both areas was the scarcity of the animals, only one specimen found at Kye Bay and three from Denman Island. It has been found in certain animals that pigment production can increase when animals are gregarious. For example, those species of locusts which were gregarious had more ommochrome pigments than solitary species (Fox & Vevers, 1960). Ommochromes are granular pigments first discovered in insect and crustacean eyes. They are thought to function as light screens. Like ommochromes, naphthaquinones production may be influenced in a similar manner.

CHAPTER II

MORPHOLOGY: TENTACLES AND OSSICLES

INTRODUCTION

The type and shape of ossicles in holothurians has been a major taxonomic character used in describing species in this class. The reliability of this character is now questionable (Swan, 1966) for as holothurians age, the shape and number of their ossicles usually changes. These changes occur either by peripheral secretion or resorption of calcite. There may also be diversity in ossicle forms within a species (Madsen, 1942) due to ecological differences in habitat.

In his original descriptions Heding (1928) did not take into account either the age of the animal or differences in habitat which might cause ossicle variations within a species. In this chapter an attempt is made to determine whether Heding's description of the ossicles of *L. clarki* and *L. transgressor* remains consistant in specimens at various stages of development and from different areas. The size of the specimens collected and the number of tentacles have also been recorded in this chapter. Though little taxonomic significance can be given to size owing to frequent contraction or autotomy of the animals, it has been shown that a size comparison of the two species is of some value.

Ossicle Types in *Leptosynapta*

The genus *Leptosynapta* contains several types of ossicles which are to be found both internally as well as in the body wall. These ossicles are calcareous structures and, depending upon their individual form, are referred to as rods, anchors and plates. A calcareous ring containing 10-12 rectangular plates joined by connective tissue supports the pharynx,

MORPHOLOGY: TENTACLES AND OSSICLES

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Juveniles may have ossicles which differ from the adult forms. Everingham (1961) found that juvenile *L. clarki* with less than 8 tentacles had ossicles unlike the adult type while definitive ossicles began to appear in the 8 tentacle stage. In the embryonic stages he found that the calcareous ring began to form in the early pentacula stage and was conpleted in the late pentacula. In the latter stage, disc-shaped perforated plates began to appear in the body wall but there were no anchors. These "primary plates" were unlike the definitive plates of the adults. Larger specimens of this stage had rods in the tentacles.

METHODS

Records were kept of the length and number of tentacles of all specimens collected each month. Two adult specimens from each station sample were examined monthly for ossicles. Small pieces of tentacular, muscle and body wall (anterior and posterior) tissues were cut, then placed on glass slides, cleared with a few drops of 90% ethyl alcohol and mounted in euparol. Ossicles were measured by using a calibrated micrometer disc. Juvenile specimens were examined whenever possible and several specimens of *L. clarki* from locations other than Station 1 were also examined.

Criteria for Immaturity

Juvenile specimens were distinguished by small size, undeveloped gonads, underdeveloped tentacles and, in the case of *L. clarki*, primary ossicles.

RESULTS

Body Length and Tentacle Number

As already mentioned, body length is not a reliable criterion to distinguish between *Leptosynapta* species. There were, however, trends in body size in *L. clarki* collections examined from various parts of the Pacific coast. Adults of this species collected locally measured 12-82mm, while specimens from Califormia were larger (80-100mm), and a specimen from the Queen Charlotte Islands was largest of all (135mm). *L. transgressor* specimens from Sooke were 10-60mm long and those from Bosun Bank were 8-103mm long with only 2 exceeding 80mm.

No correlation was apparent between length and number of tentacles in the adults of either species. A large adult of *L. transgressor* could have as few as 9 tentacles, while a small adult could have as many as 14 tentacles. In *L. clarki*, adults of varying sizes almost invariably had 12 tentacles.

A comparison of tentacle number in a large series of adults of each species indicated that the average number and the distribution of tentacle number differed significantly between the species. Table 3 shows that *L. clarki* usually has 12 tentacles, rarely 9-13, while *L. transgressor* has a variable number of tentacles (8-14) with a mean of 10.3. It is of interest to note that 84% of *L. transgressor* examined had 10 or 11 tentacles and only 6.6% had 12 tentacles.

The collection of *L. clarki* juveniles ranged in length from 1-17mm and had 5-12 tentacles. There is a significant correlation between tentacle number and length (Table 4). The few juvenile *L. transgressor* collected showed no correlation between length and number of tentacles. Three specimens 5-9mm long had 10 tentacles, some of which were short and still developing, while one specimen of 6mm had 12 tentacles.

Table 3

A Comparison of Tentacle Number in Adult *L. clarki* and *L. transgressor*.

	L. clarki		*L. transgressor*	
Number of Tentacles	Number of Animals	% of Total	Number of Animals	% of Total
8	-	-	3	1.3
9	1	0.5	13	5.7
10	-	-	147	64.5
11	3	1.0	45	19.7
12	205	98.0	15	6.6
13	1	0.5	3	1.3
14	-	-	2	0.9
Total Number of Animals	210		228	
Mean Number of Tentacles	12.0		10.3	

An unpaired t-test was significant at the .05 level (t** = 28.02, df = 436) showing that the mean number of tentacles was significantly different between species.

Table 4

Number of Tentacles Related to Body Length in 40
L. clarki Juveniles

Number of Tentacles	Number of Specimens	Range of Body Length (mm)
5	12	1.0-2.5
8	3	1.5-2.0
9	1	3.5
10	6	3.0-5.5
11	2	5.0-9.0
12	16	7.0-17.0

For linear regression coefficient βyx, t was found to be significant at the .05 level ($\beta yx \pm .057$, $t^{**} = 9.63$, df = 38). A significant correlation therefore exists between body length and number of tentacles in juvenile *L. clarki*.

Adult Ossicles

A comparison of ossicles of *L. clarki* and *L. transgressor* are presented in summarized form below. Characters common to both species are placed in the center column.

L. clarki		*L. transgressor*
	Tentacle Rods (Figs. 7A-7B)	
	1. Found along the sides of tentacle stalks and digits and the base of tentacles.	
	2. Rods slightly curved to straight.	
3. Rod edges smooth to slightly undulating		3. Rod edges distinctly undulating to knobby; sometimes smooth
4. Rod ends knobby and perforated; sometimes forked		4. Rod ends branched or wrench-shaped; sometimes perforated
5. Tentacle stem rods L:36-96μ		5. Tentacle stem rods L:40-88μ
	6. Rods in the base of tentacle stem shorter and more ornate	
	Body and Internal Rods (Figs. 7C-7D)	
1. Found in the ambulacra, interambulacra and longitudinal muscles but not always present		1. As *L. clarki*; may also be found in the polian vessicles
	2. Longitudinal muscle rods chela or ring-shaped; L:24-40μ	
	3. Ambulacra rods in 2 dense rows, similar to long. muscle rods	
4. Interambulacra rods mainly in posterior end, slightly curved to semi-circular, sometimes Y-shaped, ends branched, edges smooth, L:56-84μ		4. Interambulacra rods variable; straight, curved or chela-shaped, ends knobby or slightly branched, edges smooth or undulating, tendency to be ornate, L:32-56μ
5. No polian vessicle rods found		5. Polian vessicle rods similar to interambulacra rods; not always present

(L: Length, W: Width)

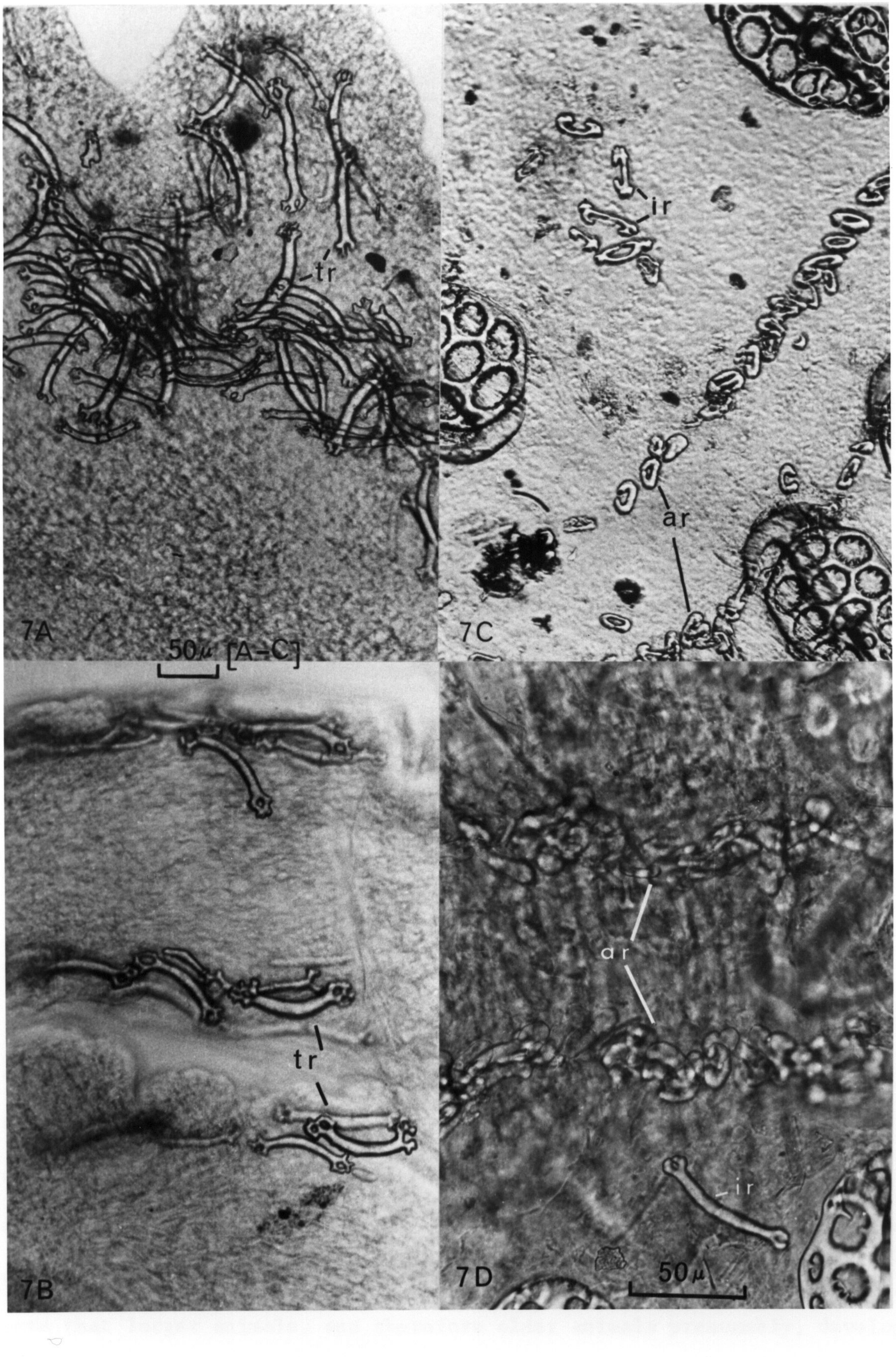
Figure 7. Tentacle and body rods.

A. Tentacle rods (tr) of *L. transgressor*, Sooke specimen.

B. Tentacle rods of *L. clarki*, Esquimalt specimen.

C. Ambulacral rods (ar) and interambulacral rods (ir) of *L. transgressor*, Bosun Bank specimen (phase contrast).

D. Ambulacral and interambulacral rods of *L. clarki*, Piper's Lagoon specimen.



tr
7A
50µ [A–C]
ir
ar
7C
tr
7B
ar
ir
50µ
7D

L. clarki	*L. transgressor*

Anchor and Plate Ossicles
(Figs. 8A-8D)

L. clarki	*L. transgressor*
1. Anterior anchors as long as, or scarcely longer than ant. plates; anchor arms short with edges smooth to slightly serrate, broad-angled L:144-184μ W:76-88μ	1. Similar to *L. clarki*, L:152-188μ W:76-112μ
2. Posterior anchors longer than posterior plates, anchor arms long, curved downward, distinctly serrate, L:184-216μ, W:72-112μ	2. Posterior anchors (a or b) (a) Tend to be similar to anterior anchors in small specimens (b) Tend to be longer than anterior anchors in large specimens; similar to *L. clarki*, L:168-192μ, W;72-120μ
3. Plates- anterior and posterior similar, roundish-oblong; large holes smooth or toothed. L:120-156μ, W;100-128μ Plates not reduced in articular end	3. Plates- similar to *L. clarki* but there is a tendency for plates to be reduced in the articular end L:112-156μ. W:88-124μ

In *L. clarki*, most specimens from Sooke lacked body rods though body and internal rods were found in specimens from Piper's Lagoon and Menzie's Bay, and were extremely prolific in specimens from the latter area. In Piper's Lagoon specimens posterior anchors were very long with narrow arms, while Sooke specimens had shorter anchors with broader arms.

In his original description of *L. transgressor*, Heding (1928) stated that this species has only one type of anchor ossicle. He claimed that anterior and posterior anchors were similar. In this study 40 specimens of *L. transgressor* varying in length from 6-103 mm, were examined for the type of anchor ossicles they possessed (see Table 5). It was observed that 15 animals (6-42 mm) had only one type of anchor while 25 animals (18-103 mm) had two types of anchors. The latter group contained only 6 individuals measuring less than 42 mm. These observations indicate that larger animals, and therefore older animals presumably, develop

Figure 8. Anchor and plate ossicles.

A. Anterior anchor and plate, *L. clarki*, Menzie's Bay specimen.

B. Posterior anchor and plate, same specimen.

C. Anterior anchor and plate, *L. transgressor*, Sooke specimen. (The anchor is not lying flat and thus appears somewhat larger than it should).

D. Posterior anchor and plate showing reduced plate in articular end, same specimen.

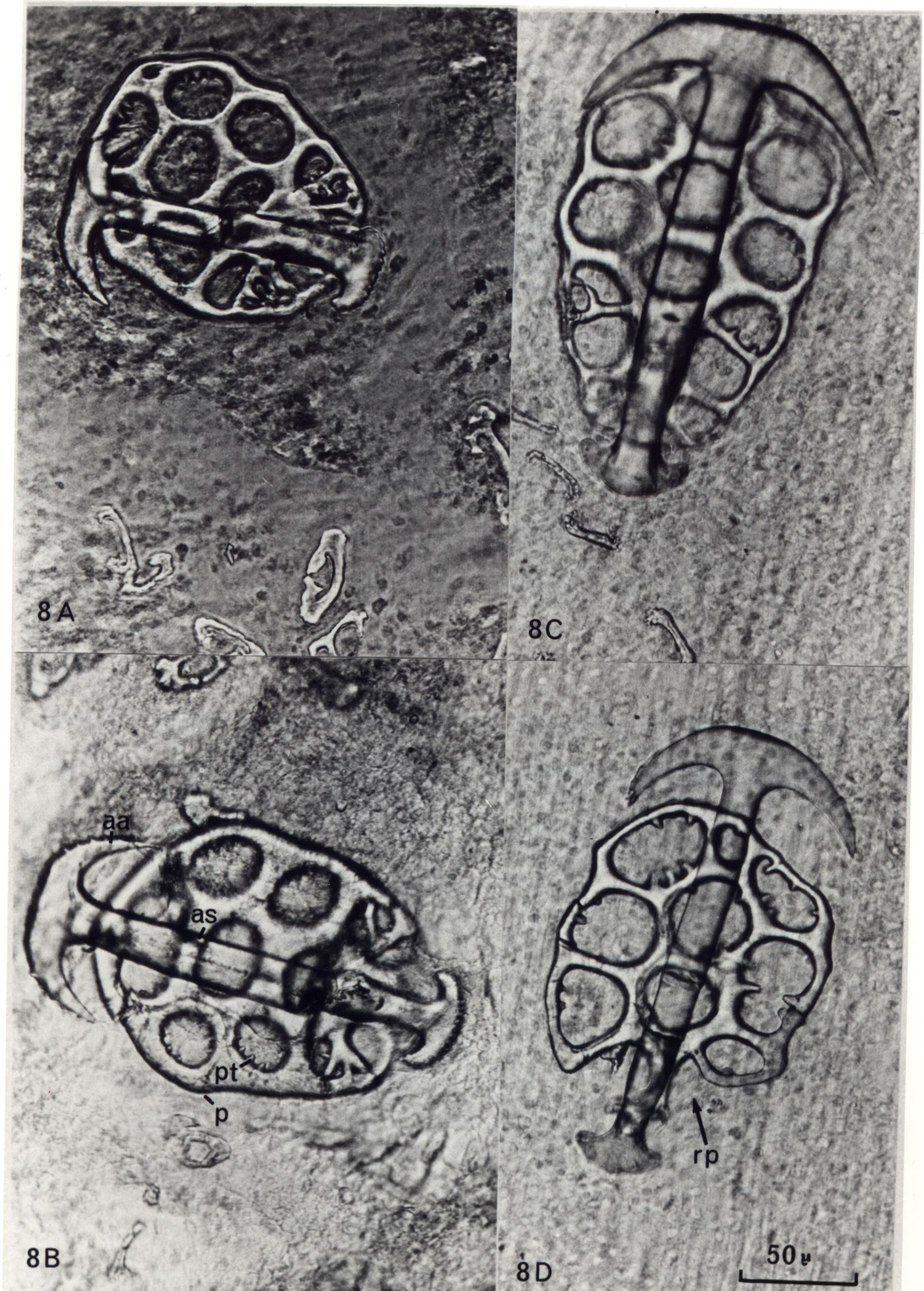
(aa) anchor arm

(as) anchor shaft

(p) plate

(pt) plate teeth

(rp) reduced plate



8A
8C
aa
as
pt
p
8B
rp
50 µ
8D

Table 5

Anchor Type* Related to Body Length in 40 Specimens of *L. transgressor*.

Body Length (mm)	Number of Specimens with Type I Anchors	Number of Specimens with Type II Anchors
Less than 15	4	-
16-25	9	2
26-35	-	2
36-45	2	4
46-55	-	9
56-65	-	4
66-75	-	1
76-85	-	1
More than 85	-	2

* Type I = Anchor Ossicles Similar in Anterior and Posterior ends of Body.

Type II = Posterior Anchor Ossicles Different to Anterior Anchor Ossicles.

posterior anchors which differ from those in the anterior and that the presence of a single type of anchor anteriorly and posteriorly in an animal indicates it is probably a young adult or, in the case of animals of less than 15 mm, is a juvenile. These results show that there are ossicle changes with age, a phenomenon that Heding, who admitted basing his description on young specimens, neglected to take into consideration.

Juvenile Ossicles - *L. clarki*

Tentacle rods (Fig. 9B)

Tentacle rods were present in both brooded and free late pentacula juveniles from Sooke. The smallest specimens of 1-1.25 mm had simple curved rods. Larger pentacula young of 2-2.5 mm from Chatham Island had rods of the adult type. Some larger specimens (3-17 mm) had rods at the tentacle base which resembled the adult type.

Body rods (Fig. 9B)

Most of the juveniles examined lacked body rods, however, one specimen of 12.5 mm from Sooke had a few rods in the ambulacra, while a specimen from Menzie's Bay had interambulacra rods similar to the adult type. Another specimen of 17 mm from Menzie's Bay had body and internal rods similar to the adults found there.

Calcareous ring (Fig. 9B)

First evidence of the calcareous ring appeared in the early pentacula stage and consisted of a ring of unconnected stellate-shaped ossicles. Completion of the ring was evident by the late pentacula stage.

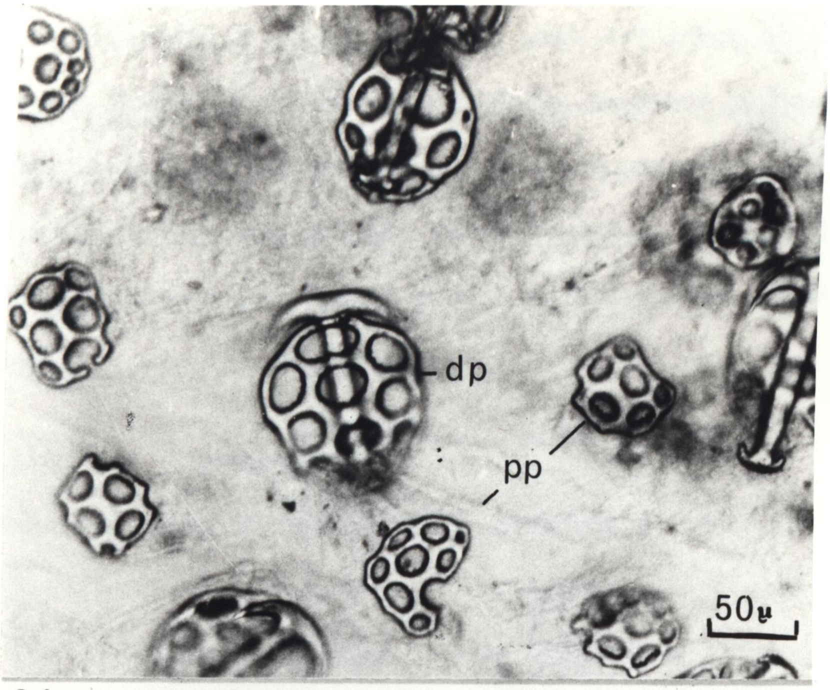
Primary plates (Figs. 9A & 9B)

Primary plates first appeared in the posterior region of late pentacula specimens both in the brooded and free state.

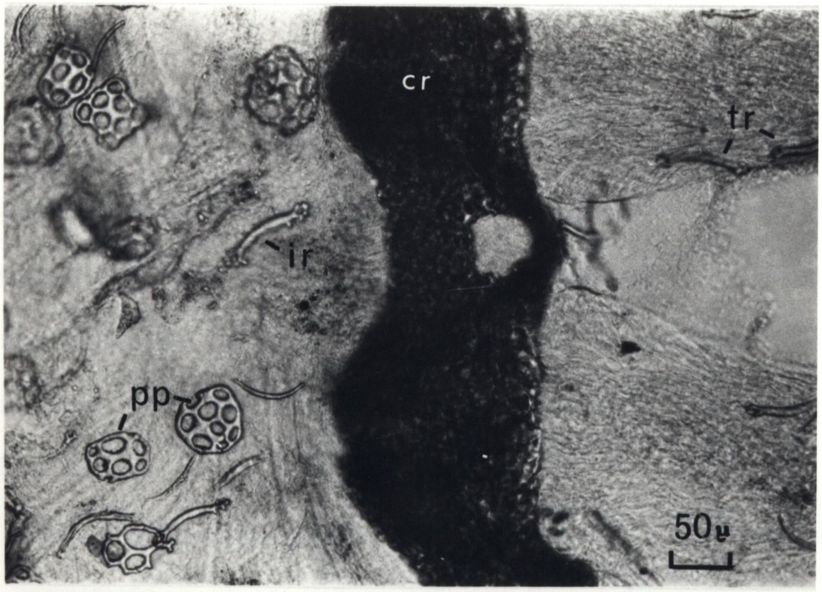
Figure 9. Ossicles of juvenile *L. clarki*.

A. Primary plates (pp) and definitive plates (dp) from the posterior end; Esquimalt Lagoon specimen, 9 tentacle stage, 3 mm. long.

B. Tentacle rods (tr), calcareous ring (cr), interambulacral rods (ir) and primary plates (pp); Menzie's Bay specimen, 11 tentacle stage, 9 mm. long.



9A



9B

All other juveniles examined had primary plates anteriorly and posteriorly though they were more abundant in the posterior end. The plates were about 40-68μ in diameter, roundish in shape with 6-9 large holes either toothed or smooth. Definitive anchor and plate ossicles appeared in specimens as small as 3 mm (10 tentacles) and were present in the larger juveniles, usually in combination with primary plates (Fig. 9A). There was an unusual persistence of primary plates in a specimen of 39 mm found at Menzie's Bay.

Juvenile Ossicles - *L. transgressor*

A comprehensive study of the juvenile ossicles of this species was not possible because of the small number of juveniles collected. Those examined had rods similar to the adult type. No primary plates were found. As already discussed under adult ossicles, small specimens, presumably juveniles, of this species tended to have only one type of anchor ossicle.

DISCUSSION

It is apparent from the results that the number of tentacles is an important, though not infallible, taxonomic character in differentiating between the two species. Still, it is important to examine a large number of specimens from one area before basing their identification on tentacle number alone.

A more complex taxonomic problem is presented by the ossicles, owing to the variability of these structures found in each species. This study has shown that the anchor ossicles are not always of one kind as Heding (1928) stated in his original description of *L. transgressor*. The ossicles showing the most marked difference between the species were the interambulacra rods but even this character was proven fallible when it was found that Menzie's Bay specimens of *L. clarki* had interambulacra rods similar to *L. transgressor*. Also many specimens of both species lacked body rods completely. The "typical" specimens of *L. clarki* as described by Heding (1928) were seldom found and the ossicles of this species varied a good deal depending on age and location. One can only say there were tendencies for certain ossicle types in both species. Because of the amount of variation that was found, however, it is the author's opinion that the ossicles alone do not give sufficient evidence to identify an adult specimen.

The presence of primary ossicles in juvenile *L. clarki* and the absence of these in *L. transgressor* made it easy to distinguish between the young of these two species. When examining small specimens, however, one should not make the mistake of assuming they contain juvenile ossicles; for there is no specific size or age known at which primary ossicles disappear in *L. clarki* or two types of anchor appear in *L. transgressor*.

Despite a certain amount of variation, there appeared to be some similarity in ossicles within a population in *L. clarki*. The Sooke population tended to lack body rods while the population at Menzie's Bay had an abundance of rods and displayed a tendency towards ornate ossicles. These differences reflect a degree of geographical variation to be found in this species. There was some evidence that sediment might be an important factor related to ossicles, particularly the anchors (see also part IV). The long, narrow posterior anchors found in the Piper's Lagoon specimens would be of benefit to this population which inhabits a sediment of coarse sand and gravel . Such anchors would have good holding power in a coarse sediment which would shift readily. The long anchors would permit deep penetration of the sediment and narrow anchor arms angled close to the shaft would grip the sediment well. The shorter-shafted, broader-angled anchor arms of the posterior anchors found in the Sooke specimens of *L. clarki* would have less holding power but would be adequate in that location where the finer, more stable sediments were further held in place by Eel Grass roots. In such a relatively protected environment, Sooke specimens would not require a great deal of body support and this could explain their lack of body rods. The abundance of rods in the Menzie's Bay specimens would afford greater body support to that population which inhabits a coarser sand sediment (Fig. 23) in an area which is buffeted by strong tidal action and heavy seasonal runoff from Mohun Creek.

Ecological reasons may also explain why juvenile ossicles differed from the adult. Very small specimens of both species were found above the sediment, *L. clarki* on the fronds of large algae or *Zostera* and *L. transgressor* on polychaete tube masses (Fig. 24). These juveniles then, would have less need for ossicles as locomotory devices and the long posterior anchors in particular would not be needed above the sediment.

To sum up, though ossicles do not present a strong taxonomic argument in differentiating between the two *Leptosynapta* species, they are, none the less, an extremely interesting feature of these animals. There is some evidence that they are related to environmental factors as well as age.

CHAPTER III

REPRODUCTION

INTRODUCTION

Reproductive studies of marine invertebrates have proven taxonomically valuable because they help to distinguish those species which might otherwise resemble each other. For example, studies by Everingham (1961) revealed that *L. clarki* was a viviparous species, brooding the young in its gonad tubules. This established it as a species separate from the oviparous *L. inhaerens* with which it was thought to be synonymous. Since virtually nothing is known about the reproductive habits of *L. transgressor*, it would be interesting to know whether this species is, like *L. clarki*, a viviparous brooder, or whether it is oviparous. In order to determine this, the reproductive cycles of both species of *Leptosynapta* have been examined and compared in this chapter.

Review of Synaptid Reproduction

In synaptid holothurians the gonad consists of two branched tubules, one on either side of the dorsal mesentary, lying free in the coelom. A number of synaptid species are hermaphroditic, producing eggs and sperm synchronously (Clark, 1907) while others are protandrous. In the case of *L. inhaerens*, Runnström (1927) found evidence that the young of this species were male, becoming female after several years.

Internal brooding of the young takes place in several species of apodous holothurians. This is a phenomenon which is especially prevelant in species inhabiting polar and northern waters. Large, yolky eggs are produced in such species and the young are incubated in incubatory sacs, in the coelom, or in the gonad tubules themselves. How fertilization occurs in these cases is largely unknown, though various suggestions have been proposed.

Synaptula hydriformis, a viviparous hermaphrodite, sheds its sperm externally and Clark (1898) believed the sperm reached the coelom by way of the anus and small openings in the wall of the rectum. There is also a possibility that the sperm could enter the ovary via the gonopore. Hermaphroditic species may, of course, be self fertile.

The embryology of holothurians includes four possible stages: (1) a uniformly ciliated post gastrula stage; (2) an auricularia stage; (3) a doliolaria stage and (4) a pentacula stage. Only four species, however, are known to pass through this complete sequence. These include the two apodous species *Labidoplax* (= Synapta) *digitata* and *Synaptula vittata*. Generally one or more stages are omitted in the majority of holothurians (Hyman, 1955).

Several accounts have been given of the embryology of Synaptidae. Clark (1898) found *Synaptula hydriformis* to be hermaphroditic and viviparous, brooding the young in its coelom. The early embryos were uniformly ciliated until they reached the pentacula stage. When the young were 5 mm. long or more and possessed the adult number of tentacles (10), they were released through a rupture in the posterior end of the adult, either through the body wall or through the walls of the rectum. The development of *Chirodota rotifer* proved to be similar except in the later embryological stages, during which bands of cilia were developed (Clark, 1910). The oviparous species, *L. inhaerens*, has a uniformly ciliated larvae which later develops into a doliolaria larvae with ciliated bands. There is no auricularia stage (Runnström, 1927).

Everingham (1961) found that the eggs of *L. clarki* were considerably larger in size than those of *L. inhaerens* but the embryonic stages were similar apart from the complete lack of cilia in *L. clarki*. Two early post gastrula stages, a pre-pentacula stage, and an early, middle and late pentacula stage were found in the embryology of *L. clarki*. A detailed description of each stage can be found in Everingham (1961). According to Hyman (1955), most holothurians are capable of an independent existence at the late pentacula (5-tentacle) stage. Everingham, however, claimed that embryos of *L. clarki* remained in the ovarian tubules until the eight to ten tentacle stage.

Several ideas have been expressed in the literature regarding the release of *L. clarki* young from the adult. Everingham (1961) suggested that the ovary wall probably ruptures and the young pass out into the somatocoel before their release through a rupture in the body wall. Anderson (1966) noted the existence of pores around the anus of *L. clarki* and confirmed this by histological preparations. He found the pores functioned as openings through which coelomic fluid escaped, thus enabling the animal to reduce its size quickly. He postulated that the release of young from the coelom might be accomplished through these pores.

METHODS

Ten specimens of *Leptosynapta* were collected monthly from each station over a period of one year. Their gonads were examined and the stage of their gamete development was recorded. When oocytes were present, a whole mount of the gonad was made and the diameters of ten of the largest oocytes were measured microscopically using a calibrated micrometer disc. the mean of the diameters were recorded for each specimen. The average and range of the means within each sample was then plotted graphically against the month.

The oocytes and sperm of each species were compared and note was taken of the development of embryos and post embryonic juvenile stages. Sectioning was carried out when necessary using techniques outlined by Humason (1967). Embedding was done in paraplast and blocks were sectioned at a thickness of 6-10 μ. Mayer's haemotoxylin and eosin were used for staining and slides were mounted with histoclad.

Sediment temperatures were recorded monthly from each station to determine if any relationship existed between temperature and gamete development.

RESULTS

Leptosynapta clarki

Development of Oocytes

Immature oocytes with an average diameter of 47μ were found in specimens from Sooke Sta. 1 in June. (The population at this station was not discovered until June, hence no May sample was obtained.) Oocyte diameters showed a slow increase during the summer months and a marked increase during the fall and winter months (Table 6, Fig. 12). Despite fluctuations, the general trend was towards an increase in oocyte size from June to March with a maximum average diameter of 404μ recorded in February. This exceeded the maximum average diameter of 272μ for *L. transgressor* oocytes recorded in March. Everingham (1961) has given a detailed description of the oocytes.

Development of Sperm

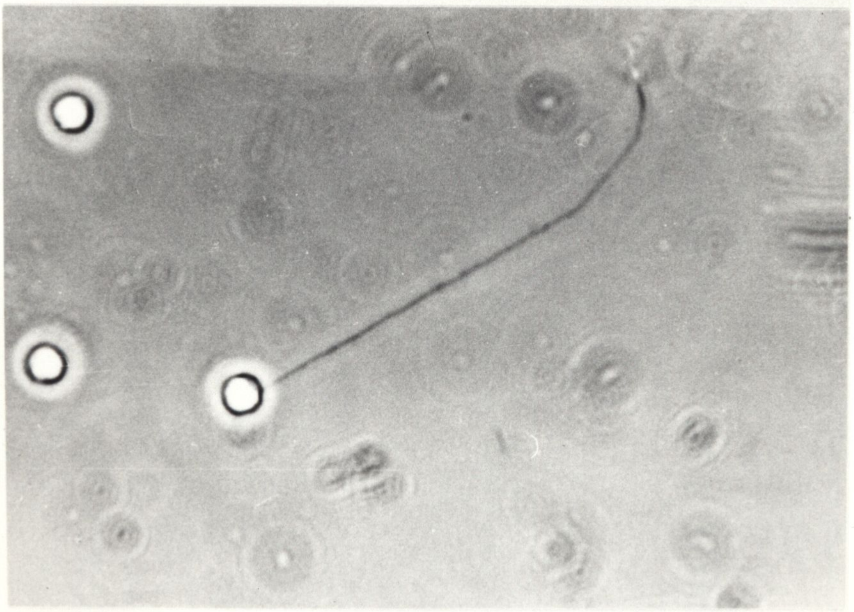
Spermatogenesis appeared to be taking place in the testes of specimens collected in July and August while ripe sperm began to appear in

Figure 10. Mature sperm of A. *L. clarki* and

B. *L. transgressor* (phase contrast).

10 A

10 μ



10 B

Figure 11. Mature sperm of A. *L. clarki* and B. *L. transgressor* showing differences in tail length.

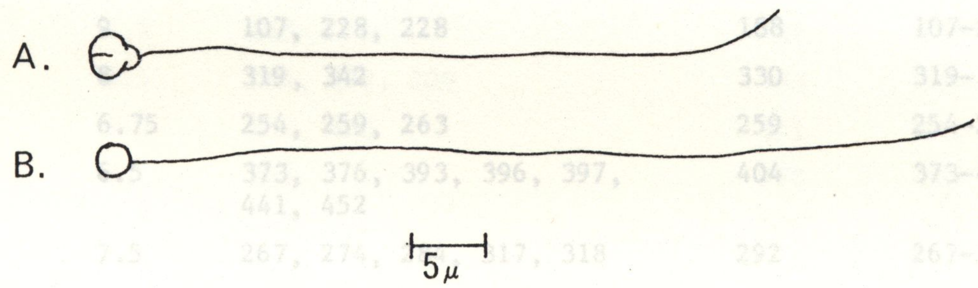

A.
B.
5μ

Table 6

Oocyte Diameters in Ovarian Tubules of Female *L. clarki*,

Sta. 1 (Sooke).

Month	Temp. °C	Mean (μ) oocyte diameters	Average μ	Range μ
Jun	15	31, 33, 57, 65	47	31-65
Jul	14	98, 100, 100	99	98-100
Aug	12	146, 152	149	146-152
Sep	10.5	137, 137, 140, 148, 154, 160	146	137-160
Oct	9.5	228, 230, 301, 311, 321	278	228-321
Nov	9	107, 228, 228	188	107-228
Dec	8	319, 342	330	319-342
Jan	6.75	254, 259, 263	259	254-263
Feb	6.5	373, 376, 393, 396, 397, 441, 452	404	373-452
Mar	7.5	267, 274, 284, 317, 318	292	267-318
Apr	9	Embryological stages		

Table 7

Oocyte Diameters in Ovarian Tubules of Female *L. transgressor*

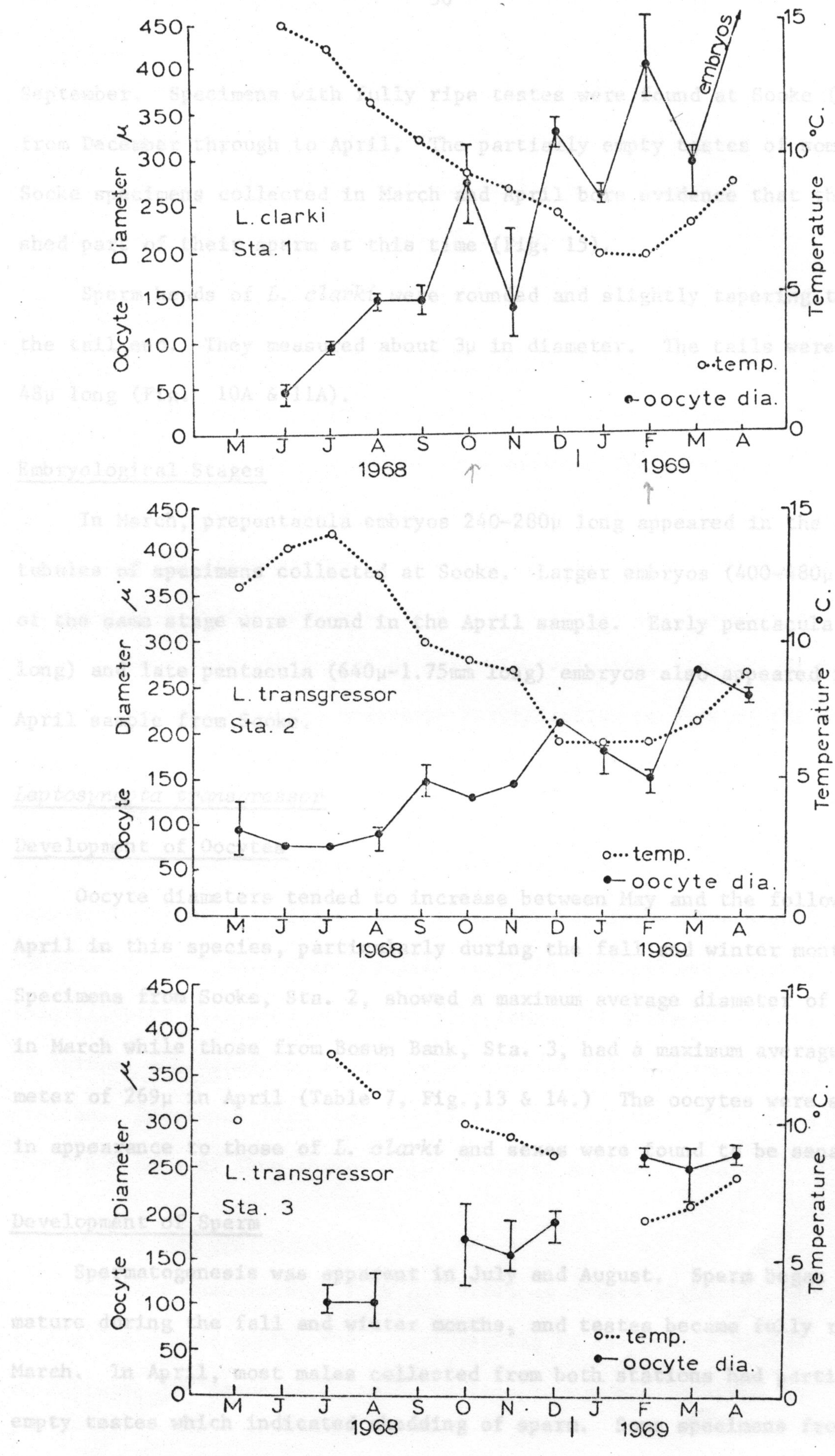
(a) Station 2 (Sooke).

Month	Temp. °C	Mean (μ) oocyte diameters	Average μ	Range μ
May	12	64, 128	96	64-128
Jun	13.5	76, 76	76	76
Jul	14	76	76	76
Aug	12.5	80, 95, 96	90	80-96
Sep	10	141, 160	150	141-160
Oct	9.5	130	130	130
Nov	9	146	146	146
Dec	6.5	218	218	218
Jan	6.5	164, 176, 208	183	164-208
Feb	6.5	142, 160	151	142-160
Mar	7.25	272	272	272
Apr	9	247, 250	248	247-250
		(b) Station 3 (Bosun Bank).		
May	10	24	24	24
Jun		no sample		
Jul	12.5	93, 100, 120	101	93-120
Aug	11	78, 96, 100, 119, 133	105	78-133
Sep	11	none with oocytes		
Oct	10	120, 158, 163, 167, 186, 187, 196, 217	174	120-217
Nov	9.5	141, 144, 146, 194	156	141-194
Dec	8.75	175, 210	192	175-210
Jan		no sample		
Feb	6.5	262, 269	266	262-269
Mar	7	209, 256, 266, 271	251	209-271
Apr	8	266, 268, 273	269	266-273

Figure 12. Average and range of mean oocyte diameters of *L. clarki* related to sediment temperature; Station 1.

Figure 13. Average and range of mean oocyte diameters of *L. transgressor* related to sediment temperature; Station 2.

Figure 14. Average and range of mean oocyte diameters of *L. transgressor* related to sediment temperature: Station 3. (No data for June, Sept. & Jan.)


Oocyte Diameter μ
Temperature °C.
L. clarki
Sta. 1
embryos
o·temp.
•oocyte dia.
M J J A S O N D J F M A
1968
1969
L. transgressor
Sta. 2
o···temp.
•—oocyte dia.
L. transgressor
Sta. 3
o···temp.
•—oocyte dia.
0
50
100
150
200
250
300
350
400
450
0
5
10
15

September. Specimens with fully ripe testes were found at Sooke (Sta. 1) from December through to April. The partially empty testes of some Sooke specimens collected in March and April bore evidence that the males shed part of their sperm at this time (Fig. 15).

Sperm heads of *L. clarki* were rounded and slightly tapering toward the tail end. They measured about 3μ in diameter. The tails were about 48μ long (Fig. 10A & 11A).

Embryological Stages

In March, prepentacula embryos 240-280μ long appeared in the ovarian tubules of specimens collected at Sooke. Larger embryos (400-480μ long) of the same stage were found in the April sample. Early pentacula (360 long) and late pentacula (640μ-1.75mm long) embryos also appeared in the April sample from Sooke.

Leptosynapta transgressor

Development of Oocytes

Oocyte diameters tended to increase between May and the following April in this species, particularly during the fall and winter months. Specimens from Sooke, Sta. 2, showed a maximum average diameter of 272μ in March while those from Bosun Bank, Sta. 3, had a maximum average diameter of 269μ in April (Table 7, Fig.,13 & 14.) The oocytes were similar in appearance to those of *L. clarki* and sexes were found to be separate.

Development of Sperm

Spermatogenesis was apparent in July and August. Sperm began to mature during the fall and winter months, and testes became fully ripe in March. In April, most males collected from both stations had partially empty testes which indicated shedding of sperm. Some specimens from

Sooke, Sta. 2, had full ripe testes in May, 1969.

Sperm heads of *L. transgressor* were rounder than those of *L. clarki* with no obvious tapering towards the tail. Heads measured about 2μ in diameter and tails about 60μ long (Fig. 10B & 11B).

Embryological Stages

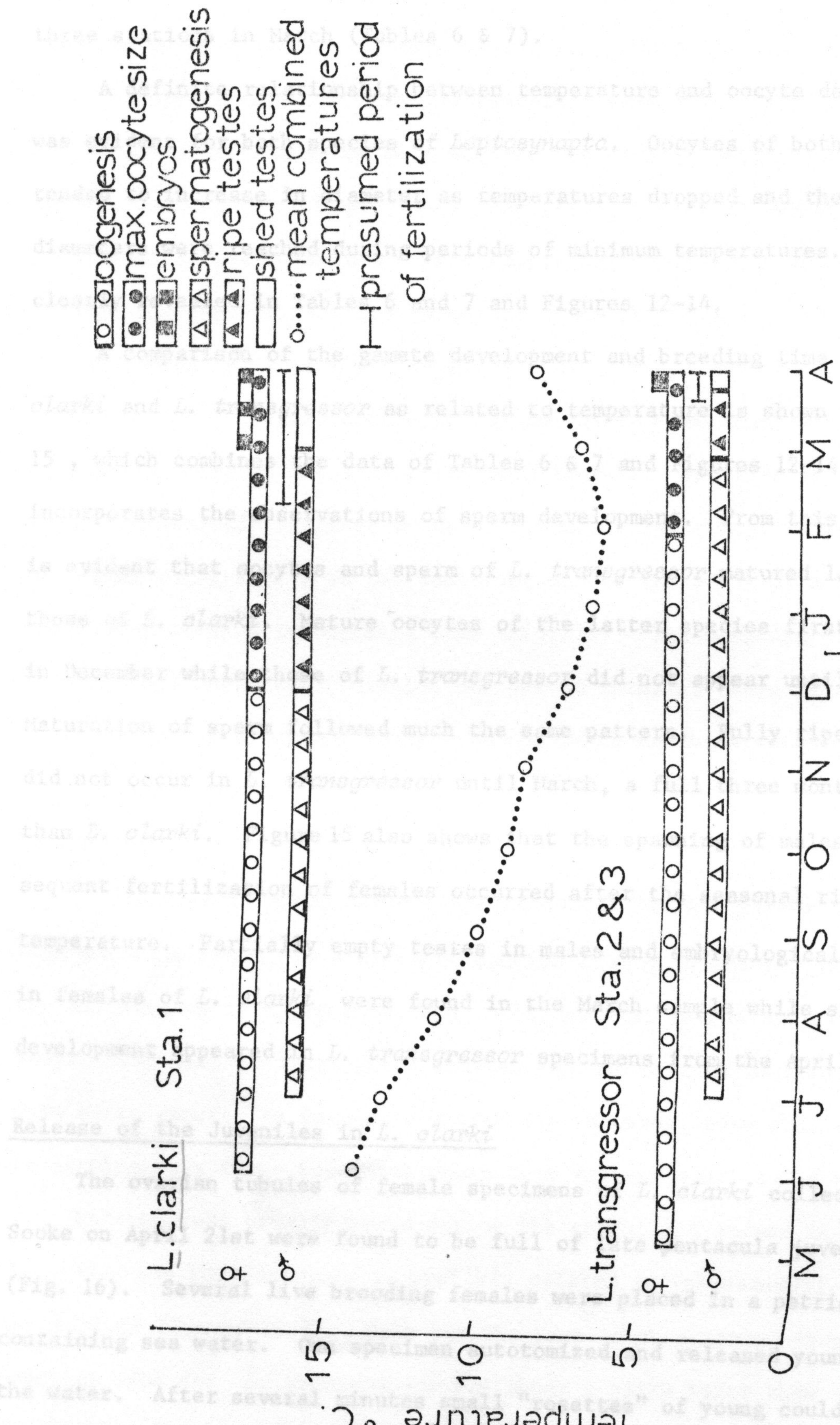
No fertilized eggs or embryological stages were seen in *L. transgreesor* before April. Only one female specimen was present in the April sample from Sta. 2. The gonad of this specimen had oocytes and also what appeared to be fertilized eggs in the gastrula stage. The oocytes averaged 225μ in diameter and the embryos (?) were about 250μ long.

The four female specimens from Sta. 3 in the April sample all contained either fertilized eggs or early embryological stages. It was assumed that the eggs had undergone fertilization because of the disappearance of the germinal vessicle and the formation of a membrane about 8μ thick around the circumference of the egg. The egg surface was dense and granular, and the round eggs measured about 275μ in diameter. Attempts to culture fertilized eggs from live specimens were unsuccessful. Embryos in these specimens appeared to be in the post gastrula stages, were slightly ellipsoid and about 288μ long. No cilia were noticable. No further embryological stages were found in this species from either station. Specimens from Sta. 3 were collected in May, 1969, as a further check but none of them showed well developed gonads, (females).

Temperature and Reproduction

Sediment temperatures from all stations showed highest readings during the summer months and lowest reading in the winter from December to February. A seasonal increase in temperature was recorded from all

Figure 15. A summary of the reproductive cycles of *L. clarki*, Station 1., and *L. transgressor*, Station 2 & 3, related to mean sediment temperatures. (Combined data of Figs. 12 to 14).


L.clarki Sta.1
♀
♂
L.transgressor Sta.2&3
♀
♂
oogenesis
max oocyte size
embryos
spermatogenesis
ripe testes
spent testes
....mean combined temperatures
presumed period of fertilization
Temperature °C
15
10
5
0
M J J A S O N D J F M A
1968
1969

three stations in March (Tables 6 & 7).

A definite relationship between temperature and oocyte development was evident for both species of *Leptosynapta*. Oocytes of both species tended to increase in diameter as temperatures dropped and their maximum diameters were reached during periods of minimum temperatures. This is clearly revealed in Tables 6 and 7 and Figures 12-14.

A comparison of the gamete development and breeding time of *L. clarki* and *L. transgressor* as related to temperature is shown in Figure 15 , which combines the data of Tables 6 & 7 and Figures 12-14 and incorporates the observations of sperm development. From this figure it is evident that oocytes and sperm of *L. transgressor* matured later than those of *L. clarki*. Mature oocytes of the latter species first appeared in December while those of *L. transgressor* did not appear until February. Maturation of sperm followed much the same pattern. Fully ripe testes did not occur in *L. transgressor* until March, a full three months later than *L. clarki*. Figure 15 also shows that the spawning of males and subsequent fertilization of females occurred after the seasonal rise in temperature. Partially empty testes in males and embryological stages in females of *L. clarki* were found in the March sample while similar development appeared in *L. transgressor* specimens from the April sample.

Release of the Juveniles in *L. clarki*

The ovarian tubules of female specimens of *L. clarki* collected at Sooke on April 21st were found to be full of late pentacula juveniles (Fig. 16). Several live brooding females were placed in a petrie dish containing sea water. One specimen autotomized and released young into the water. After several minutes small "rosettes" of young could be seen around the posterior ends of the other specimens (Fig. 17). When examined under a dissecting microscope, the young were seen emerging through pores around the anus. Peristaltic waves from posterior to anterior took place along the body wall of the adult, forcing the gonad tubules downward at a rapid rate. The pressure exerted was apparently sufficient to cause rupture of the tubule walls and force the young through the anal pores. The juveniles frequently became looped around two anal pores so that their anterior and posterior ends would both protrude (Fig17b). This phenomenon was confirmed when several juveniles were pulled through the anal pores with forceps. The five anal pores would consequently often be occupied by only three young. After emerging, the young would adhere to the posterior end of the adult by a mucous-like web before eventually becoming free (Fig.17c). The complete process of "birth" took about 15-20 minutes per set of animals. This is the first time release of *L. clarki* young from the adult has been witnessed.

DISCUSSION

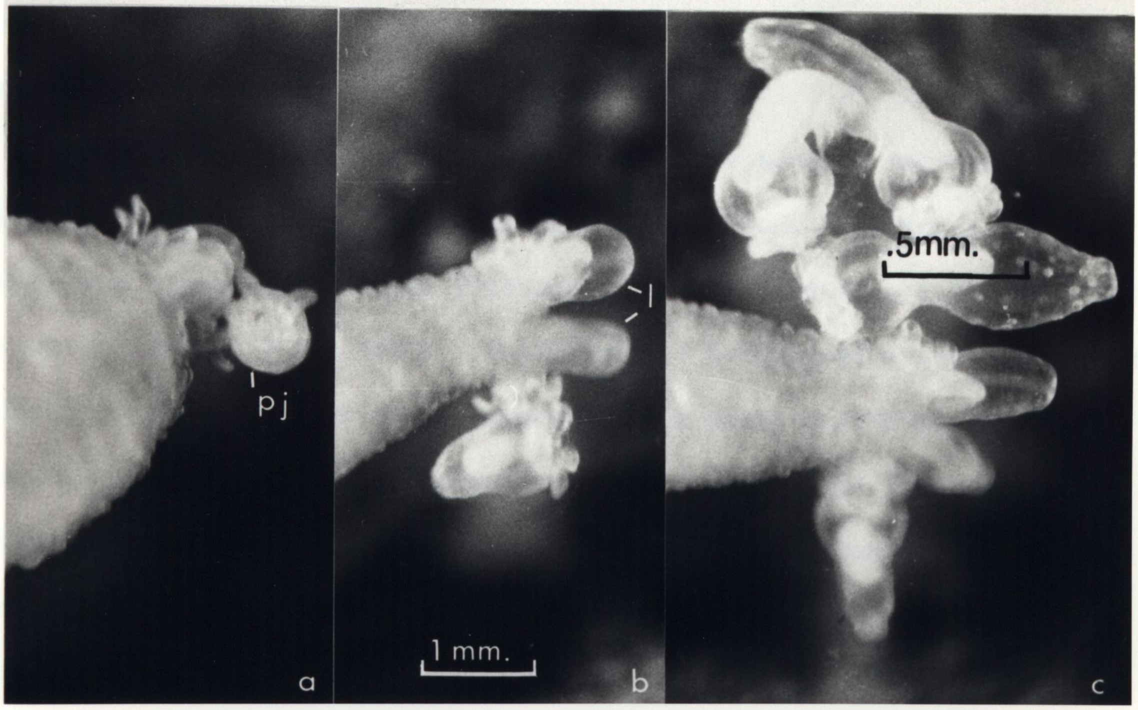
Difference in sperm shape helped convince Dunnill (1968) that the clam *Macoma elimata* was a distinct species from *M. calcarea*. Similarly, the differences in sperm shape and length, as well as the differences in oocyte size help to distinguish between the two species of *Leptosynapta*. When using gametes as taxonomic characters, however, care should be taken to examine only those specimens whose eggs and sperm are fully mature.

Holothurians usually breed during the spring and summer in temperate latitudes and this tendency is probably related to the seasonal rise in temperature (Hyman, 1955). Such was the case in this study, though spawning time for *L. transgressor* was about a month later than that for *L. clarki*. This is of little significance, for breeding time in the

Figure 16. Dissection of brooding female of *L. clarki* showing juveniles in ovarian tubules (ot).

Figure 17. Sequence (a-c) showing release of pentacula juveniles (pj) through the anal pores of *L. clarki*. Notice looping (l) of the young in (b). Free juveniles in (c) have floated to the top.

16



spring may vary from year to year depending upon temperature, weather conditions and location. Breeding time and release of young might also be influenced by the abundance of appropriate food for the juveniles. At Sooke very young specimens of *L. clarki* were found on the surface of large seaweeds during the late spring and summer months. A laboratory examination revealed that these animals had diatoms in their gut.

There was an obvious relationship between temperature and gamete development for both species. Oogenesis appeared to be stimulated by an increase in temperature and presumably took place right after the young were released, although whether this occurred in the same individuals which had just produced young has not been proven. Specimens collected in May might have been young adults just beginning gamete development, or previously protandric males. There is also a probability that once the young are released or gametes shed, the adult dies. This latter point might explain the decrease in population of *L. transgressor* at Bosun Bank after the summer months. Oocytes increased in size as temperatures dropped throughout the fall and winter.

Spermatogenesis was not noticable until the maximum temperature was reached and sperm developed as temperatures dropped. Expulsion of sperm began soon after the seasonal rise in temperature, between 6.5° and 7.5°C for *L. clarki* and 7-9°C for *L. transgressor*. Orton (1920) noted a similar situation in perch, i.e. spermatogenesis took place at the beginning of the temperature drop and sperm developed as temperatures dropped while expulsion of sperm began at the onset of the seasonal temperature rise. Thorson (1946) also found that certain Danish marine invertebrates which were non-pelagic in development, spawned during the first small rise in water temperature.

According to Hyman (1955), holothurians may spawn at intervals instead of discharging all their gametes at one time. This appeared to be the case in males of both species of *Leptosynapta*. In *L. clarki* the evidence of exuded sperm indicated that fertilization first occurred between February 16th and March 20th. Fertilization time may also be calculated from the timing of embryological stages as observed by Everingham (1961). It was assumed that females with late pentacula young in April (21st) had been fertilized the third week of February at the latest as the embryos need at least two months to arrive at this stage. Therefore, fertilization must have occurred during the last two weeks of February and was still continuing well into April, as some specimens contained prepentacula embryos on April 21st and these take 1-2 weeks to develop.

Evidence of fertilization first occurred in *L. transgressor* females by the appearance of eggs which showed characteristics of having been fertilized. According to Balinsky (1965), cortical reactions take place on the surface of the egg cytoplasm after fertilization. The germinal vessicle disappears and a fertilization membrane forms, often lifting well above the egg surface. Eggs in the April specimens showed these various characters. Assuming the eggs were indeed fertilized, such fertilization must have occurred between March 19th and April 21st, a considerably later breeding period than that of *L. clarki*. Ecological differences such as deeper habitat, lower light intensity, and lower oxygen levels might account for a later breeding period in this species. Temperature differences were not significant enough to be the cause of the later breeding period in *L. transgressor*.

Because of the smaller size of oocytes in *L. transgressor*, this species was suspected of being oviparous. The evidence obtained in this study indicates that fertilization is internal and young are probably brooded, at least in the very early stages of development. It is probable that the young are released when they have developed further and are motile, however, there is as yet no evidence of this nor can we say the species is definitely a brooding species.

Although they are believed to be hermaphroditic (Everingham, 1961) sexes of *L. clarki* appeared to be separate in this investigation; in no instances were sperm and eggs found in the same individual. Everingham points out that the synchronous development in the ovary tends to support the idea of cross fertilization. If such is the case, sperm must enter the gonopore of the female. The exact method of fertilization remains an enigma until more conclusive evidence is found.

Fertilization time may vary within a specific area. For example, Everingham (1961) found embryological stages in females of *L. clarki* collected at False Bay from November through until May. Sooke (Sta. 1) specimens however, showed a high degree of synchrony in this respect. This could be attributed to their high population density within a small area where ecological conditions would be uniform and close proximity of the sexes would facilitate cross fertilization.

The method of the release of the young of *L. clarki* has now been witnessed for the first time and Anderson's (1966) theory, that the young may be released from the coelom through the anal pores, has now been confirmed. Everingham (1961) found that juveniles in the gonad tubules of specimens found late in the spring at False Bay had eight or more tentacles. Those juveniles seen being released from the Sooke adults were all in the five-tentacle stage and no other juveniles found in ovarian tubules of B.C. specimens had more than five tentacles.

Release of *L. clarki* young in the laboratory might have been a premature event which may not have coincided with such an occurrence in the natural environment. A sudden increase in temperature in the water or the presence of young from the autotomized adult may have presented physical or chemical stimuli which might have triggered release of the young. However, the very small juveniles (1.5-4mm long with from 8-10 tentacles) which were found at Sooke and nearby areas in late May and June indicate that the release of pentacula young took place naturally in April and May.

CHAPTER IV

ECOLOGICAL ASPECTS

INTRODUCTION

This chapter deals with some of the physical and biological aspects of the ecology of the two species under review. The physical factors, exclusive of temperature (see Chapter III), which have been examined are depth of water, intertidal position, exposure to wave action and grain size of sediment. In the case of biological factors, the faunal communities with which each species is associated have been compared and predation and mutualistic relationships have been presented. The geographical distribution of each species on the Pacific Coast has been outlined.

Sediment

The anchor and plate ossicles of *Leptosynapta* have a locomotory function (p. 2) and the shape and size of these ossicles appear to be related to environmental factors (see Chapter II), particularly the physical nature of the sediment. In order to determine if there is any difference in the type of sediment preferred by each of the two species, analyses of sediment from each station have been undertaken. These analyses deal with the range of grain size and the classification of sediment. The sediments from Bosun Bank are classified as sand and have been described in detail by Dunnill (1968) and McCallum (unpublished manuscript). Those of Sooke Harbour have a large percentage of silt from alluvial deposition and appear to be rich in organic matter. The sediments there are classified as sandy silt (Fig. 23).

Community and Mutualism

The mutualistic relationships which have been recorded for the genus *Leptosynapta* are mainly internal and include two parasitic bivalves and three commensal peritrichous ciliates. Wass (1968) also found a species of *Pinnixa*, the pea crab, commensal with *Leptosynapta crassipatina* in Florida. The occurrence of a commensal polychaete, *Malmgrenia nigralba*, inhabiting the burrows of "*L. inhaerens*" was recorded by Berkeley (1924) in Piper's Lagoon. Heding (1928) later discovered the synaptids in that lagoon were *L. clarki* and the polynoid has since been renamed *Malmgrenia lunulata* (Delle Chiaje). Few cases of predation have been recorded in the literature. Demersal fish probably feed on a small number of subtidal forms, while gulls and crabs consume some intertidal species (Pawson, 1966).

Because the two species were found to be ecologically isolated, it was suspected that each species might be associated with a distinct community of animals. In this chapter animals collected from the three stations as well as other locations have been listed and compared.

METHODS

In addition to the collecting methods already described (p. 6), the exposure and intertidal positions of the habitats were noted. Subtidal depths were recorded by echo sounder at Bosun Bank and by weighted rope at Sooke Harbour. Intertidal sampling was carried out from Victoria north to Menzie's Bay on Vancouver Island and also on the Queen Charlotte Islands. Subtidally, dredgings were taken from Victoria north to Nanoose Bay. Marine stations and universities from Alaska to California were contacted for information on the distribution of *Leptosynapta*. Animals collected for community studies were identified as accurately as possible by individuals familiar with the particular taxonomic groups.

Sediment Analyses

Three sediment samples were analysed from each of the three stations under study and a representative sample from each station was plotted as a histogram. Several samples were analysed from other locations where *L. clarki* was found in order to determine the range of sediments this species would inhabit. Analysis was carried out using a method modified from Folk (1965) whereby the clay and silt fractions were determined by hydrometry and the various sand fractions were arrived at by seive analysis. Cumulative curves were drawn for each sample by plotting the percentage passing against grain size (App. I-III) and histograms were constructed based on these curves (Figs. 20-22). Various fractions of sand and silt were based on the Wentworth Grade Scale (Folk, 1965), and nomenclature was determined by plotting the percentages of the three main fractions (sandy silt and clay) on the triangle proposed by Shepard (1954).

RESULTS

Geographical Distribution (Fig. 18, Table 8)

L. clarki

In this study *L. clarki* has been recorded from the Queen Charlotte Islands south to Pacific Grove, California. This species has been taken along the east coast of Vancouver Island from Menzie's Bay south to Victoria and Sooke. It also occurs on Stephen's Island and some of the Gulf Islands. No records have been received between Puget Sound and Tomales Bay, California.

Figure 18. Records of *L. clarki* and *L. transgressor* collected in British Columbia.

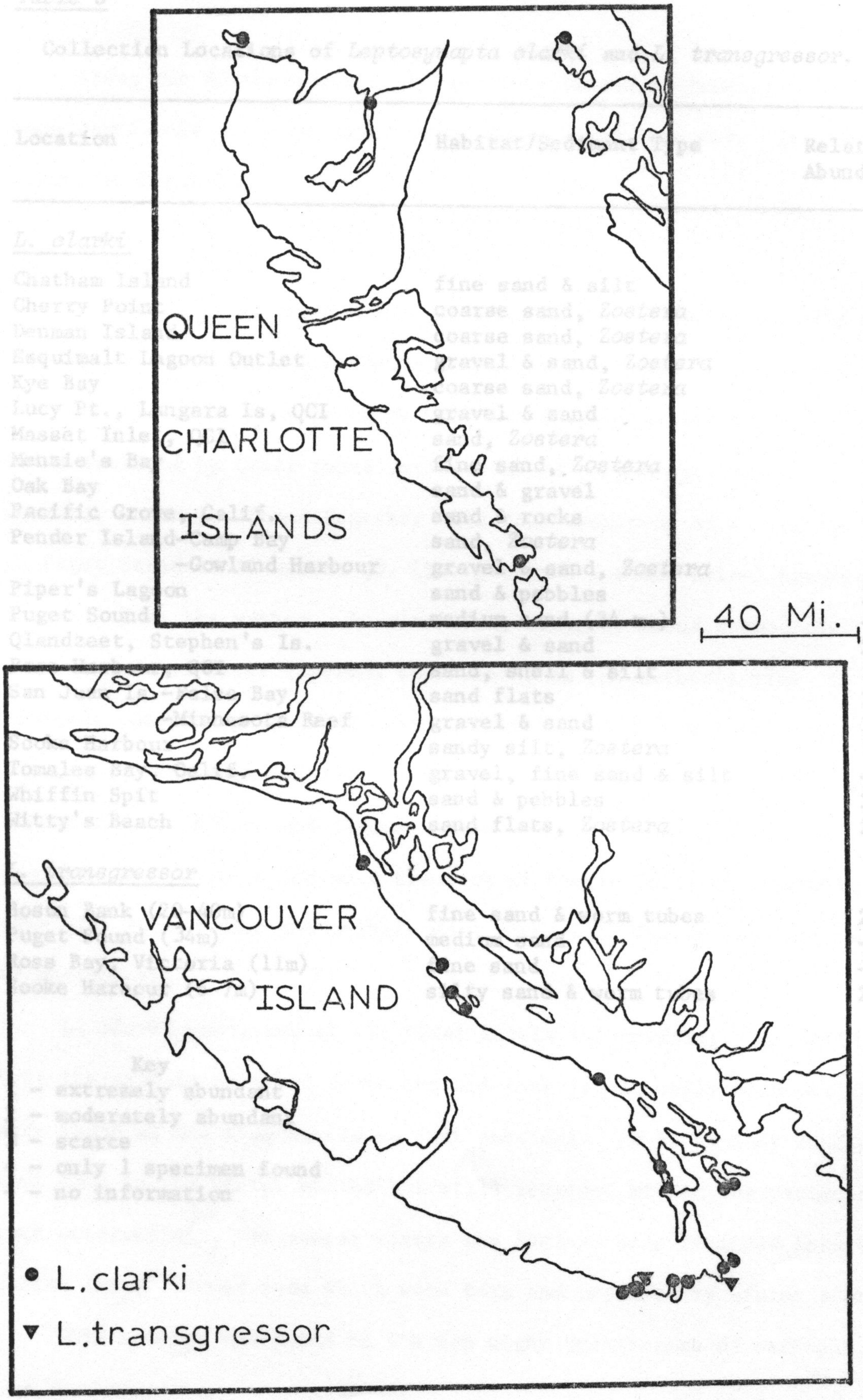

QUEEN
CHARLOTTE
ISLANDS
40 Mi.
VANCOUVER
ISLAND
L.clarki
L.transgressor

Table 8

Collection Locations of *Leptosynapta clarki* and *L. transgressor*.

Location	Habitat/Sediment Type	Relative Abundance
L. clarki		
Chatham Island	fine sand & silt	2
Cherry Point	coarse sand, *Zostera*	2
Denman Island	coarse sand, *Zostera*	3
Esquimalt Lagoon Outlet	gravel & sand, *Zostera*	2
Kye Bay	coarse sand, *Zostera*	4
Lucy Pt., Langara Is, QCI	gravel & sand	3
Masset Inlet, QCI	sand, *Zostera*	2
Menzie's Bay	fine sand, *Zostera*	2
Oak Bay	sand & gravel	2
Pacific Grove, Calif.	sand & rocks	-
Pender Island-Camp Bay	sand, *Zostera*	3
-Gowland Harbour	gravel & sand, *Zostera*	3
Piper's Lagoon	sand & pebbles	2
Puget Sound	medium sand (34 m.)	-
Qlandzeet, Stephen's Is.	gravel & sand	2
Rose Harbour, QCI	sand, shell & silt	1
San Juan Is.-False Bay	sand flats	2
-Minnesota Reef	gravel & sand	3
Sooke Harbour	sandy silt, *Zostera*	1
Tomales Bay, Calif.	gravel, fine sand & silt	-
Whiffin Spit	sand & pebbles	2
Witty's Beach	sand flats, *Zostera*	2
L. transgressor		
Bosun Bank (20-40m)	fine sand & worm tubes	2
Puget Sound (34m)	medium sand	-
Ross Bay, Victoria (11m)	fine sand	-
Sooke Harbour (6-7m)	silty sand & worm tubes	2

Key

1 - extremely abundant
2 - moderately abundant
3 - scarce
4 - only 1 specimen found
- - no information

L. transgressor

Along the southern coast of Vancouver Island *L. transgressor* has been found only at Bosun Bank, Sooke Harbour and Ross Bay. It also occurs in Puget Sound.

Habitat of *L. clarki*

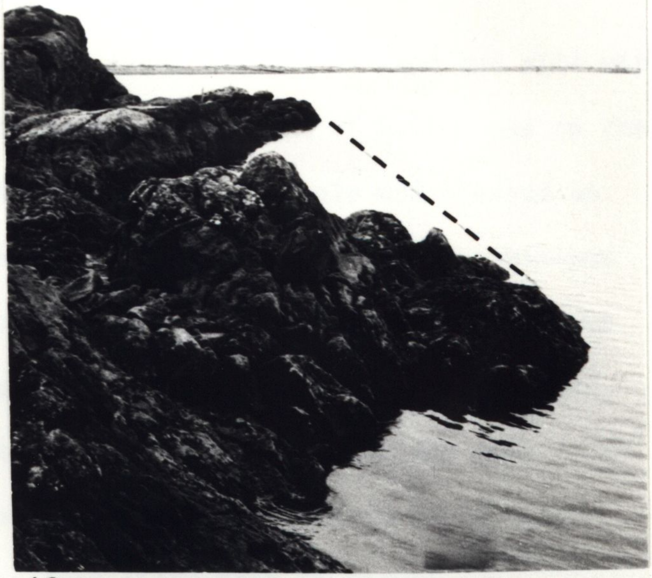
In the sampling carried out in B.C., *L. clarki* was found only intertidally or just barely subtidally and did not inhabit deeper waters as was the case in *L. transgressor*. The two species appear to be ecologically isolated by their zonal preference. A single specimen of *L. clarki*, however, which was found together with four specimens of *L. transgressor* in Puget Sound at 34 meters (U. Lie, personal communication) has been identified by the author. *L. clarki* was commonly found around the rhizomes of Eel Grass (*Zostera* sp.) where, in the author's view, it receives adequate protection against shifting sand; and less frequently under rocks or large pebbles. This species appeared to favour the protected waters of bays and lagoons which were often bounded by rock outcroppings. Samples taken on a transect at Cherry Point and Sooke Harbour (Fig. 19A) showed that the animals were found only inside the influence of outcroppings.

L. clarki was found at all tidal levels intertidally. At Whiffin Spit the animals occurred above the mid-tide level, while at Sooke they were found at low tide levels to just subtidal. At Sooke they tended to move down in the winter months but still remained within the protective rock outcroppings. In deeper waters the *Zostera* beds remained intact, unlike the shallower beds which were torn and unrooted by winter storms.

This species was found in the top eight centimeters of sediment,

Figure 19. A. Sooke Harbour, Station 1. *L. clarki* was only found within the protection of the rock outcroppings as indicated by the broken line.

B. Closer view of Station 1 showing *Zostera* (Z) blades.



19 A

19 B

at times just under the surface as indicated by small holes in the sand, or encased in tubes of sand which were apparently formed by a secretory adhesive. At Sooke small juveniles were found on *Zostera*, algal fronds or diatom clumps.

Sediment

At Station 1 there was an almost equal mixture of sand and silt with 50.5 and 41.5% respectively. The median grain size of 0.05mm was in the coarse silt fraction (Fig. 20, App. I) and the sample was classed as sandy silt (Fig.23). Samples analysed from other locations contained a high percentage of sand, especially in the fine and medium fractions (Table 9). The Denman Island sample contained slightly coarser sand, while the sample from Menzie's Bay had mostly fine sands and silt. Unanalysed sediments from other locations are described in Table 8 and include a wide range of sediments from silty sand to gravel.

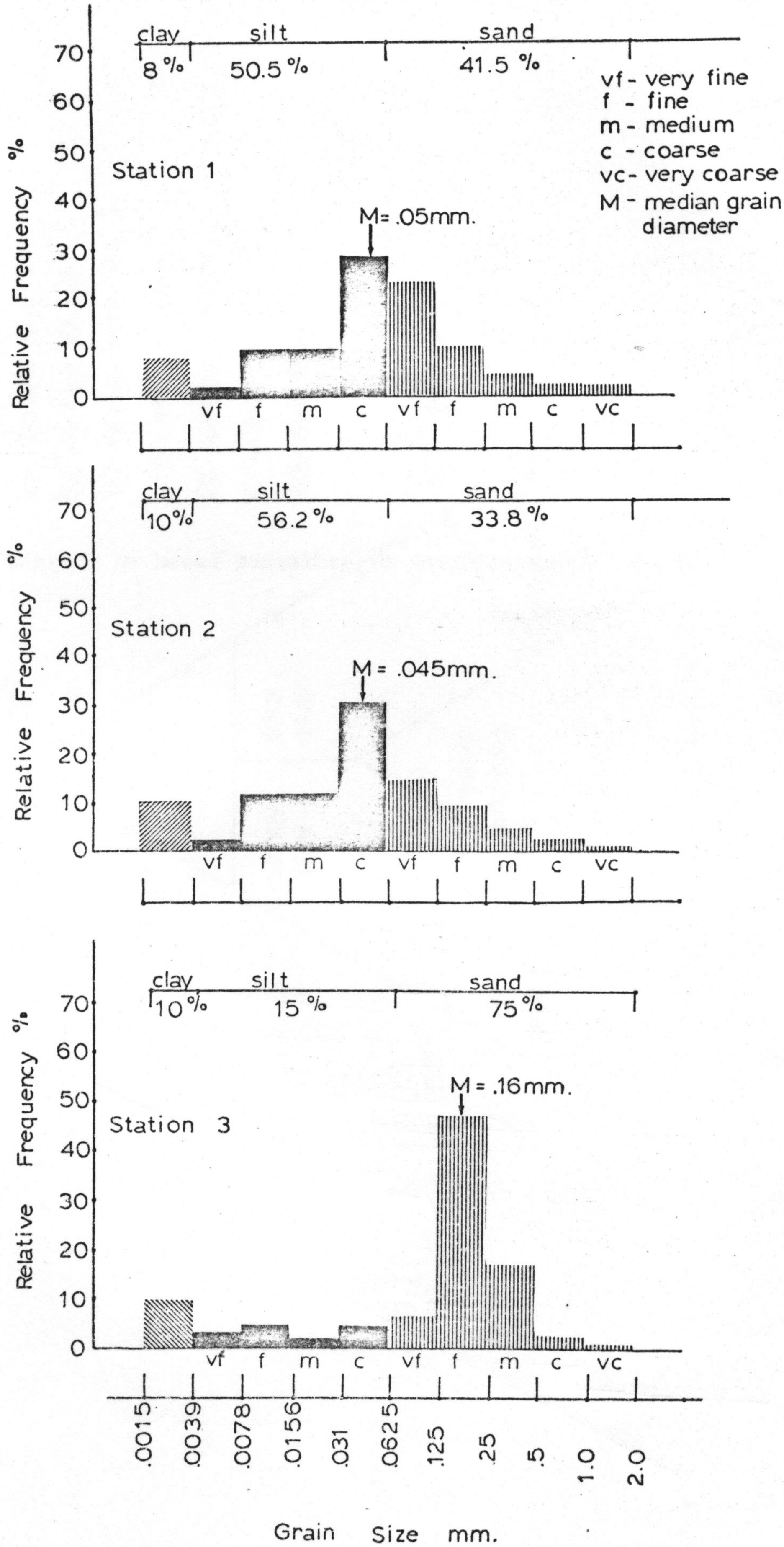
Habitat of *L. transgressor*

L. transgressor is a subtidal species, occurring only in bays and channels of inside waters at depths of 6 to 40 meters. Large specimens were found a few centimeters under the sediment while small specimens were frequently entwined around the tube masses (Fig. 24) of the polychaete worm, *Phyllochaetopterus prolifica* at Bosun Bank and around the tubes of the polychaete *Prionospio* sp. at Sooke. Some seasonal variation in numbers was observed at Bosun Bank and dredging was carried out in slightly deeper waters after August. The animals apparently either moved into deeper waters during the cooler months of the year or the population from the first area simply died out.

Figure 20. Size distribution of sediments: Sooke Harbour, Station 1.

Figure 21. Size distribution of sediments: Sooke Harbour, Station 2.

Figure 22. Size distribution of sediments: Bosun Bank, Station 3.



clay
silt
sand
8 %
50.5 %
41.5 %
vf - very fine
f - fine
m - medium
c - coarse
vc - very coarse
M - median grain diameter
Station 1
M= .05mm.
Relative Frequency %
70
60
50
40
30
20
10
0
vf
f
m
c
vf
f
m
c
vc
clay
silt
sand
10%
56.2 %
33.8 %
Station 2
M = .045mm.
clay
silt
sand
10 %
15 %
75 %
Station 3
M = .16mm.
.0015
.0039
.0078
.0156
.031
.0625
.125
.25
.5
1.0
2.0
Grain Size mm.

Figure 23. Classification of sediments based on Shepard's (1954) triangle.

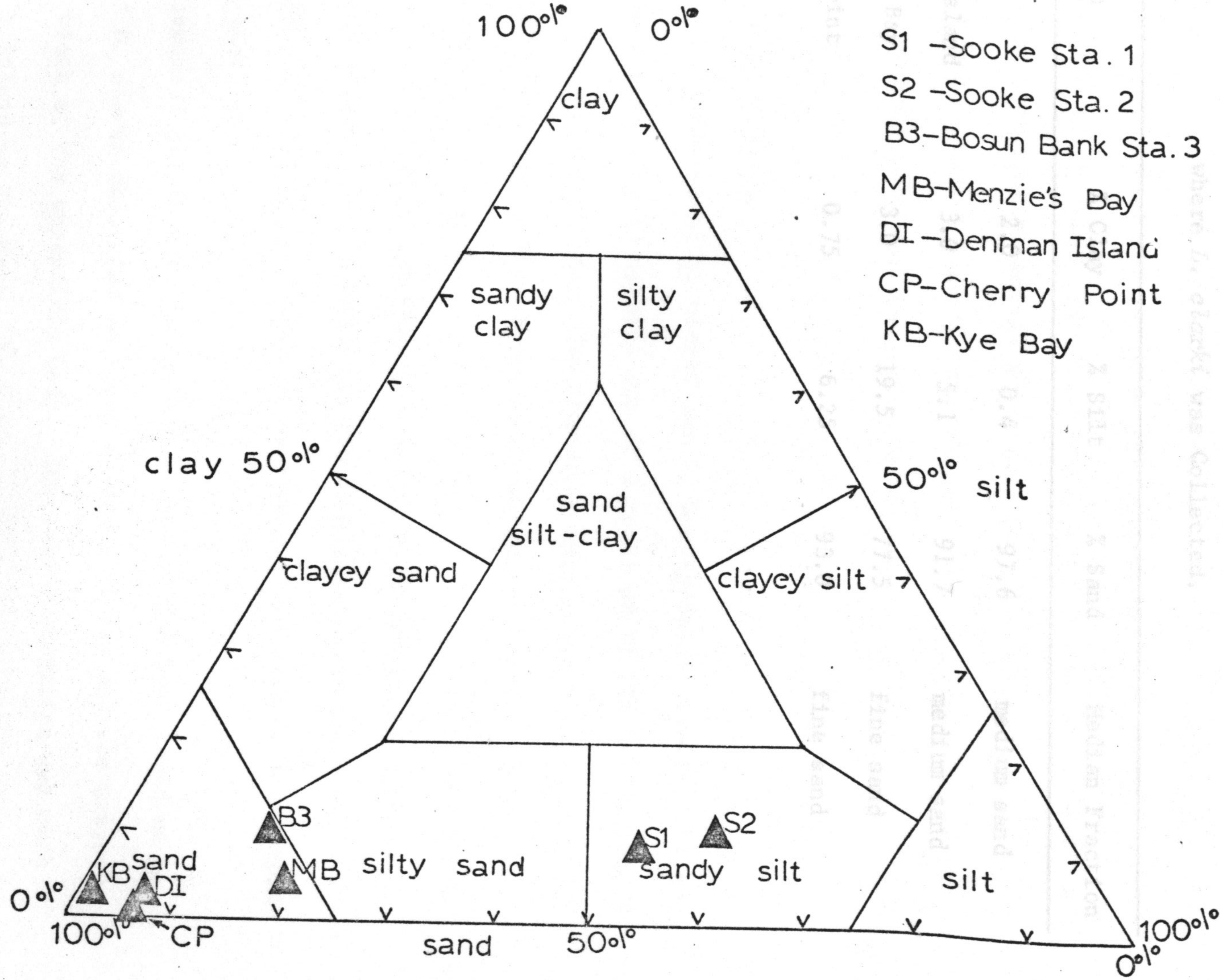

100%
0%
clay
sandy clay
silty clay
clay 50%
sand silt-clay
50% silt
clayey sand
clayey silt
B3
S1
S2
KB
sand
DI
MB
silty sand
sandy silt
silt
0%
100%
CP
sand
50%
100%
0%
S1 -Sooke Sta. 1
S2 -Sooke Sta. 2
B3-Bosun Bank Sta. 3
MB-Menzie's Bay
DI -Denman Island
CP-Cherry Point
KB-Kye Bay

Table 9

Analyses of Sediments from Some Areas on Vancouver Island where *L. clarki* was Collected.

Location	% Clay	% Silt	% Sand	Median Fraction
Kye Bay	2.0	0.4	97.6	medium sand
Denman Island	3.0	5.1	91.7	medium sand
Menzie's Bay	3.0	19.5	77.5	fine sand
Cherry Point	0.75	6.25	93.0	fine sand

Sediment

Sediment from Station 2 consisted mainly of silt and fine sand with 56.2% of the sample in the silt range. The medium grain size of 0.045mm was in the coarse silt fraction. According to Shepard (1954) (Fig. 23), this sediment is classified as sandy silt and although it bears the same classification as sediment from Station 1, it has a lower sand fraction and slightly more fine sediments (Fig, 21, App. II).

Station 3 sediments were classed as sand (Fig. 23) with 75% of the sample in the sand range and the median of 0.16mm in the fine sand fraction (Fig. 22, App.III).

Community and Mutualism

Faunal communities of *L. clarki* and *L. transgressor* were for the most part distinct with only 13 species out of a total of 71 (Table 10, App.IV) common to both Leptosynaptids. The community associated with *L. transgressor* was by far the richer of the two, yielding 55 species while only 29 species were found associated with *L. clarki*. An ophiuroid (*Amphiodia occidentalis*) and a pelecypod (*Macoma nasuta*), both intertidal species, were commonly found with *L. clarki* and subtidal species of the same groups (*Amphiodia urtica* and *Axinopsis* sp.) were found with *L. transgressor* at both stations.

The commensal polychaete, *Malmgrenis lunnulata* has been found on a specimen of *L. clarki* collected at Piper's Lagoon (1968) and was wrapped around the width of the synaptid's body. The same observation was made at Stephen's Island by Dr. Hart. It has also been found with *L. transgressor* at Station 2. The commensal crab, *Pinnixa schmitti* has been found for the first time in the burrows of *L. clarki* at Oak Bay (Dr. Hart, personal communication) and Cherry Point. Several specimens of

Table 10

A Summary of the Faunal Communities Associated with *L. clarki* and *L. transgressor*.

Taxonomic Group*		No. of Species associated with *L. clarki*	No. of Species associated with *L. transgressor*	No. of Species common to both
Polychaetes	(27)	12	20	5
Echinoderms	(8)	3	7	2
Arthropods	(7)	6	3	2
Pelecypods	(21)	4	19	2
Gastropods	(8)	4	6	2
Total	71	29	55	13

* List of species in Appendix IV

Figure 24. A dredging from Bosun Bank, Station 3, showing tube masses of the polychaete *Phyllochaetopterus prolifica* found above the sediment. Small specimens of *L. transgressor* were found among the tubes.

L. clarki were found on top of the sand at False Bay while the tide was low. They had been partly eaten by gulls or crows.

DISCUSSION

The most obvious ecological factor which appears to separate the two species, at least locally, is depth of water. Though *L. clarki* is usually found intertidally, it cannot be said that the species is entirely intertidal as Heding (1928) claims to have dredged it in Roxton Passage at 15-25 fathoms while Lie (1969) found one specimen in 34 meters at Puget Sound. Dredgings undertaken by the author have failed to reveal any specimens deeper than the subtidal fringe. *L. transgressor* has not been found intertidally but occurs in depths of 6-40 meters. The two species may then be ecologically separated by their depth ranges but more specifically by the fact that *L. transgressor* is not an intertidal species.

In both species the young apparently feed on diatoms which grow on algal and *Zostera* fronds or on polychaete tubes above the sediment. The adults on the other hand, are found a few centimeters below the sediment surface. Meadows and Anderson (1968) found that most diatoms, bacteria and blue-green algae occurred in the top 5 cm. of sand, while Johnson (1967) found that the average median depth of infauna of a sand flat was 8 cm. The availability of food in these upper layers is obviously the main factor in the within-sediment distribution of these animals.

With respect to the physical nature of the sediment, *L. clarki* was the more adaptable of the two species, tolerating a wide range of sediments from sandy silt to gravel. *L. transgressor* was found in finer sediments ranging from sandy silt to medium sand but the complete range of sediments cannot be established because this species has only been found in a small number of locations. Nevertheless, these results do indicate that sediment might be considered as an ecological factor when differentiating between the two species.

The extreme abundance of *L. clarki* found at Sooke Station 1 is, in the author's opinion, due to two factors which contribute to optimum conditions for the growth of this species: first, the high productivity which is evident and is probably related to extensive *Zostera* beds and prolific diatom growth and secondly, the protection afforded by the vigorous, dense growth of *Zostera* (Fig.19B). The protection given by eel grass or rocks is important to this species. Tests of the burrowing time of *L. clarki* carried out by Anderson (1965) indicated that the animals were unable to burrow fast enough to escape shifting sand caused by wave action during a combination of incoming tides and storms. Once out of the sediment the animals, because of their lightness, may be easily carried along by currents.

The faunal communities, because they are fairly distinct, do, when taken as a whole, show a difference in the ecology of the two Leptosynaptids and therefore should be taken into account when differentiating between species of *Leptosynapta*.

SUMMARY

1. The pigments of *L. clarki* and *L. transgressor* are believed to be naphthaquinone.

2. In life each species has a distinct external pigment pattern.

3. 98% of adult *L. clarki* examined had 12 tentacles (range 9-13) while 65% of adult *L. transgressor* had 10 tentacles (range 8-14).

4. Ossicles varied with age and location of specimens and were not reliable for distinguishing between the species in adult specimens.

5. Immature *L. clarki* had primary ossicles which were lacking in immature *L. transgressor*.

6. Small specimens (6-42 mm) of *L. transgressor* tended to have one type of anchor while larger specimens (18-103 mm) had two types of anchors similar to *L. clarki*.

7. There was some evidence that ossicles were related to environmental factors such as substrate and exposure.

8. Oocytes of both species increased in diameter as sediment temperatures dropped in the fall and winter, and reached their maximum size during minimum temperatures (Dec.-Feb.).

9. Mature oocytes of *L. clarki* were larger (average 404μ) than those of *L. transgressor* (average 272μ) and there were slight differences in sperm shape and length.

10. Fertilization occurred after the first seasonal rise in sediment temperature in the spring, though *L. transgressor* was a month later (April) in breeding time than *L. clarki* (March).

11. *L. clarki* broods its young until at least the pentacula stage.

12. Evidence that *L. transgressor* is a brooder is inconclusive. Only fertilized eggs and very early embryological stages have been found

in the females.

13. Juveniles of *L. clarki* are released through the five anal pores of the adult and this phenomenon has been witnessed for the first time.

14. *L. clarki* occurs from Queen Charlotte Islands to central California, while *L. transgressor* has only been found from southern Vancouver Island to Puget Sound.

15. *L. clarki* is mainly intertidal and often found in *Zostera* beds. It inhabits a wide range of sediments from sandy silt to gravel.

16. *L. transgressor* is subtidal only (6-40 meters) and inhabits finer sediments ranging from sandy silt to medium sand.

17. The faunal communities associated with each species are, for the most part, distinct.

18. The commensal polychaete *Malmgrenia lunulata* has been found for the first time with *L. transgressor* and is therefore common to both species of *Leptosynapta*.

19. The commensal crab, *Pinnixa schmitti*, has been found for the first time in the burrows of *L. clarki*.

20. These results indicate that *L. transgressor* may now be considered a distinct species from *L. clarki*.

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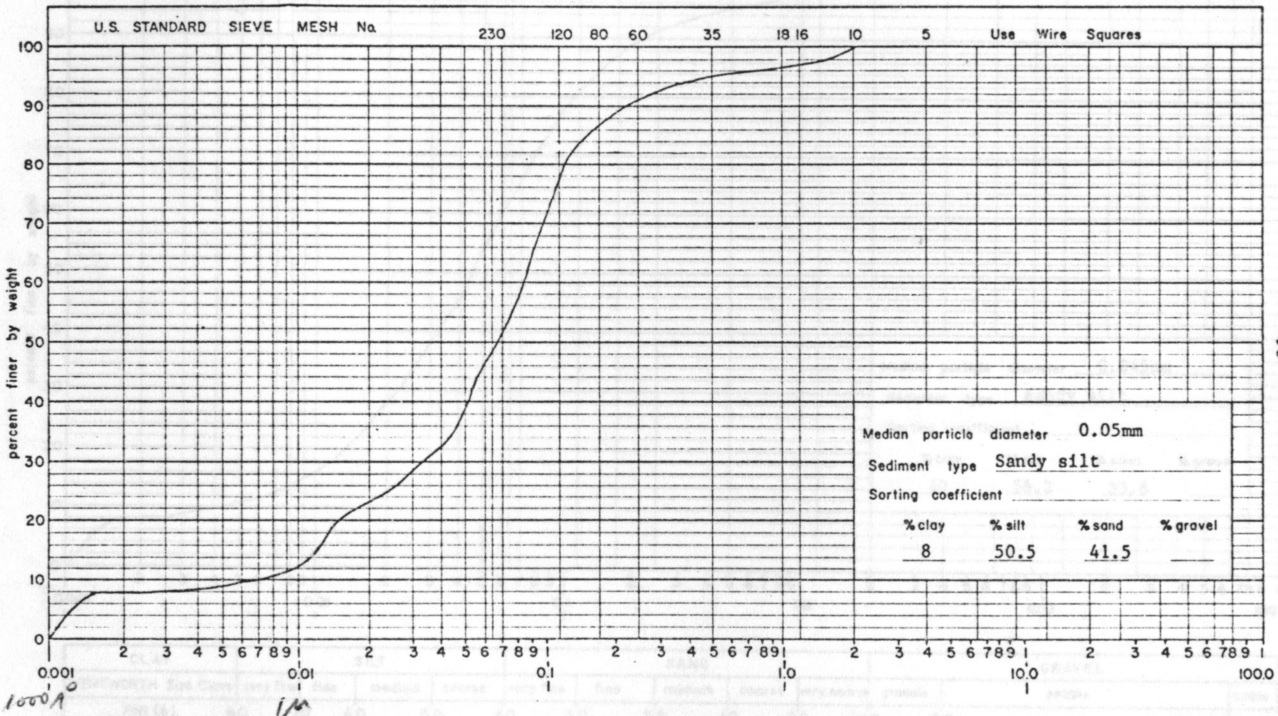
APPENDIX

APPENDIX I

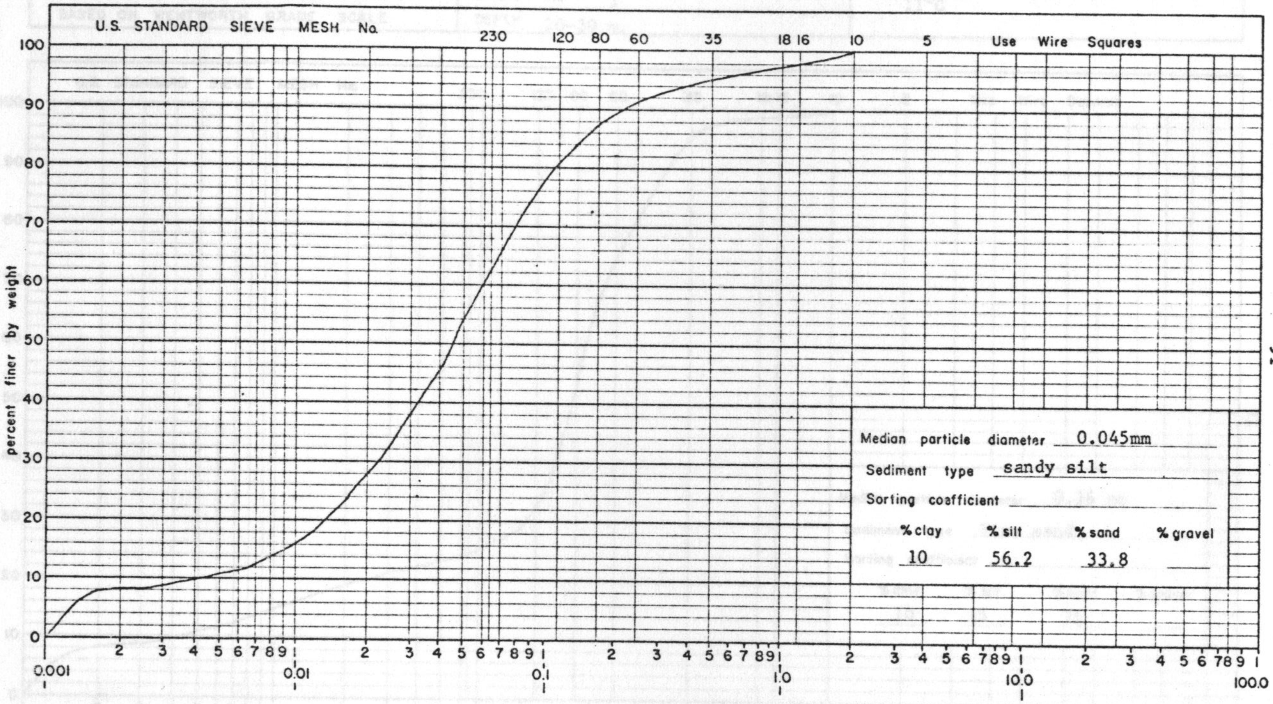
CUMULATIVE CURVE BASED ON WENTWORTH GRADE SCALE	TESTED BY LOCATION Sooke STATION No. Sta. 1 DEPTH 0 m.	DATE Sept. 24/68 DATE TESTED 10.5°C



CLAY	SILT				SAND					GRAVEL		
WENTWORTH Size Class	very fine	fine	medium	coarse	very fine	fine	medium	coarse	very coarse	granule	pebble	coble
PHI (φ)	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0	-1.0	-2.0	-6.0
GRAIN SIZE (mm)	.0039	.0079	.0156	.031	.0625	.125	.25	.50	1.0	2.0	4.0	

APPENDIX II

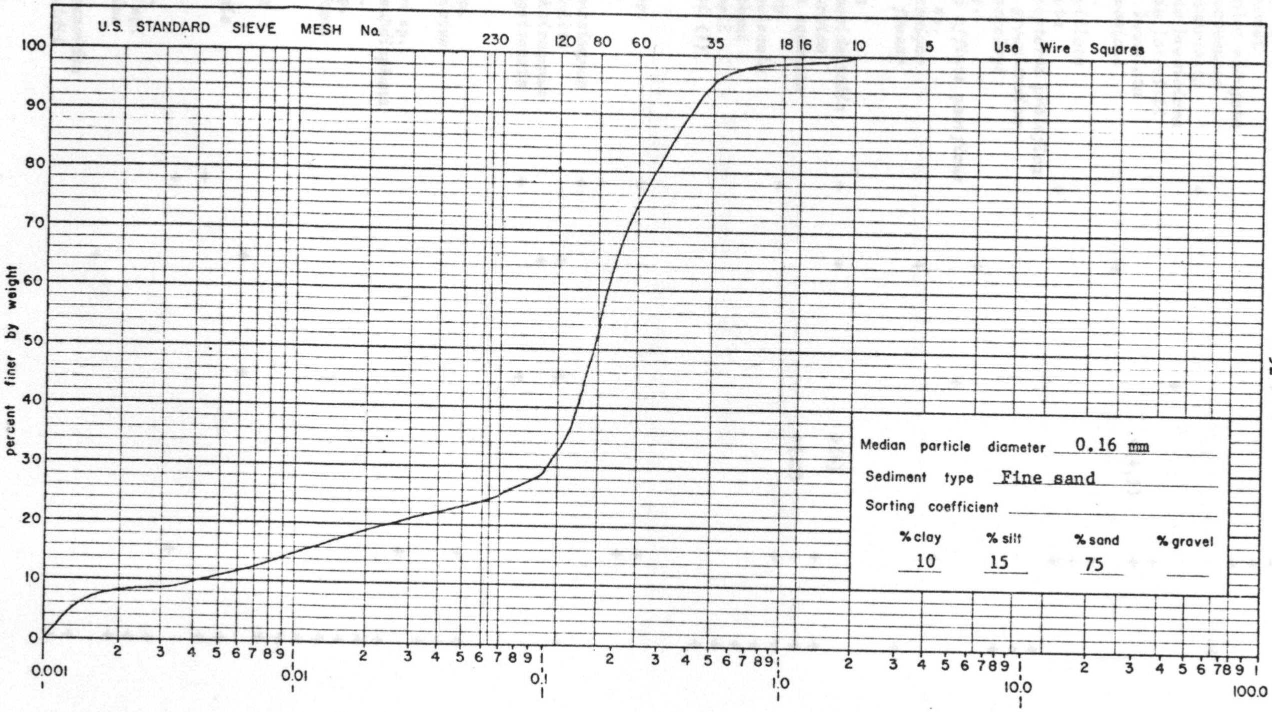
CUMULATIVE CURVE BASED ON WENTWORTH GRADE SCALE	TESTED BY LOCATION Sooke STATION No. 2 DEPTH 8 m.	DATE Sept. 24/68 DATE TESTED 10°C



CLAY	SILT				SAND					GRAVEL		
WENTWORTH Size Class	very fine	fine	medium	coarse	very fine	fine	medium	coarse	very coarse	granule	pebble	coble
PHI (Φ)	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0	-1.0	-2.0	-6.0
GRAIN SIZE (mm)	.0039	.0079	.0156	.031	.0625	.125	.25	.50	1.0	2.0	4.0	

APPENDIX III

CUMULATIVE CURVE BASED ON WENTWORTH GRADE SCALE	TESTED BY	DATE Aug. 17/68
	LOCATION Bosun Bank	DATE TESTED
	STATION No. 3	11°C
	DEPTH 20-30 m.	



CLAY	SILT				SAND					GRAVEL		
WENTWORTH Size Class	very fine	fine	medium	coarse	very fine	fine	medium	coarse	very coarse	granule	pebble	coble
PHI (φ)	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0	-1.0	-2.0	-6.0
GRAIN SIZE (mm)	.0039	.0079	.0156	.031	.0625	.125	.25	.50	1.0	2.0	4.0	

APPENDIX IV

A Comparison of the Fauna Associated with *L. clarki* and *L. transgressor*.

Species	*L. clarki* stations				*L. transgressor* stations	
	Sooke	Cherry Pt.	Oak Bay	Other	Sooke	Bosun Bank
POLYCHAETES						
Abarenicola pacifica					+	
Ampharete arctica					+	
Arabella tricolor		+				
Armandia brevis			+			+
Diopatra ornata						+
Dorvillea sp.	+				+	
Glycera americana						+
Glycinde armigera			+		+	
Goniada brunnea					+	
Haploscoloplos elongata					+	
Harmothoe extenuata						+
Harmothoe imbricata	+					
Lumbrinereis brevicirra			+			
Lumbrinereis latreilli					+	
Malmgrenia lunnulata				+(4,5)	+	
Nepthes caecoides		+				
Nepthes sp.					+	
Pherusa papillata					+	
Pholoe minuta	+				+	
Phyllochaetopterus prolifica						+
Phyllodoce groenlandica						+
Pilargis berkeleyi						+
Polydora sp.		+				
Praxillella affinis pacificus			+			
Prionospio sp.					+	
Scoloplos armiger		+				
Sternaspis fossor						+
ECHINODERMS						
Amphiodia occidentalis	+	+		+(6)		
Amphiodia urtica					+	+
Amphipholis squamata				+(13)	+	+
Chiridota sp.	+				+	+
Henricia leviscula						+
Ophiura lutkeni						+
Ophiura sarsii						+
Thyone benti (?)						+
ARTHROPODS						
Amphipoda sp.	+				+	
Cragnon sp.					+	
Nebalia sp.	+					
Pagurus granosimanus	+					
Pinnixa schmitti		+	+			+
Telmessus cheiragonus		+				
Upogebia pugettensis	+		+			
PELECYPODA						
Axinopsis sp.					+	+
Chlamys hericius						+
Chlamys sp.						+
Clinocardium sp.					+	
Compsomyax subdiaphana						+
Crenella sp.						+
Humilaria sp.						+
Macoma alaskana						+
M. calcarea						+
M. elimata						+
M. incongrua						+
M. nasuta		+	+			
M. yoldiformis						+
Macoma sp.	+					+
Mysella sp.	+				+	
Nuculana sp.						+
Protocardia sp.						+
Solen sicarius						+
Tellina sp.		+				
Thracia trapezoides						+
Thyasira gouldii						+
GASTROPODA						
Bittium sp.	+					
Eulima micans						+
Lacuna sp.		+			+	+
Margarites pupillus						+
Nassarius sp.	+				+	+
Transennella tanti				+(2)		
Trichotropis cancellata						+
Turbonilla sp.					+	

Key to Other Localities
1........Denman Island
2........False Bay
3........Kye Bay
4........Piper's Lagoon
5........Stephen's Island
6........Whiffin Spit