SPIROCHETES OF THE GENUS CRISTISPIRA IN BIVALVE MOLLUSCS OF EASTERN CANADIAN WATERS

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by

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ABSTRACT

Seven species of bivalve molluscs, from waters of eastern Canada, were examined for the presence of *Cristispira* spp. during the years 1968-1969. Field investigations revealed that, of these seven species, only *Crassostrea virginica* Gmelin harboured this large spirochete.

Laboratory investigations into the source of these spirochetes and their mode of infection included examination of water and sediment, removal of spirochetes from infected oysters, and subsequent attempts to re-infect them. The life-history of these spirochetes was found to involve transverse binary fission.

The occurrence and degree of infection of *Cristispira* spp. in a population of artificially-reared *C. virginica* was studied. Examination of styles and digestive contents indicates that, in these oysters, the occurrence of *Cristispira* spp. may be seasonal and releated to the reproductive cycle of the oysters.

Possible factors influencing the incidence of *Cristispira* spp. in bivalve molluscs are discussed.

ACKNOWLEDGEMENTS

A study such as this is seldom the undertaking of a single individual. The observations and the work are my own, but I must acknowledge the assistance and co-operation of others. I would like to express my sincere appreciation to my supervisor, Dr. George Moskovits of the Department of Biology, Memorial University of Newfoundland, under whose direction this work was done.

I would also like to thank Dr. F. A. Aldrich, Dean of Graduate Studies, Memorial University of Newfoundland, for suggesting the problem and for giving helpful information.

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INTRODUCTION

The crystalline style was first recorded in a species of Mytilus by de Heide (1683), who called it the "stylus crystallinus". Certes (1882) reported a large, spiral organism inhabiting the crystalline style of the oyster. Since that time, investigators such as J. Nelson (1890, 1891, 1892, 1893, 1904, 1905), Lustrac (1896), Perrin (1906), Dobell (1911), Berkeley (1923, 1935, 1933, 1959, 1962) and others have described these spiral microorganisms. Morton (1960) stated that, "The bacteriology of the crystalline style deserves much closer study. In many species of bivalves the style harbours a pure culture of a species of the spirochete genus Cristispira".

To date, little information has been published on the biology of these spiral microorganisms . Most of the available literature deals with their morphology. Nothing is known regarding the source of these spirochetes, the manner in which they gain entry into the molluscan host, or the nature of their relationship with the mollusc. These basic questions must be answered before any advance can be made in our knowledge of the biology of these microorganisms.

The purpose of the present investigation was to ascertain the source of the spirochetes and to determine their mode of transmission to bivalve molluscs.

Historical Review

Spirochetes in the crystalline style of bivalve molluscs were first recorded by Certes (1882) from oysters he studied at the Collège de France. Möbius (1883) reported that he had observed the organisms in 1869 in the styles of oysters from Schleswig-Holstein. Because of the possession of an undulating membrane, Certes thought that these organisms were trypanosomes, and consequently established the name Trypanosoma balbianii, in honour of his teacher and colleague at the Collège de France. Certes made numerous observations on the movement and structure of these organisms. He also mentioned longitudinal division as the normal method of multiplication. J. Nelson (1889, 1890) reported these spirochetes in Crassostrea virginica Gmelin taken from New Jersey. He called them "cytohelminths" because, even though they possessed an undulating membrane, he did not feel certain they were trypanosomes. In 1893, J. Nelson described rod-shaped granules or spores occurring in these microorganisms. Lustrac (1896) gave a more detailed account of longitudinal division and described the role of the membrane in multiplication. Laveran and Mesnil (1901) described the main characteristics of this organism and stated that it was more closely related to the bacteria, specifically to Spirochaetaceae and Spirilla, than to Trypanosomatidae. J. Nelson (1904, 1905) figured these bacteria with diffuse chromatin granules in bands (personal communication from Dr. F. A. Aldrich, from notes taken during a course on molluscs given by T. C. Nelson at Rutgers University, 1953). Perrin (1906) chose to

retain the organism which he referred to as a parasite, in the Trypanosomatidae and described in great detail a life history which included polymorphism, gamete formation, and encystment. Keysselitz (1906) discovered and described a new species, Spirochaeta anodonta, from the crystalline style of the freshwater clam, Anodonta mutabilis.

Fantham (1908) described the structure and movement of the spirochete from both the oyster and the freshwater clam, as well as their sites of infection. Gross (1910), at the Stazione Zoologica in Naples, placed both Trypanosoma balbianii and Spirochaeta anodonta in the genus Cristispira and described two new species, C. pectinis and C. interrogationis, both from Pecten jacobaeus L.

Bosanquet (1911) made observations on what he identified as S. anodonta and confirmed that these spiral organisms were bacteria. Dobell (1911) found a large species of Cristispira in the crystalline style of the clam, Venus (Meretrix) castra Chem., from the salt water Lake Tremblegan, Ceylon. Noguchi (1921) studied the spirochetes of the molluscs at Woods Hole, Massachusetts, and Long Island Sound, New York. He suggested that the classification of spirochetes found in the alimentary tracts of bivalve molluscs be based on the presence or absence of the crista, the shape of the cell ends, the width and the length of the cell, the spiral amplitude, and the width of the spiral.

More recently, Berkeley (1923, 1933, 1935, 1959, 1962) investigated the relationship of Cristispira to the crystalline style

of various molluscs and the possible factors determining the occurrence of these spirochetes in the style. Ryter and Pillot (1965) investigated the ultrastructure of C. balbianii.

The Crystalline Style - A Review

I. Adult Oyster

The crystalline style is found in all bivalve molluscs, although in some of the more primitive genera it is very small (Yonge, 1932). Form-wise, it is a tapered hyaline rod (Fig. 1) and when present always occupies the same relative position in the body. The style lies in a digestive diverticulum which is in free communication with the intestine via a common side. According to Yonge (1932) this is probably the primitive condition, although malacologists differ on this point in their interpretation of phylogeny. In some advanced bivalves the style sac becomes completely separated from the intestine during the development of the animal (Yonge, 1932).

In the oyster Crassostrea virginica, the style sac communicates with the intestine except at the entrance to the stomach where the two structures are separate. The style sac is slightly twisted around the midgut and occupies a somewhat dorsal position. Food particles which have been carried down the intestine by ciliary currents may enter the sac, become entangled in the substance of the style, and thus be carried back to the stomach (T. C. Nelson, 1918). Cilia lining the epithelium of the sac rotate the style on its axis, while

a tract of longer cilia push it forward into the stomach so that it rubs against the gastric shield and is slowly dissolved. This rotary motion was first observed by T. C. Nelson in 1918. The crystalline style is not a permanent structure. In oysters that have ceased feeding or have been taken from, and kept out of, water, the style dissolves in a short period of time. A number of theories concerning the role of the crystalline style have been put forth and are summarized by T. C. Nelson (1925). It is generally accepted today that the role of the style is that of a carrier of digestive enzymes, although its rotary motion probably aids the passage of food through the alimentary tract (Yonge, 1932).

The style shows two main regions. The outer one, incorporating approximately one-third of the diameter of the style, tends to be of a firm consistency, while the inner two-thirds are less viscous. When a style is cut transversely it is seen to be composed of a series of concentric layers (Fig. 2), found most clearly defined in the outer, firm area. Yonge (1932) attributed these concentric rings to the result of the rotary motion of the style during deposition.

The styles of some species are characteristically of a firmer consistency throughout than those of other species, with some genera possessing softer, less viscous styles. Generally speaking, the former have been produced in a separate style sac, the latter within a style sac connected with the intestine.

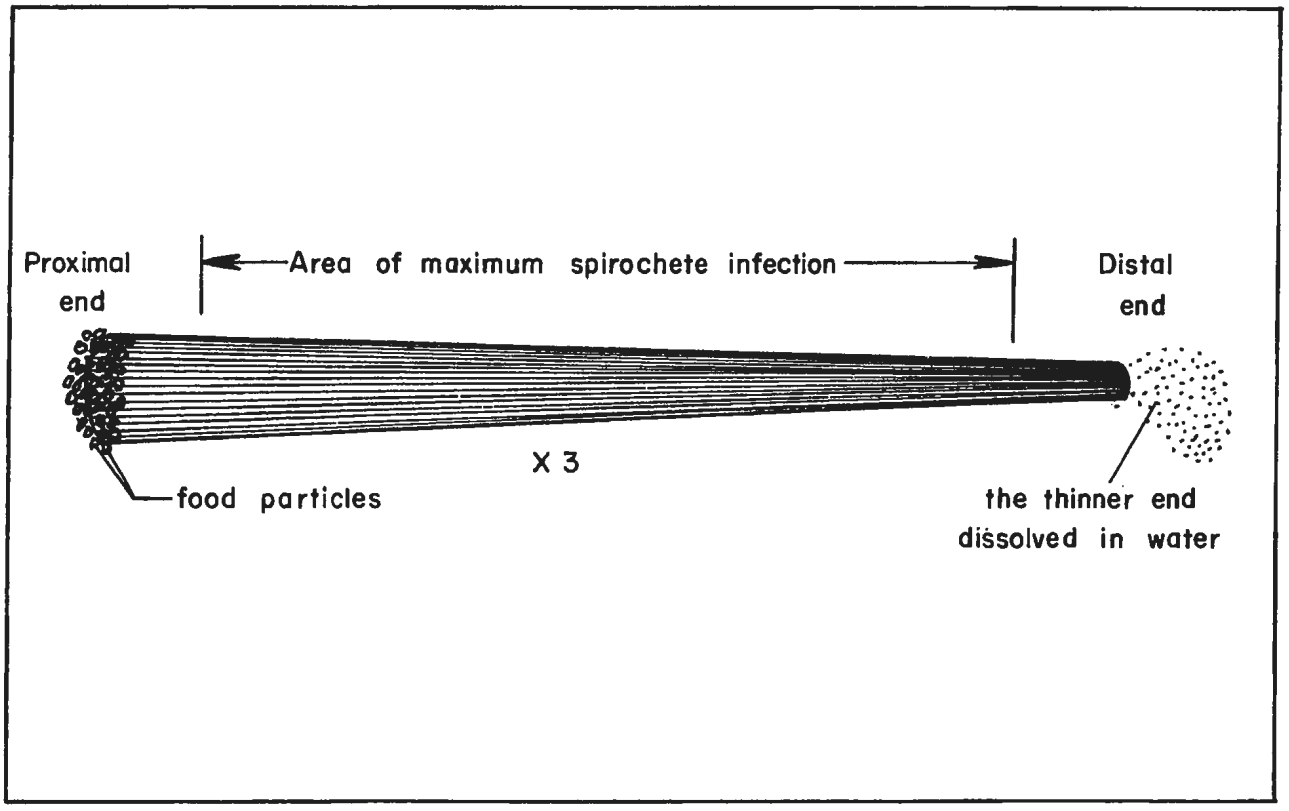


FIG. 1. DRAWING OF THE CRYSTALLINE STYLE OF Crassostrea virginica GMELIN.

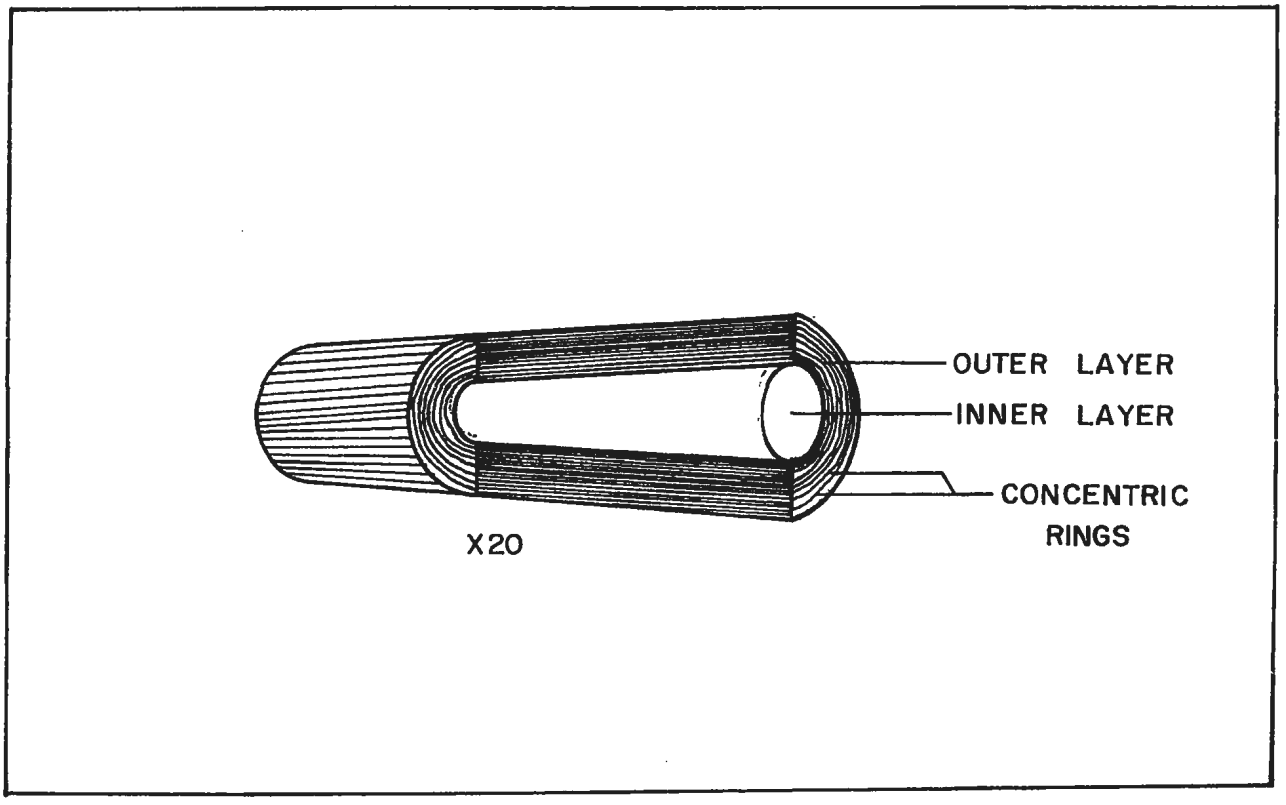


FIG. 2. DRAWING OF A CUT-AWAY SECTION OF THE CRYSTALLINE STYLE OF Crassostrea virginica SHOWING INNER AND OUTER LAYERS AND CONCENTRIC RINGS.

II. The Style in Young Oysters

The only account of the style in the young oyster *C. virginica* is that of T. C. Nelson (1925) who indicated that in an oyster 4 mm in diameter, the digestive organs were well developed and a distinct style was present. The style was comparatively short, thick, and transparent, showing the same twisted inner core as found in the adult. The style of a 7 mm oyster was about 1.2 mm in length and 0.3 mm in diameter at its widest point.

III. Chemical Composition

The presence of digestive enzymes in the crystalline style was first reported by Coupin (1900), who found an amylase and a weak invertase in the style of *Cardium* spp. This was confirmed by Mitra (1901), who found amylase and glycogenase in *Anodonta* spp., and by T. C. Nelson (1918), and Yonge (1932) who found amylase and glycogenase in the style of *Mya* spp. Berkeley (1923) reported an oxidase and a dehydrogenase in the styles of several bivalve molluscs. Analysis of the styles of *Cardium* spp. gave the following composition: water, 87.11%; solid organic matter, 12.03%; solid inorganic matter, 0.86% (Barrois, 1889, 1890). The organic component of the style was considered to be a globulin, with traces of a mucin or a chondrin-like substance. Berkeley (1935) demonstrated that the styles of *Crassostrea gigas* (Thunberg) *Mya arenaria* L., *Schizothaerus nuttallii* Conrad, and *Saxidomus giganteus* Deshayes, yielded on acid hydrolysis, in addition to protein: glucuronic

and sulphuric acids and a hexosamine, the essential constituents of both mucin and chondrin. Variations in the solubility and in the quantitative differences in the chemical composition of the styles suggested that the less readily soluble styles contained larger amounts of mucin (Berkeley, 1935).

The digestive enzymes of the crystalline style are liberated on solution. Of the enzymes present, amylase and glycogenase are the most active (Galtsoff, 1964). Mitra (1901) found that an aqueous solution of styles caused a rapid conversion of starch to sugar, with a dextrin-like intermediate product. He also indicated that the activity of the style solution toward glycogen was similar to that of ptyalin. Mitra (1901) thought the style represented a mass of enzymes, but T. C. Nelson (1918) advanced the view (which proved to be correct) that the enzymes are adsorbed on the surface of albuminoid substances.

MATERIALS AND METHODS

1. Field Investigations

(a) Locations Sampled. The investigations were carried out in Newfoundland, Nova Scotia, New Brunswick, and Prince Edward Island. Collection locales are given in Table 1.

(b) Molluscs Sampled. Seven different species of bivalve molluscs were collected and examined for the presence of *Cristispira* spp. in their crystalline styles and digestive tracts. *Mya arenaria* L. was collected from sandy, intertidal areas. *Mytilus edulis* L. was removed from rocks in the intertidal zones, usually near freshwater run-off. *Volsella (Modiolus) modiolus* L. was collected from a depth of 20 to 30 feet of water by diving. *Venus mercenaria* L. was dug from sand and mud bottoms. *Crassostrea virginica* was either dredged from shallow water or collected from sand bottoms by diving. *Ensis directus* Conrad was collected at low tide from sand bottoms. The numbers of each species examined are given in Table 1.

(c) Methods of Examination

(1) Style Removal. The right valve of the molluscs was removed with great care to avoid damaging the soft parts. This was done by prying the valves apart and carefully cutting the adductor muscles with a scalpel. Styles were extracted from an animal by making an incision into the stomach through the body wall. Slight pressure on the body caused the style to project through the incision (Fig. 3). In some

TABLE 1

Presence of *Cristispira* spp. in bivalve molluscs from various locales

Location Sampled	Mollusc	Average Water Temperature °C	Numbers		
			Examined	Style Present	*Cristispira* Present
Portugal Cove, Conception Bay, Nfld.	*Mytilus edulis*	7.0	11	10	0
	Volsella (Modiolus) modiolus		23	23	0
Witless Bay, Nfld.	*Mytilus edulis*	4.3	36	36	0
Bay Bulls, Nfld.	*Mytilus edulis*	4.5	22	19	0
Petty Harbour, Nfld.	*Mytilus edulis*	4.5	10	10	0
Salmonier Arm, Nfld.	*Mytilus edulis*	5.0	42	42	0
Swift Current, Placentia Bay, Nfld.	*Mytilus edulis*	8.2	10	10	0
	Mya arenaria		52	52	0
Bellevue Beach, Trinity Bay, Nfld.	*Crassostrea virginica*	8.0	42	31	34
St. Andrews, N.B.	*Mytilus edulis*	14.0	19	19	0
	Mya arenaria		34	34	0
	Pecten spp.		2	2	0
Sandy Point, N.B.	*Mytilus edulis*	14.5	7	7	0
	Volsella (Modiolus) modiolus		23	23	0

TABLE 1, continued

Location Sampled	Mollusc	Average Water Temperature °C	Numbers		
			Examined	Style Present	*Cristispira* Present
Oak Bay, N.B.	*Mya arenaria*	19.0	12	12	0
	Crassostrea virginica		4	4	4
Shediac, N.B.	*Venus mercenaria*	15.0	24	24	0
Green Park, P.E.I.	*Mytilus edulis*	24.7	20	20	0
	Mya arenaria		5	5	0
	Crassostrea virginica		63	62	47
Grand River, P.E.I.	*Mytilus edulis*	14.1	22	22	0
	Mya arenaria		12	12	0
Ellerslie, P.E.I.	*Volsella (Modiolus) modiolus*	12.3 - 27.3	15	15	0
	Mya arenaria		5	5	0
	Venus mercenaria		11	11	0
	Crassostrea virginica		427	381	317
Linkletter Beach, P.E.I.	*Ensis directus*	27.0	37	32	0
Cape John, N.S.	*Mytilus edulis*	18.2	15	15	0
	Crassostrea virginica		4	4	4

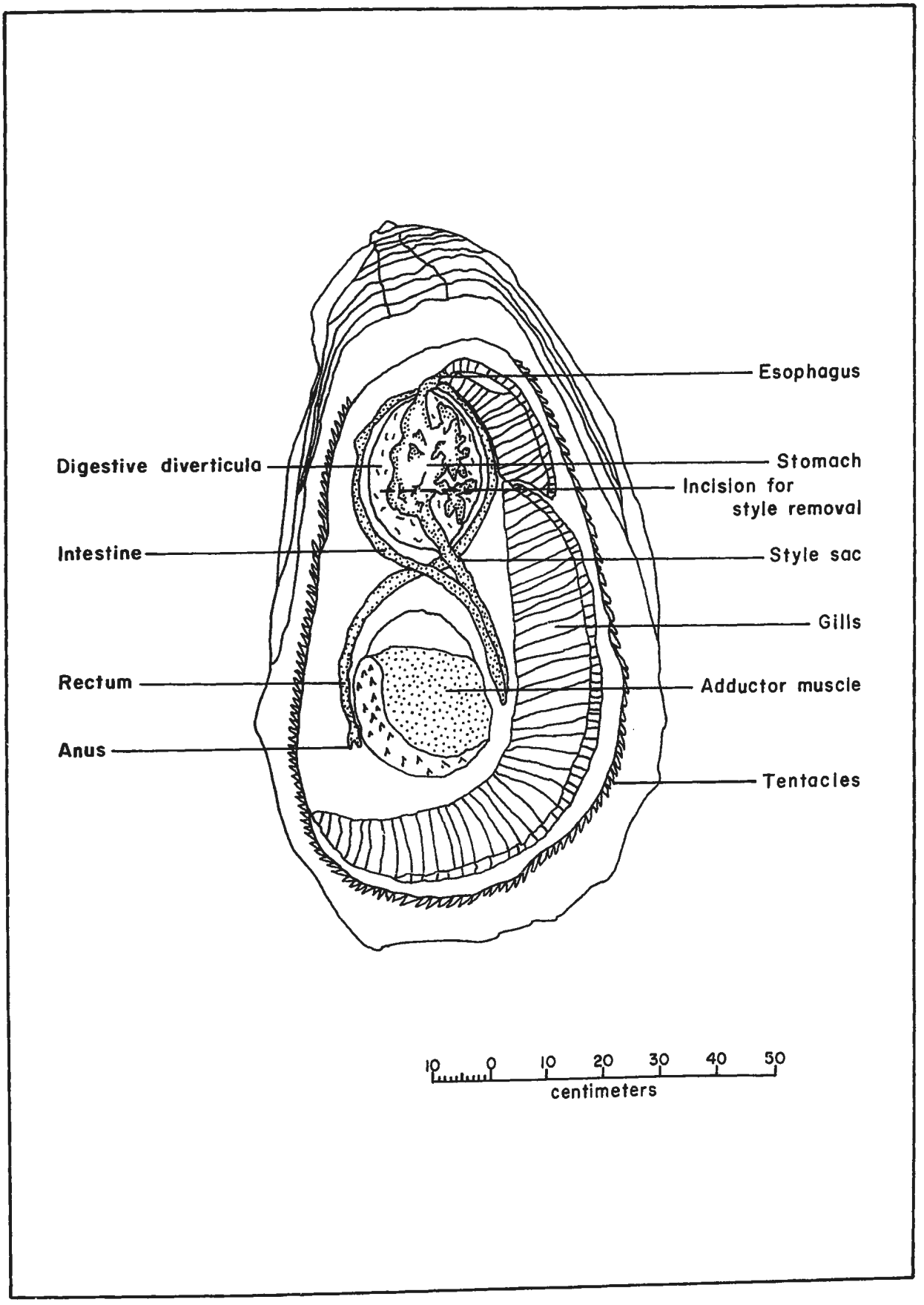


FIG. 3. DIGESTIVE SYSTEM OF *Crassostrea virginica*

cases it was necessary to probe for the style with forceps.

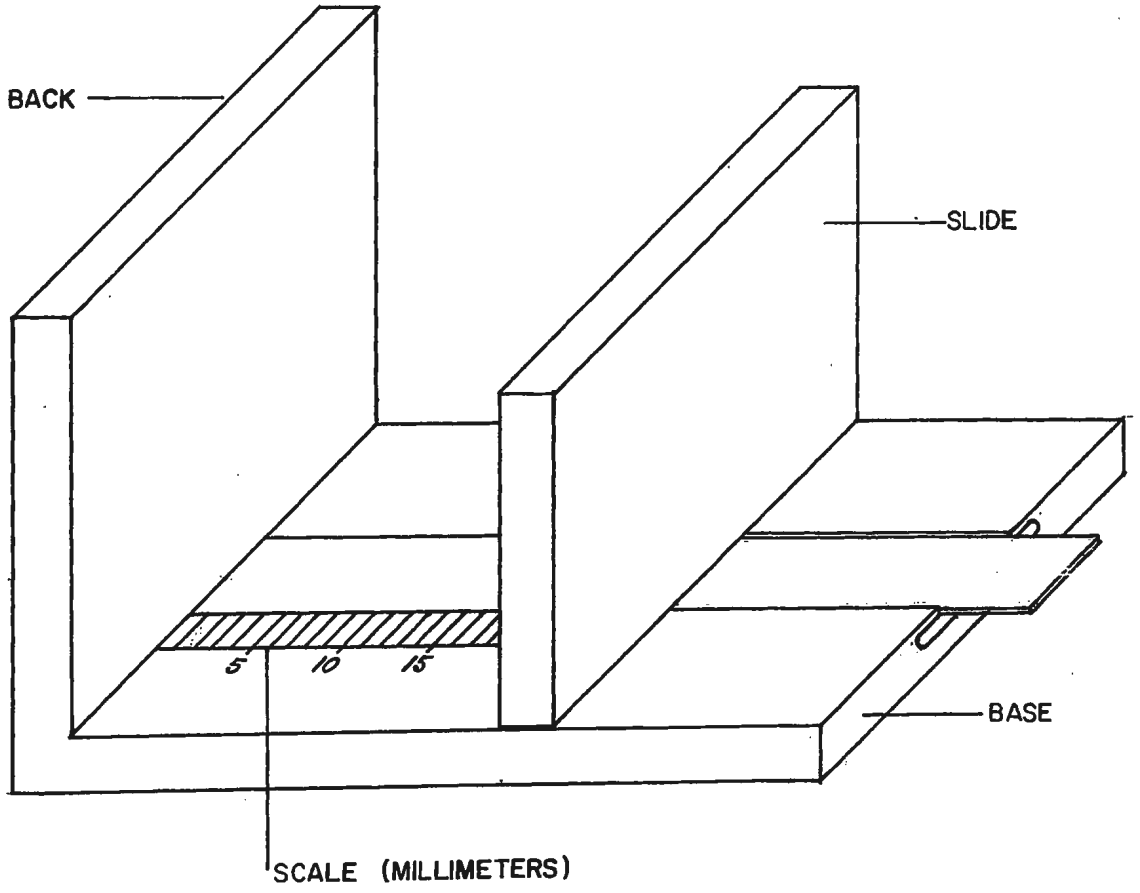
(ii) Examination. The anterior 2 or 3 mm (grinding end) was cut off to remove debris accumulated there. Further cleaning of the style was carried out by washing in saline. Unstained wet mounts in sea water were prepared. Where the styles were large and firm and did not dissolve readily, as in Mya arenaria and Volsella (Modiolus) modiolus, they were cut into small sections and crushed on the slide. The slides were examined for spirochetes with a Nikon model H field microscope at magnifications of 100x and 400x.

When no spirochetes were observed in the extracted styles, the stomach fluid was removed with a Pasteur pipette and examined.

(iii) Size measurements. Shell lengths and widths of the molluscs were measured before the organisms were opened. The measuring device (Fig. 4) used was designed by Mr. R. P. Scaplen of the Marine Sciences Research Laboratory, Memorial University of Newfoundland. Styles were measured on a glass slide with an attached millimeter scale (Table 3, Appendix).

(d) Other Parameters Investigated

Immediately prior to sampling a population of molluscs, water temperatures and salinities were determined. Water temperatures were taken with a centigrade thermometer as close as possible to the sampled population. Salinities were measured with a hydrometer.



NOTE:
This drawing is not to scale

FIG. 4. DEVICE USED FOR MEASURING SHELL LENGTH AND WIDTH OF BIVALVE MOLLUSCS.

Water and sediment samples were collected from Bellevue Beach, Newfoundland; Oak Bay, New Brunswick; and several locales in Prince Edward Island. These samples were collected as close as possible to the populations being sampled and returned to the laboratory for examination.

(e) Transportation of Molluscs to the Laboratory

Animals collected in the field were returned to the laboratory by placing them in plastic bags containing sea water. These bags were maintained at approximately 0°C by surrounding them with ice in a cooler chest.

2. Laboratory Investigations

(A) Examination of Style Material

(i) Live material. Wet mounts of freshly removed styles were prepared as for the field investigations. These were first examined under the dissecting microscope (80x) and then by phase contrast (100x, 400x and 600x). When more styles were available, five to ten of these styles were kept in boiled and cooled sea water in order to reduce the oxygen tension and maintain the spirochetes alive longer. The style suspension was then examined by phase contrast or fixed and stained.

(ii) Preparation of stained material. Perrin (1906), Noguchi (1921) and Dimitroff (1926) outlined a number of methods for fixing and staining these spirochetes. Several of their methods were tested

and evaluated. These procedures were as follows:

(a) Air-dried style smears were heat-fixed and stained with Ehrlich's hematoxylin for 15 to 30 minutes and washed in distilled water.

(b) Air-dried smears were heat-fixed and stained with Giemsa (pH 6.0) overnight, and rinsed in distilled water.

(c) Air-dried films were fixed with absolute methanol, stained with Ehrlich's hematoxylin for 15 to 30 minutes, and washed with distilled water.

(d) Air-dried films were fixed with absolute methanol for 30 minutes, stained with Giemsa (pH 6.0) overnight and washed in distilled water.

(e) Air-dried films were fixed with 5% glutaraldehyde in phosphate buffer (pH 7.4). Twenty ml of a 25% glutaraldehyde solution in sea water were added to 100 ml of 0.2 M phosphate buffer. Barium carbonate was added to precipitate glutarates and the solution filtered through a No. 2 Whatman filter. After fixation, the smears were stained with Ehrlich's hematoxylin or Giemsa, as before.

(B) Examination of Gut Contents

Whether or not spirochetes were found in the styles, the gut contents of the molluscs were also examined for Cristispira spp. This was done by flushing out the digestive tract, from the anus to the mouth, by the method of Savage (1925) as modified by G. Moskovits (personal communication).

The flushing apparatus is diagrammed in Figure 5. It consisted of a reservoir containing sea water connected to a B-D observation tube by a piece of rubber tubing. The side arm of the reservoir was connected to a source of compressed air. A breather tube enabled regulation of air pressure and therefore of water flow from the reservoir. One end of the observation tube was fitted with a 20 gauge hypodermic needle with the bevel ground off.

A mollusc was carefully opened and the right valve discarded. The organism in the remaining half shell was fastened to two glass supporting rods using spring clothespins so that the rectum was positioned in a superior position. The hypodermic needle was then inserted into the rectum. A trough-shaped piece of aluminum foil was then inserted between the viscera and a funnel to divert the flushed-out gut contents from the mouth into the collecting beaker. The rate of water flow from the reservoir through the digestive tract was maintained by finger pressure on the breather tube. The apparatus worked well in practice.

The gut contents were then examined by phase contrast microscopy. When spirochetes were not observed by this method, concentration by filtration through a 0.45 μ porosity membrane filter was carried out. The filters were stained with Ehrlich's hematoxylin for 15 minutes, washed with water, dehydrated in an ethanol series of 70%, 85%, 95%, 100% and 100% for two minutes in each concentration, air-dried and cleared with immersion oil.

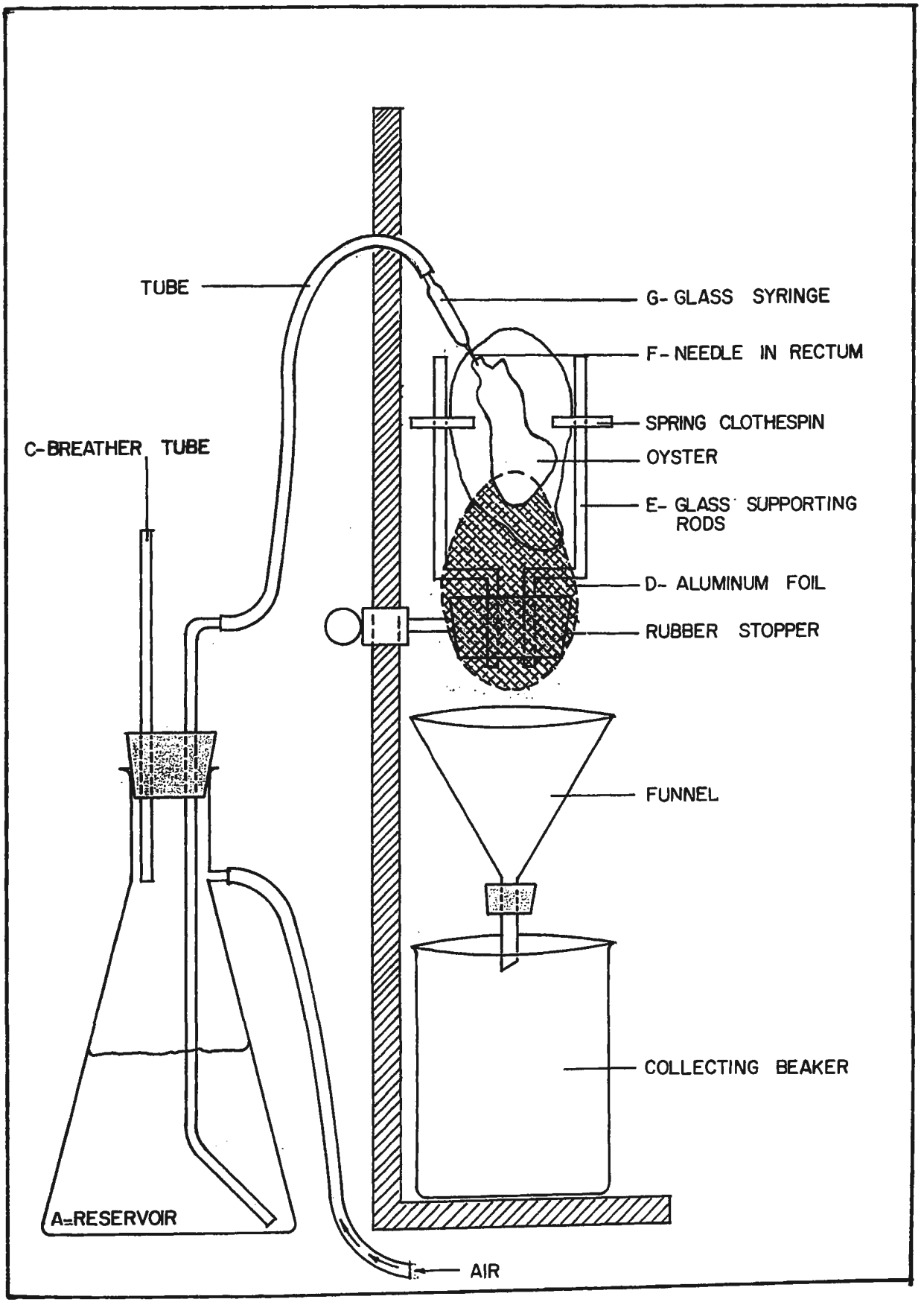


FIG. 5. APPARATUS USED FOR FLUSHING DIGESTIVE CONTENTS FROM BIVALVE MOLLUSCS.

(C) Examination of Sediment and Water Samples

Sediment and water, collected at the sampling sites, were examined for the presence of Cristispira spp. This was done to determine if these organisms could be found in the free-living state.

Fifteen grams of wet sediment were shaken in 25 ml of sea water for approximately 1 minute. After standing for 2 to 3 minutes, to allow the larger particles to settle, the supernatant was filtered through a 0.45 μ porosity membrane filter. The filter was then processed as in the examination of gut contents. Water samples were similarly treated. Examinations were also carried out by phase-contrast microscopy.

3. Experimental Work

The experimental work was carried out at the Fisheries Research Board of Canada, Oyster Unit, Ellerslie, Prince Edward Island. The oysters (C. virginica) used were taken from a population reared in tanks and out of contact with bottom sediments since being spawned. They were artifically reared by Mr. Roy Drinnan and were the off-spring of a pair of oysters he induced to spawn in 1967. Size-wise, they ranged from 4 to 10 cm in length. These oysters, held in tanks of non-sterilized running sea water filtered through a 60 μ core filter, were found to be heavily infected with the spirochetes. When experiments were carried out in closed systems, the oysters were contained in plastic trays each measuring 18" x 14" x 2 1/2", and

containing 8 liters of filtered (60 μ core filter) ultraviolet-sterilized sea water. To each tray 1500 ml of a mixed algal culture was added twice daily. Whether in closed or open systems, all experiments were carried out over a period of 11 days with examination at daily intervals, except where otherwise noted.

(A) Removal of Spirochetes

Three trays with 20 oysters each were prepared. The oysters in the first tray had been scrubbed with a wire brush and rinsed in ultraviolet-sterilized sea water to remove detritus adhering to the shells. Streptomycin was then added to the tray water to give a final concentration of 0.06 g/l. The oysters in the second tray were similarly treated, but no streptomycin was added to the water. The third tray contained unscrubbed oysters in sea water. No streptomycin had been added. Oysters from the original tank-reared population served as control organisms.

Two oysters from each tray were examined daily for the presence of Cristispira spp. in their styles or gut contents 45 minutes after feeding.

(B) Infection Experiments

Experiment 1: Twenty scrubbed, streptomycin-treated oysters were held in a tank of running sea water filtered through a 60 μ core filter and ultraviolet sterilized.

Experiment 2: Twenty streptomycin-treated oysters were held in a plastic tray of running, filtered, ultraviolet-sterilized sea water to which sediment from an outside oyster bed had been added.

Experiment 3: Five infected oysters, taken from the control population, were held with twenty streptomycin-treated oysters in a closed system of ultraviolet-sterilized sea water with aeration.

Experiment 4: A suspension of Cristispira spp. was made by dissolving the styles of five infected oysters in 20 ml of ultraviolet-sterilized sea water. Five ml of this suspension was added to twenty streptomycin-treated oysters, in a closed system, at the time of each algal culture feeding. Following administration of the suspension the oysters were examined at intervals of 0.5, 2 and 8 hours.

Experiment 5: Twenty streptomycin-treated oysters were relocated to the edge of an oyster population in four feet of water. They were placed unconfined on the bottom and the site marked with a stick.

Experiment 6: Twenty streptomycin-treated oysters were held in a tray of unfiltered, unsterilized running sea water.

(C) Examination of Oysters for Degree of Infection

Tank-reared oysters were examined at frequent intervals from May 21 to August 25, 1969, for the presence of Cristispira spp. in their styles or digestive tracts. Water temperature and salinity were

recorded prior to each examination and the degree of infection was recorded as none, light, or heavy. A Petroff-Hausser counting chamber was used, but accurate counts could not be obtained, so that the degrees of infection as given are only relative. Light infections were estimated at less than 50 spirochetes per style, medium infections from 50 to 200, and heavy infections more than 200 per style.

Larval oysters and newly set spat were examined directly under the light microscope and by phase-contrast. The spat were collected on glass microscope slides which had been cleaned and then soaked in sea water for three days. Spat settled readily on these slides, and this technique enabled their examination while still alive.

RESULTS

1. Field Investigations

Occurrence of Cristispira spp.

Of the seven different species of bivalve molluscs examined for the presence of Cristispira spp. in their crystalline styles and digestive tracts, only C. virginica was found to harbour these large spirochetes. Oysters examined in New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland, were found to be infected with apparently the same species of Cristispira. A rod-shaped bacterium was found in the styles of Mya arenaria, but the styles of the other molluscs examined were, by the methods used, found to be devoid of all bacteria. Data on the incidence of the spirochetes in the molluscs examined are found in Table 1.

2. Laboratory Investigations

(A) Examination of Live Material

Fresh styles of C. virginica, when examined by phase-contrast (600x), also showed large numbers of a small, spiral bacterium. These organisms could be observed in the styles of those oysters which were also infected with Cristispira spp.

The length of most of the Cristispira found in these oysters varied from 55-75 μ (average: 63.5) for living or unfixed specimens. Individuals as short as 30 μ also occurred, but only rarely. Widths ranged from 1 to 2 μ. The organisms had blunt rounded ends, toward

which the diameter gradually diminished. An undulating membranous structure, the crista, coiling obliquely and spirally along the cell was seen in unstained specimens under phase-contrast.

The organisms move in a characteristic corkscrew fashion, with the path of movement either in a straight line or more or less in a circle. Movement is by a series of "waves" or undulations, with characteristically six demonstrable apices along the cell. Lashing movements are also observed. In the case of slowly moving specimens, the organism moves forward while turning on its long axis. The movements occur in jerks. The organism may suddenly come to an abrupt halt, or just as suddenly proceed more slowly. The organism was capable of suddenly reversing its direction of movement and returning on its own path, with either end moving foremost.

These organisms are capable of very rapid movement. In a firm substrate such as the crystalline style, the organisms move more slowly. However, when retracing their paths of movement they are capable of fairly rapid motion. In freshly removed styles, tunnel-like tracings may be observed, and it is in these that the spirochetes are capable of rapid movement. In the more fluid inner core of the styles, spirochetes were observed to move very rapidly. As the style liquefied, the speed of movement of the spirochetes increased and, upon liberation to the surrounding sea water medium, the spirochetes moved away very rapidly. Movement in liquid media appeared to differ from that in

solid media; the spiral movement was not observable and the organisms appeared to move by undulating the whole body.

The species of Cristispira studied in this work reproduced by transverse binary fission. Upon reaching a length of 65 to 75 μ the organisms began to divide (as observed in a crystalline style under phase-contrast). The point of division of binary fission was at the third apex. At this point a light hyaline band appeared around the body. This stage lasted approximately 5 minutes, during which time the hyaline band became more clearly defined and two resultant organisms became visible. The spirochetes were able to move backwards as well as forwards up until the actual division. In most cases separation was accomplished by one half turning back upon the other. This was attempted several times before complete separation occurred. They frequently remained in this doubled-up state for some time, up to 20 minutes.

As soon as separation occurred, the two daughter cells intertwined and moved backwards and forwards against one another. In a few cases they separated and "paired up" with other cells of the same size; usually two organisms of the same size comprised a pair. The spirochetes often moved freely, paired, then moved individually again. Generation time was estimated to be 45 to 60 minutes.

(B) Examination of Stained Material

The cytoplasm of these organisms stained uniformly and was apparently homogeneous. Specimens stained with hematoxylin demonstrated

a blue cytoplasmic mass with the crista staining lighter blue, the outer edge of which, being thicker, stained more darkly. Cross striations appeared at regular intervals, seeming to divide the body into chambers or sections. This was most clearly observed in forms which were turned back upon themselves.

Of the five methods of fixation and staining utilized, those with methanol gave the best results. The formation of salt crystals from the sea water posed a problem in each method. Alcohol fixation caused slight shrinkage, whereas fixation by heat rendered the membrane indiscernable. Glutaraldehyde produced slight shrinkage, but salt crystal formation was especially bad by this method. With Giemsa's stain the cross striations were easily detected, but it did not stain the crista as well as hematoxylin. For routine staining, methanol and hematoxylin proved to be the best combination of fixative and stain.

(C) Examination of Molluscan Gut Contents

When large numbers of spirochetes were encountered in the gut, examination of the flushed contents was relatively easy. However, when concentration by membrane filtration was necessary, difficulty was encountered because of the large amounts of detritus present.

Spirochetes were found in the gut contents of all oysters whose styles were infected. Where styles were uninfected, no spirochetes were found in the gut content. Where well developed styles were found to be infected, relatively few Cristispira were found in the gut. In

oysters with developing styles, and especially in those whose styles had recently dissolved, large numbers of living Cristispira spp. were found in the stomach contents.

(D) Examination of Sediment and Water Samples

No spirochetes were observed in any of the sediment or water samples examined. Phase-contrast microscopy was used for the examination of sediment samples because the membrane filters became clogged and staining rendered examination almost impossible.

3. Experimental Work

(A) Removal of Spirochetes

Streptomycin proved effective, at the concentration used, for the removal of Cristispira spp. from C. virginica. The data, Table 2, show that no spirochetes were found in the oysters held in sea water with streptomycin after 1 day, while all other oysters, including the control organisms, remained infected. The small spiral organisms or spirilla found in the style were also removed by the streptomycin.

(B) Infection Experiments

Data for these experiments are given in Table 3.

Experiments 1 and 2: (Conditions repeated). No spirochetes or spirilla were observed in the oysters after 11 days.

Experiment 3: None of the streptomycin-treated oysters developed an infection with Cristispira spp. either in the style or gut. The

Key to Table 2

[1]Degree of infection indicated by figures:
0 - no infection
1 - light infection (less than 50 spirochetes per style)
2 - medium infection(50-200 spirochetes per style)
3 - heavy infection (more than 200 spirochetes per style)

[2]Two oysters examined each day

[3]Five oysters examined at time 0; two oysters examined each day thereafter.

[4]There were no styles in these oysters

Table 2

Effects of streptomycin on the degree of infection of *Cristispira* spp. in experimentally maintained *Crassostrea virginica*

Days following start of experiment	Degree of Infection[1]			
	Streptomycin (0.062 g/l)[2] in sea water	Scrubbed oysters[2] in sea water	Unscrubbed oysters[2] in sea water	Control oysters[3]
0	-	2	-	3
1	0	2	3	3
2	0	3	3	3
3	0	3	3	3
4	0	3	3	3
5	0	2	3	3
6	0	3	2	3
7	0	3	3	0[4]
8	0	2	3	3
9	0	3	3	3
10	0	3	3	3

Key to Table 3

[1]Experiments: (1) Streptomycin-treated oysters in filtered, ultraviolet-sterilized sea water.
(2) Streptomycin-treated oysters in filtered, ultraviolet-sterilized sea water plus sediment.
(3) Streptomycin-treated oysters in filtered, ultraviolet-sterilized sea water held with five infected oysters.
(4) Streptomycin-treated oysters in filtered, ultraviolet-sterilized sea water; style suspension added daily.
(5) Streptomycin-treated oysters relocated to outside oyster bed.
(6) Streptomycin-treated oysters in running, raw sea water.
Controls are oysters from the original tank-reared population.

* 0 - no infection
1 - light infection (less than 50 spirochetes per style)
2 - medium infection (50-200 spirochetes per style)
3 - heavy infection (more than 200 spirochetes per style)

** Two oysters examined each day.

*** One oyster examined prior to feeding, 0.5, 2, and 8 hours after feeding and addition of style suspension for first three days, one oyster prior to feeding, and one at êight hours thereafter.

† Five oysters examined at time 0; two oysters examined each day thereafter.

†† The five infected oysters were found to have a medium infection at this time.

Table 3

Experimentally induced infection of Crassostrea virginica by Cristispira spp.

Days following start of experiment	Degree of Infection*									
	Experiment[1]									
	1**	2**	3**	4***				5**	6**	Control Oysters
				prior to feeding	0.5 hr.	2 hr.	8 hr.			
0	-	-	-	0	0	0	0	-	-	3†
1	0	0	0	0	0	1	1	0	0	3
2	0	0	0	0	0	1	1	0	0	3
3	0	0	0	0	0	1	2	0	0	3
4	0	0	0	0	-	-	1	0	0	3
5	0	0	0	0	-	-	1	0	0	3
6	0	0	0	0	-	-	2	0	0	3
7	0	0	0	0	-	-	1	0	0	3
8	0	0	0	0	-	1	1	0	0	3
9	0	0	0	-	-	-	-	0	0	3
10	0	0	0††	-	-	-	-	0	0	3

five infected oysters from the original population examined after 11 days remained infected with both Cristispira spp. and spirilla.

Experiment 4: After feeding and addition of the style suspension, examination of the experimental oysters was carried out at 0.5, 2 and 8 hours. These examinations revealed light infections of Cristispira spp. When examined the following morning, prior to feeding, no infection could be observed. A new suspension of infected style material had to be added each day to maintain the infection. Generally, the longer the period of time which elapsed between addition of the suspension and examination, the greater was the degree of infection. Again, no spirilla were found in any of the streptomycin-treated oysters.

Experiment 5: No spirochetes were observed in any of the streptomycin-treated oysters relocated to the outside.

Experiment 6: Examination of these oysters proved negative for both spirochetes and spirilla.

(C) Examination of Tank-Reared Oysters for Degree of Infection

Table 2 (Appendix) presents temperature, salinity, and infection data for the population of tank-reared oysters examined from 21 May to 25 August, 1969. No infection of Cristispira spp. was found until 7 June when the water temperature reached 17.9 C, at which time slight infections were observed. By 21 June, when the water temperature reached 20.3 C, medium infections were observed. On 24 June, the water temperature was 20.7 C and the first heavy infection was encountered. From 27 June

(water temperature: 20.2 C), until the end of the observation period, the infections remained heavy.

Large numbers of the small spirilla mentioned previously were not observed until shortly before the first slight spirochete infection was detected. These organisms appeared quite suddenly, in the styles, on 4 June 1969.

No infections with spirochetes were observed in wild populations of oysters examined in the field (Ellerslie, Green Park, and Grand River, P.E.I.), until several days after infections were observed in the laboratory.

No spirochetes were observed in larval oysters or newly set spat. Styles could only be recovered from oysters 7 mm long or larger. These had the same degree of infection as the styles of larger oysters examined at the same time.

DISCUSSION

The results of the present study differ in some respects from those published previously. *Cristispira* spp. have been recorded from a large number of bivalve molluscs (Table 1, Appendix), but the results of this investigation indicate that only *Crassostrea virginica* was infected.

This may in part be attributed to the small numbers of some of the species examined. However, this seems unlikely in cases such as the clam, *Venus mercenaria*, which was collected in close proximity to populations of infected oysters. No spirochetes were observed in the 35 clams examined.

Accurate counts of the spirochetes were unobtainable because these organisms moved very rapidly and because facilities for measuring accurate volumes of small amounts of style material were not available in Prince Edward Island. Thus, the numbers obtained with the Petroff-Hausser counting chamber were not considered to be reliable. Artificial cultivation of these microorganisms would greatly facilitate the determination of their numbers in bivalve molluscs.

It was suggested by Noguchi (1921) and Edmonson (1920) that the degree of solidity of the crystalline style might be partly or chiefly responsible for the presence or absence of *Cristispira* spp. in bivalve molluscs. Indeed, it seems that oysters (*C. virginica*) offer the optimum

conditions for the habitation of the organism and in fact possess the softest styles of the molluscs examined.

The softness of the style of a mollusc depends upon the type of style sac it possesses. As mentioned, those styles formed in incompletely-separated style sacs are softer than those formed in a style sac completely separated from the intestine. It is reasonable, assuming that the route of infection is either through the mouth or anus, that the style lying in a sac which is incompletely separated would be most accessible to spirochete infections than one which has no connection with the gut except where the style projects into the stomach. This agrees well with the data collected on the various molluscs examined, and it may well prove that both style consistency and accessibility are factors governing the occurrence of these spirochetes.

The chemical composition of the different styles and their action on various food elements may also play a role in the occurrence of Cristispira spp. The latter seems more probable, since Bailey and Warbouys (1960) and Berkeley (1935) found in their analyses that the styles of the different species of bivalves do not differ greatly except in the amount of mucin present. In addition, even in the styles of C. virginica, none or extremely few spirochetes are ever found in the anterior 2 or 3 mm of a fully formed and functioning style. Physical forces resulting

from the rotary motion of the style may be responsible for this repulsion of spirochetes. Berkeley (1933) has suggested that some factor, resulting from oxidation of food material, might be responsible for the lack of Cristispira spp. at the head of the style. For example, he stated that the only substance which has been recognized as probably resulting from the oxidizing activity of the crystalline style system is glucosone, obtained by reacting it with glucose. Berkeley found that the addition of 0.5% of glucosone to an active suspension of Cristispira spp. killed the organisms in 1 hour. He further stated that the production of glucosone by the united action of food material and style has been found to operate only in the presence of glucose as substrate, and there is no evidence of the breakdown of complex polysaccharides to the hexose stage by style activity. Yonge (1926) found that among those he studied (including pectin, glycogen, starch, lactose, maltose, raffinose, sucrose, and cellulose), only starch and glycogen were degraded to the hexose stage by the style of Ostrea edulis. Lavine (1946) in Mya arenaria and Mactra solidissima Dillwyn, and Newell (1953) in Ostrea edulis L. and Mytilus edulis found that the style enzymes degraded cellulose, and the former found glucose in the digestive contents after several days. It is conceivable, therefore, that the action of the style enzymes of some molluscs produce substances which are toxic to Cristispira spp. These toxic substances may be responsible for the lack of spirochetes in certain species of bivalves.

The molluscs that were studied intensely in this work were those which had been tank-reared at Ellerslie, Prince Edward Island. When these oysters were first examined they were found to be devoid of Cristispira. When the temperature approached 18 C the spirochetes appeared quite suddenly, and their numbers increased (Table 2, Appendix). These observations led to the belief that there was a direct relationship between the presence of Cristispira spp. and temperature. However, since the oysters at Bellevue Beach, Newfoundland, collected from waters at 8 C and maintained under similar laboratory conditions, were infected with spirochetes, other possible explanations were sought.

Temperature affects the life of the oyster by controlling the rate of water transport, feeding, respiration, gonad formation and spawning. It is conceivable that one or more of these factors may influence the presence or absence of Cristispira spp. The rate of water transport, feeding, and respiration can possibly be ruled out because the Bellevue Beach oysters were infected with Cristispira spp., even though these factors were reduced as compared to those for oysters from Prince Edward Island. The remaining two factors, gonad formation and spawning, have a definite relation with stored tissue glycogen, and therefore have some possible significance in the occurrence of Cristispira spp.

The gonads of oysters from Prince Edward Island begin to develop in the temperature range of 16 to 18 C, and spawning occurs at 22 to 24 C. This is precisely the temperature range at which Cristispira spp. was first

observed in these oysters. The oysters with the heaviest population of spirochetes also appeared to have the most well developed gonads. J. Nelson (1891) also recorded similar observations. This leads to the suggestion that glycogen content of the oysters may have some significance concerning the presence of Cristispira spp.

If this is correct, then the lack of the spirochetes before gonad ripening may be due to glucosone formation, as mentioned before. Kuhn, Lasnik, and Rubenstein (1968), in an abstract of their work, reported no seasonality in the occurrence of Cristispira spp. There was no reply to correspondence sent them. Other workers, Perrin (1906) and Berkeley (1959), have made similar observations. However, the molluscs examined by them were taken from warmer waters that do not have the great seasonal fluctuations encountered in oyster areas of eastern Canada. Oysters from warmer waters feed all year and are known to spawn several times during the year. They do not require large amounts of stored glycogen for reserve food material. Consequently, the glycogen content of their tissues is not high over a long period of time. This may account for the lack of seasonality reported.

The ability to accumulate various elements present in sea water at very low concentrations is common to many marine invertebrates. Of particular interest is the ability of many bivalves to accumulate heavy metals such as zinc, copper, manganese, lead, and arsenic. The concentration of these metals was found to be seasonal in oysters taken from Long Island Sound, being highest in summer and declining in the fall and winter (Galtsoff, 1964). The concentrations of these heavy metals

could possibly have some relationship to the presence of spirochetes in bivalve molluscs and could warrant investigation.

Another possible explanation for the presence or absence of Cristispira lies in the observation that in all cases, except the experimentally infected oysters, a thin spiral microorganism was found associated with Cristispira in the styles of oysters. This spiral organism may be Spirillum ostreae which Noguchi (1921) first reported, although he did not find it in all cases of spirochete infections. It has been found that some strains of spirochetes are nutritionally dependent upon the bacteria with which they are normally associated. Evidence to this effect has long been available (Rosebury and Foley, 1941; Wichelhausen and Wichelhausen, 1942; Kast and Kolmer, 1940; Neven, Hamp, and Dewey, 1960; Hardy, Lee, and Nell, 1963) and this may well be the case with Cristispira spp. These spirilla may be subject to the above environmental and physiological conditions, and their presence may prove to be the factor controlling the occurrence of Cristispira spp.

The results of the experiments involving removal of spirochetes from infected oysters with streptomycin and subsequent attempts at re-infection lend some possible support to this last suggestion. In addition to removing the spirochetes the antibiotic also removed all other micro-organisms associated with the style. The more highly motile Cristispira spp. were able to re-infect the style but were unable to maintain the infection. This was perhaps due to the absence of the smaller spirilla

which may not have been able to gain entry into the style. If this is true, it would explain why no infection was found in oysters which were relocated to the outside or placed in trays with infected oysters.

Berkeley (1959), working with Saxidomus giganteus, studied the relationship of Cristispira spp. to the regeneration of the crystalline style. He found that normal styles were developed 48 hours after the molluscs were returned to a normal environment, but that few, if any, spirochetes were present at this stage. It took about two weeks for the population of spirochetes to return to normal. The oysters used in the present work developed complete styles in 20 to 30 minutes after feeding.

When the oysters were not treated with streptomycin, both spirochetes and the other spiral microorganisms reappeared in normal numbers when the style was fully formed. The styles of streptomycin-treated oysters became infected only if a suspension of spirochetes was added to the sea water in which they were held, and this infection was of short duration. No spirochetes were observed in the gut contents of the oysters treated with antibiotics, and this suggests that, in nature, Cristispira spp. remains in the gut of the oysters after the style disappears and that re-infection was not possible.

It is possible that the experimentally induced infection could not be maintained because the lack of the small spirillum, as discussed

previously, may have prevented the establishment of the infection. A second explanation is that enough streptomycin may have remained in the tissues of the oysters to prevent the habitation of the style by the spirochetes. Also, the infective dose may have been too low and the condition of the oysters may not have been such as to allow infection to occur.

It appears, however, that infection of the style is initiated by spirochetes which are already in the digestive tract. Whether or not Cristispira spp. remain in the digestive tracts of bivalve molluscs for a long period of time in a morphologically different form, or whether they are reintroduced by the feeding habits of the mollusc is open to speculation. The former suggestion is unlikely, however, since no encysting or comparable stage was ever observed during the growth and division of the microorganisms, or in dying spirochetes. Since the oysters reared in tanks had never come in contact with bottom sediments, indications are that infection takes place directly from the sea, or from close contact with oysters already infected.

Discussion of Reproduction

The method of division in spirochetes is generally accepted to be transverse. Bergey's Manual (7th edition) records no method of division for the genus Cristispira and there has been much dispute as to whether it is transverse or longitudinal. Laveran and Mesnil (1901)

maintained it was transverse, while Certes (1882), Lustrac (1896) and Fantham (1908) found that both modes of division occurred. Fantham (1908) reported longitudinal division as the usual method and that division usually occurred in the crystalline style, but also less frequently in the gut.

Only transverse division was observed in the species of Cristispira studied in the present work. As mentioned, just prior to the separation the daughter cells bend back across one another and often remain in this position for quite a long time. Possibly, this position may have led other investigators to assume longitudinal division occurred. Fantham (1908) states, "the daughter cells resulting from fission remain attached to one end, often for a long time". It is conceivable that even such astute observers as Certes, Perrin and Fantham could have mistaken organisms in this position to be completing longitudinal division. Indeed, were it not for the fact that the whole process was observed under the microscope in this study, it would be easy to assume that longitudinal division took place.

Fantham (1908) observed somewhat long spirochetes with the membrane discontinuous in the center, where a vacuole-like space occurred; the edges of this space were sharp, not torn, while the periplast appeared just continuous over the gap. These long forms were observed in the present study just prior to division.

The significance of the "pairing up" phenomenon which was observed to take place between daughter cells, and other cells of the same dimensions, is not at present understood. Possibly, it is what Perrin (1906) referred to as conjugation. No polymorphism was observed in any of the hundreds of specimens examined, and it is felt that Perrin's male, female, and indifferent forms merely represent differences within the limits of variation. No evidence of encystment was found in this investigation, and the life cycle of these organisms appears to involve only growth and division.

Specific Identity of Cristispira Studied

Seven species of Cristispira, all from Crassostrea virginica, are described by Dimitroff (1926). He found that the type species, C. balbianii (Certes) Gross (Appendix I) occurred most commonly and regarded two others, C. pectinis Gross and C. veneris Dobell as synonyms of C. balbianii. Dimitroff's descriptions are summarized by Bergey (1957) and these are inadequately described, as also are a number of other species in the older literature.

Classification of these microorganisms has been based on mainly: (a) body length, (b) body width, (c) distance between the apices of the waves, (d) depth of the waves, and (e) number of turns of the crista around the body length. The body length of most of the Cristispira found in the style of C. virginica vary from 55 to 75 μ. The width varies from 1.0 to 2.0 μ. The number of waves varied from 2 to 6, depending on the

fluidity of the medium. The depth of the waves and the number of turns of the crista around the body were too variable for satisfactory determination.

The characters of the Cristispira found in C. virginica, so far as determined, agree most closely with the descriptions of either C. balbianii or C. modiolae (Schellack) Noguchi, two species that seem to come so close as to bring them within the probable limits of variation (Berkeley, 1959). This seems to apply to the descriptions of many of the species given in the literature, and separation of valid species in the group will remain difficult until methods of artificial culture can be devised. Under these circumstances, it seems best to be non-committal as to the species dealt with in this report.

CONCLUSIONS

1. Infection of bivalve molluscs by spirochetes of the genus Cristispira appears to take place from the sea.

2. The mode of infection is most likely from mollusc to mollusc either through the mouth or anus, with infection of the style initiated by spirochetes already in the digestive tract.

3. Multiplication in Cristispira spp. is definitely transverse, involving only growth and division.

4. The occurrence of Cristispira spp. in Prince Edward Island oysters is seasonal and seems to correspond with the spawning of these oysters.

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APPENDICES

APPENDIX I

The classification of bacteria of the type species of the genus Cristispira, as found in Bergey's Manual of Determinative Bacteriology, 7th ed., is as follows:

Division: Prophyta

Class: Schizomycetes

Order: Spirochaetales

Family: Spirochaetaceae

Genus: Cristispira

Species: C. balbianii (Certes) Gross

APPENDIX II

Appendix Table 1

Bivalve molluscs from which *Cristispira* spp. has been recorded

Crassostrea virginica	Noguchi (1921)
Ostrea edulis	Certes (1882), Fantham (1908)
Crassostrea gigas	Berkeley (1959)
Saxidomus giganteus	Berkeley (1959)
Paphia staminea	Berkeley (1959)
Venus castra	Dobell (1911)
Venus (Mercenaria) mercenaria	Noguchi (1921)
Pecten jacobaeus	Gross (1910)
Volsella (Modiolus) modiolus	Noguchi (1921)
Anodonta mutabilis	Keysselitz (1906)
Anodonta cygnea	Fantham (1908)
Macoma spp.	Rittenberg (unpublished, from Berkeley, 1959)
Tivela stultorum	Rittenberg (unpublished, from Berkeley, 1959)
Solen spp.	Rittenberg (unpublished, from Berkeley, 1959)
Soletellina acuminata	Dobell (1911)
Venerupis japonica	Berkeley (personal communication)
Ostrea lurida	Berkeley (personal communication)
Cryptomya californica	Berkeley (personal communication)
Entodesma saxicola	Berkeley (personal communication)

Appendix Table 1, continued

Composmyax subdiaphena	Berkeley (personal communication)
Pecten caurinus	Berkeley (personal communication)
Serripes groenlandicus	Berkeley (personal communication)
Bankia setacea	Berkeley (personal communication)
Ostrea belcheri	Laird (1961)

Appendix Table 2

Occurrence of *Cristispira* spp. in a population of tank-reared oysters at Ellerslie, Prince Edward Island, 1969

Date	Temp. °C	Salinity/oo	No. exam.	No. with Styles	No. with *Cristispira*	Degree of Infection
May 1	5.0	25.1	-	-	-	-
2	5.8	26.7	-	-	-	-
3	5.2	22.3	-	-	-	-
4	5.3	25.3	-	-	-	-
5	5.0	25.9	-	-	-	-
6	4.8	25.9	-	-	-	-
7	5.2	26.5	-	-	-	-
8	6.0	21.1	-	-	-	-
9	7.3	24.1	-	-	-	-
10	8.3	23.9	-	-	-	-
11	9.0	24.0	-	-	-	-
12	9.9	23.3	-	-	-	-
13	9.6	22.7	-	-	-	-
14	9.9	24.0	-	-	-	-
15	10.9	24.7	-	-	-	-
16	11.2	22.4	-	-	-	-
17	12.7	27.0	-	-	-	-
18	11.4	26.1	-	-	-	-
19	11.3	25.6	-	-	-	-
20	13.0	25.0	-	-	-	-

Appendix Table 2, continued

Date	Temp. °C	Salinity/oo	No. exam.	No. with Styles	No. with Cristispira	Degree of Infection
May 21	12.4	24.9	5	5	0	-
22	12.0	21.0	4	2	0	-
23	12.2	22.0	5	5	0	-
24	12.3	21.3	-	-	-	-
25	13.0	23.5	-	-	-	-
26	12.3	25.3	5	5	0	-
27	12.0	24.4	5	5	0	-
28	12.1	25.8	5	5	0	-
29	12.5	27.8	5	5	0	-
30	11.9	26.9	5	5	0	-
31	12.7	28.1	-	-	-	-
June 1	12.7	27.0	-	-	-	-
2	13.8	26.2	5	5	0	-
3	14.6	26.8	5	5	0	-
4	17.3	26.7	5	3	0	-
5	17.2	26.4	5	4	0	-
6	17.0	26.6	5	5	0	-
7	17.9	25.6	5	5	4	slight
8	18.0	25.9	-	-	-	-
9	19.5	25.4	10	10	6	slight
10	18.1	26.2	5	3	4	slight
11	18.6	26.0	5	2	3	slight

Appendix Table 2, continued

Date	Temp. °C	Salinity/oo	No. exam.	No. with Styles	No. with *Cristispira*	Degree of Infection
June 12	20.3	26.1	6	0	2	slight
13	22.0	24.8	5	4	4	slight
14	22.9	26.5	-	-	-	-
15	21.3	26.3	-	-	-	- -
16	19.2	26.3	3	0	1	slight
17	18.3	22.7	4	1	2	slight
18	19.1	20.6	6	6	6	slight
19	20.7	26.0	3	3	3	slight
20	20.0	26.2	5	5	5	slight
21	20.3	26.4	5	5	5	2 slight 3 medium
22	20.1	26.6	-	-	-	-
23	20.4	25.7	10	10	10	slight to medium
24	20.7	26.1	8	8	8	heavy
25	19.4	26.0	10	10	10	6 heavy, 4 light,
26	18.5	26.6	5	5	5	1 medium 4 heavy
27	20.2	26.6	5	5	5	heavy
28	22.7	26.8	10	8	10	heavy
29	22.1	26.6	-	-	-	-
30	22.3	27.2	6	6	6	heavy

Appendix Table 2, continued

Date	Temp. °C	Salinity/oo	No. exam.	No. with Styles	No. with *Cristispira*	Degree of Infection
July 1	22.0	26.3	5	5	5	heavy
2	21.5	26.5	5	5	5	heavy
3	22.2	26.5	5	5	5	heavy
4	22.8	26.8	5	5	5	heavy
5	22.0	26.4	-	-	-	-
6	20.7	26.2	-	-	-	-
7	19.5	25.8	5	5	5	heavy
8	19.3	26.5	-	-	-	-
9	19.4	26.3	10	7	7	heavy
10	21.9	26.7	6	4	6	heavy
11	20.8	26.7	10	10	10	heavy
12	19.9	26.7	-	-	-	-
13	19.3	26.2	-	-	-	-
14	18.1	26.0	15	13	15	heavy
15	17.4	25.8	5	4	4	heavy
16	19.6	23.7	5	5	5	heavy
17	21.3	26.8	7	7	7	heavy
18	21.3	26.0	5	4	4	heavy
19	20.6	26.5	-	-	-	-
20	18.8	26.8	-	-	-	-
21	21.8	26.7	-	-	-	-
22	21.5	27.0	-	-	-	-

Appendix Table 2, continued

Date	Temp. °C	Salinity/oo	No. exam.	No. with Styles	No. with Cristispira	Degree of Infection
July 23	22.9	26.5	-	-	-	-
24	22.9	26.4	-	-	-	-
25	24.0	26.9	-	-	-	-
26	24.3	26.5	-	-	-	-
27	22.3	26.1	-	-	-	-
28	20.8	25.6	-	-	-	-
29	21.1	26.7	10	10	10	heavy
30	22.6	26.4	5	5	5	heavy
31	23.8	26.4	5	5	5	heavy
Aug. 1	23.5	26.8	4	4	4	heavy
2	23.7	27.2	5	5	5	heavy
3	24.1	27.3	-	-	-	-
4	22.7	27.2	5	5	5	heavy
5	22.8	27.0	8	7	8	heavy
6	21.8	26.4	9	8	9	heavy
7	22.0	26.2	10	10	10	heavy
8	22.1	26.4	8	8	8	heavy
9	21.6	26.9	6	6	6	heavy
10	22.3	26.6	-	-	-	-
11	22.7	27.3	5	5	5	heavy
12	23.5	26.8	4	4	4	heavy
13	23.3	26.7	-	-	-	1 heavy 3 mediu

Appendix Table 2, continued

Date	Temp. °C	Salinity/oo	No. exam.	No. with Styles	No. with *Cristispira*	Degree of Infection
Aug. 14	21.4	27.0	4	4	4	heavy
15	21.3	27.0	7	5	7	-
16	21.3	26.8	-	-	-	-
17	22.0	27.3	-	-	-	2 heavy, 1 medium
18	22.6	26.8	3	2	3	heavy
19	22.6	27.2	5	5	5	heavy
20	-	-	5	5	5	heavy
21	-	-	11	10	11	heavy
22	-	-	5	5	5	heavy
23	-	-	2	2	2	heavy
24	-	-	2	2	2	heavy
25	-	-	2	2	2	heavy
TOTALS			398	358	312	

Appendix Table 3

Mean shell length, width, and style length of bivalve molluscs examined for *Cristispira* spp.

Organism	Source	Shell Length (cm)	Shell Width (cm)	Style Length (cm)
Crassostrea virginica	St. Andrews, New Brunswick	8.0	5.1	3.2
	Bellevue Beach, Newfoundland	7.3	4.9	3.3
	Tanks at Ellerslie, Prince Edward Island	6.4	3.7	3.1
	Ellerslie, Prince Edward Island	9.6	6.3	4.7
	Cape John, Nova Scotia	14.3	9.1	4.7
Mytilus edulis	Newfoundland	4.3	2.6	1.7
	New Brunswick	5.2	3.6	2.4
	Nova Scotia	7.2	4.2	3.6
	Prince Edward Island	4.7	2.9	2.0
Volsella (Modiolus) modiolus	Newfoundland	13.0	7.2	5.3
	New Brunswick	11.9	6.1	4.2
	Prince Edward Island	10.5	6.4	3.6
Ensis directus	Prince Edward Island	12.6	4.2	3.9
Venus mercenaria	New Brunswick	6.1	4.6	3.8
	Prince Edward Island	7.3	5.6	4.1
Mya arenaria	Newfoundland	7.5	3.3	2.7
	New Brunswick	9.2	4.6	3.8
	Prince Edward Island	5.1	2.6	1.8
Pecten spp.	Newfoundland	about 12 cm in diameter		
	New Brunswick			