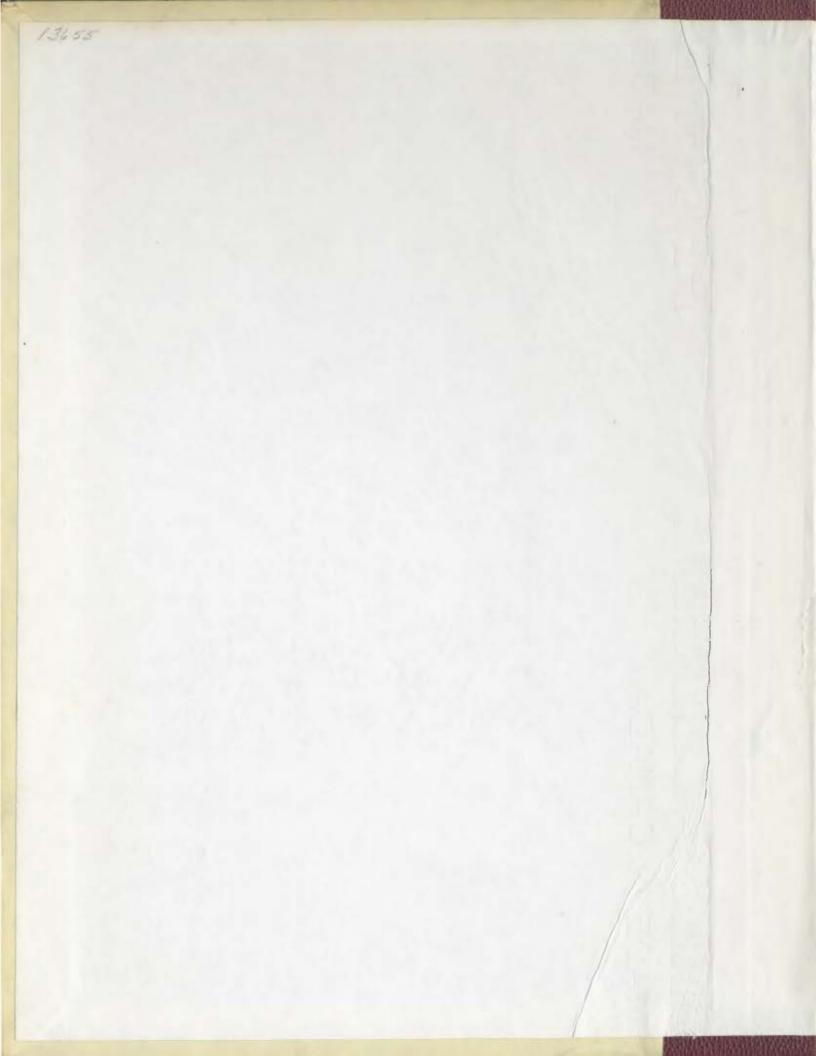
CESTODE PARASITES OF ILLEX ILLECEBROSUS ILLECEBROSUS (LeSUEUR) (DECAPODA: CEPHALOPODA)

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ELIZABETH LOUISE BROWN



CESTODE PARASITES OF <u>ILLEX ILLECEBROSUS</u> <u>ILLECEBROSUS</u> (LeSUEUR) (DECAPODA: CEPHALOPODA)

by

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A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT

Ommastrephid squid of the subspecies Illex illecebrosus
illecebrosus
(LeSueur) were obtained during the months of July through
September, 1966 and July through November, 1967, from numerous areas
around the east coast of the province of Newfoundland. The specimens
were obtained by jigging and immediately upon capture they were placed
in a styrofoam container of crushed ice and so transported to the Laboratory
for examination. An incision was made in the median ventral mantle wall
exposing the internal organs which were then examined for parasites.
Helminths found were relaxed in 1 percent urethane (ethyl carbamate) and
preserved in 70 percent ethyl alcohol, prior to staining with acid carmine.
Histological sections of the parasites were made in situ, and stained
with Mallory's Triple Stain. Measurements of total length and standard
(mantle) length of the squid were made, and the sex and maturity of each
were recorded.

Helminths of five genera were found, namely, Phyllobothrium sp. and Dinobothrium plicitum (both of which had previously been recorded),

Pelichnibothrium speciosum, Scolex polymorphus and Nybelinia sp. The last three represent new records from this Ommastrephid. All species of helminths were found free in the caecum of the host, except D. plicitum which was normally found encysted in the walls of the caecum and intestine.

The incidence of these parasites was tested for correlation with standard length, and sex of the host animal, as were annual, and seasonal variations in degree of infestation.

Also found in the various internal organs of the squid were nematodes of the genus $\underline{\text{Contracaecum}}$.

The incidence of these parasites is discussed in terms of what is known of the biology of the short-finned squid, as are postulated life-cycles for their helminth fauna.

Part of the information contained in this thesis was used, with the express permission of the Committee on Graduate Studies of this University, in the preparation of a paper entitled: "Cestode fauna of the squid <u>Illex illecebrosus illecebrosus</u> (LeSueur) in Newfoundland waters", for presentation before the American Society of Zoologists in New York, December, 1966. An abstract of this paper, authored by Elizabeth L. Brown, W. Threlfall and F. A. Aldrich appears in the American Zoologist, Vol. 6, No. 4.



Frontispiece. Acid carmine stained specimen of plerocercoid of Phyllobothrium sp.

"We must possess more knowledge concerning the diseases of the lower animals because it is of prime importance in all conservation programs, in that it may be helpful in preventing great losses of animals which are beneficial to man". - N. Fasten, 1922.

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 cooperation of others. First and foremost I must express my sincere appreciation to Dr. F. A. Aldrich, Director of the Marine Sciences Research Laboratory, who in addition to suggesting the problem in the first instance, made funds available from his research grant NRC-A-1368 that made possible the securing of squid. Of equal importance in this study has been the direction of my supervisor, Dr. W. Threlfall of the Department of Biology, Memorial University of Newfoundland.

Valuable assistance has been afforded by Messrs. C. C. Lu, M. J. Mercer and Mrs. P. C. Yorke of the Marine Sciences Research Laboratory.

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	
LIST OF PLATES	iii
LIST OF FIGURES	ν
LIST OF TABLES	vi
INTRODUCTION	1
BIOLOGY OF THE SQUID	6
Taxonomy	6
General Description	7
Distribution	10
Internal Anatomy	11
Food	14
Life History	14
MATERIALS AND METHODS	16
Cestodes	25
Nematodes	28
Dogfish and Skates	28
RESULTS	30
Order Tetraphyllidea	30
Phyllobothrium sp	
Pelichnibothrium speciosum	34
Scolex polymorphus	41



	Page
Statistical Analysis of Data on the Genus Phyllobothrium	41
Dinobothrium	56
Statistical Analysis of Data on the Genus <u>Dinobothrium</u>	56
Order Trypanorhyncha	80
Nybelinia	80
Statistical Analysis of Combined Data on Cestode Parasites	85
Nematodes	94
Dogfish and Skates	97
	400
DISCUSSION	100
CONCLUSIONS	120
REFERENCES CITED	122
APPENDIX	129

LIST OF PLATES

			<u>Pages</u>
Plate	1.	The short-finned squid <u>Illex illecebrosus</u> illecebrosus. The smallest and largest squid obtained during 1967 sampling period. The smaller squid shown measured 120 mm. in mantle length	8-9
Plate	2.	The Neyle Patent hand jigger. Total length, 8.5 cms	18-19
Plate	3.	The Japanese mechanized jigger	20-21
Plate	4.	Measuring board used for determining the mantle length of the squid examined	22-23
Plate	5.	Photograph of the internal organs of the short-finned squid. Appended to this plate is a keyed diagram indicating the organs examined for their parasite burden.	26-27
Plate	6.	Photograph of the scolex and part of the pars metabothridial of a plerocercoid of Phyllobothrium sp. from the caecum of Illex illecebrosus illecebrosus	is 31-32
Plate	7.	Photograph of an atypical plerocercoid of $\underline{Phyllobothrium}$ sp. from the caecum of \underline{Illex} $\underline{illecebrosus}$ $\underline{illecebrosus}$ -	36-37
Plate	8.	Photograph of the scolex and portion of the pars both idialis of Pelichnibothrium speciosum from the caecum of Illex illecebrosus illecebrosus	39-40
Plate	9.	Photograph of the larval form <u>Scolex polymorphus</u> from the caecum of <u>Illex illecebrosus</u> illecebrosus	43-44
Plate	10.	Photograph of a plerocercoid of <u>Dinobothrium plicitum</u> removed from a cyst in the intestine of <u>Illex</u> <u>illecebrosus</u> illecebrosus. Note the hooks on the scolex.	58-59
Plate	11.	Photograph of a transverse section through the intestine of <u>Illex illecebrosus illecebrosus</u> showing encysted plerocercoids of <u>Dinobothrium plicitum</u> in the wall.	64-65
Plate	12.	Photograph of a longitudinal section through a plerocercoid of Dinobothrium plicitum in the wall of the intestine of Illex illecebrosus illecebrosus	66-67

		<u>Pages</u>
Plate 13.	Photograph of a transverse section of an encysted plerocercoid of <u>Dinobothrium plicitum</u> in the wall of the intestine of <u>Illex illecebrosus</u> illecebrosus	68-69
Plate 14.	Photograph of a transverse section through a cyst of Dinobothrium plicitum in the wall of the intestine of Illex illecebrosus illecebrosus	70-71
Plate 15.	Photograph of the post-larva of Nybelinia sp. from the caecum of Illex illecebrosus illecebrosus	82-83
Plate 16.	Photograph of a portion of an unidentified trypanorhynch from the spiral caecum of the spiny dogfish, Squalus acanthias	98-99

1

LIST OF FIGURES

		Page
Figure 1.	Diagram of generalized decapod alimentary system (modified from Bidder, 1950)	13
Figure 2.	Map of the Avalon Peninsula and adjacent areas of Newfoundland showing sampling stations	17
Figure 3.	Scolex and portion of the pars metabothridialis of plerocercoid of Phyllobothrium sp. from caecum of Illex illecebrosus illecebrosus	33
Figure 4.	Scolex of atypical specimen of plerocercoid of Phyllobothrium sp. from caecum of Illex illecebrosus illecebrosus.	35
Figure 5.	Scolex and portion of pars metabothridialis of a plerocercoid of Pelichnibothrium speciosum from the caecum of Illex illecebrosus illecebrosus.	38
Figure 6.	Scolex and portion of the pars metabothridialis of specimen designated as Scolex polymorphus from the caecum of Illex illecebrosus illecebrosus.	42
Figure 7.	Plerocercoid of <u>Dinobothrium</u> plicitum removed from cyst in <u>wall of intestine of Illex</u> <u>illecebrosus</u> .	57
Figure 8.	Post-larval form of Nybelinia sp. from the caecum of Illex illecebrosus illecebrosus	81
Figure 9.	Portion of proboscis of <u>Nybelinia</u> sp. from caecum of Illex illecebrosus illecebrosus	84

LIST OF TABLES

			Page
Table	I.	Summary of the parasites recovered from squids of the Family Ommastrephidae	2
Table	II.	Distribution of plerocercoid larvae of Phyllobothrium spp. in I. i. illecebrosus, 1966 and 1967	45
Table	III.	Numerical distribution of plerocercoids of Phyllobothrium spp. in infected squid, I. i. illecebrosus, 1966 and 1967	46
Table	IV.	Degree of infestation with plerocercoids of Phyllobothrium spp. in male squid, <u>I. i.</u> illecebrosus, 1966.	48
Table	٧.	Degree of infestation with plerocercoids of Phyllobothrium spp. in female squid, I.i. Illecebrosus , 1966	49
Table	VI.	Degree of infestation with plerocercoids of Phyllobothrium spp. in male squid, I.i. illecebrosus , 1967	50
Table	VII.	Degree of infestation with plerocercoids of Phyllobothrium spp. in female squid, I.i. Illecebrosus , 1967	51
Table	VIII.	Percent infestation with plerocercoids of Phyllobothrium spp. with increasing mantle length in I. i. illecebrosus , 1966	53
Table	IX.	Percent infestation with plerocercoids of Phyllobothrium spp. with increasing mantle length in I.i. illecebrosus, 1967	54
Table	х.	Annual variation in number of squid, <u>I. i.</u> <u>illecebrosus</u> parasitized by plerocercoids of <u>Phyllobothrium</u> spp., 1966 and 1967	55
Table	XI.	Distribution of plerocercoids of <u>D. plicitum</u> in <u>I. i. illecebrosus</u> , 1966 and 1967	60
Table	XII.	Sites involved in multiple infestation by <u>D. plicitum</u> in individual squid, <u>I. i.</u> <u>illecebrosus</u> , 1966 and 1967	62

		Page
Table XIII.	Percentage infection with <u>D. plicitum</u> with increasing mantle length, in <u>I. i.</u> illecebrosus 1966	72
Table XIV.	Percent infection with <u>D. plicitum</u> with increasing mantle length in <u>I. i.</u> illecebrosus, 1967	73
Table XV.	Annual variation in number of squid, <u>I. i.</u> <u>illecebrosus</u> , parasitized by plerocercoids of <u>D. plicitum</u> , 1966 and 1967	74
Table XVI.	Number of male and female squid, <u>I. i.</u> <u>illecebrosus</u> , infested with plerocercoids of <u>D. plicitum</u> , 1966 and 1967	75
Table XVII.	Degree of infestation with plerocercoids of D. plicitum in male squid, I. i. illecebrosus, 1966	76
Table XVIII.	Degree of infestation with plerocercoids of <u>D. plicitum</u> in female squid, <u>I. i. i.lecebrosus</u> , 1966	77
Table XIX.	Degree of infestation with plerocercoids of <u>D. plicitum</u> in male squid, <u>I. i. illecebrosus</u> 1967	78
Table XX.	Degree of infestation with <u>D. plicitum</u> in female squid, <u>I. i. illecebrosus</u> , 1967	79
Table XXI.	Monthly variation in rate of infestation by cestode parasites in <u>I. i. illecebrosus</u> , July-September, 1966 and 1967	86
Table XXII.	Monthly variation in rate of infestation by cestode parasites in <u>I. i. illecebrosus</u> , July-November, 1967.	87
Table XXIII.	Seasonal variation in degree of infestation by plerocercoids of cestodes in female squid, <u>I. i. illecebrosus</u> , 1966	88
Table XXIV.	Seasonal variation in degree of infestation by plerocercoids of cestodes, female squid, I. i. illecebrosus, 1967	90

		Page
Table XXV.	Seasonal variation in degree of infestation by plerocercoids of cestodes in male squid, <u>I. i. illecebrosus</u> , 1966	91
Table XXVI.	Seasonal variation in degree of infestation by plerocercoids of cestodes in male squid, <u>I. i. illecebrosus</u>	92
Table XXVII.	Degree of infestation by cestode plerocercoids with increasing mantle length in <u>I. i.</u> <u>illecebrosus</u> , 1966	93
Table XXVIII	Degree of infestation by cestode plerocercoids with increasing mantle length in <u>I. i.</u> <u>illecebrosus</u> , 1967	95
Table XXIX.	Site of infestation by larvae of <u>Contracaecum</u> sp. in <u>I. i. illecebrosus</u> , 1966 and 1967	96

INTRODUCTION

The study of parasitism involves essentially two environments described by Pavlovski (1934) as the micro-environment, or the immediate environment of the parasite within the host, and the macro-environment, or the external environment of the host, which in the case of decapod cephalopods is the ocean. It is natural that parasites are influenced directly by the micro-environment and indirectly by the macro-environment, as it is this which influences the life processes of the host animal. It is therefore reasonable to assume that the micro-environment and the parasitic fauna may be considered as an ecological unit (Dogiel, 1962). The term parasitocoenosis was erected by Pavlovski (1937) to cover the complex of parasites inhabiting one host and the relationship between the parasites and their environments. The ecological relationship between parasites and their host organism has been discussed by numerous authors: Pavlovski (1934, 1935), Filipchenko (1937), Noble and Noble (1961), Hegner (1927), Baer (1952), and many others.

According to Clarke (1966) few parasites of oceanic squids have been described and those that have, have been collected mainly from Ommastrephids. Metazoan parasites, have, however, been described from other Cephalopods. In 1847, Gros described larval tetraphyllideans from Sepia, while Legendre (1934) recovered parasites from the digestive tract of Sepia officinalis L. fillouxi Alex Lafont, as did Nouvel (1934) and Theodorides (1954). Several species of cestodes have also been collected from octopods (Eledone spp.) by Nouvel (1934, 1935).

Several authors have recovered parasites from oceanic squids of the Family Ommastrephidae, and these are summarized in Table I.

Table I. Summary of the parasites recovered from squids of the Family Ommastrephidae.

Illex illecebrosus illecebrosus (LeSueur, 1821):

Phyllobothrium sp.

Leidy, 1890, U.S.A. Linton, 1922, U.S.A. Frost and Thompson, 1932, Newfoundland Squires, 1957, Canada Aldrich, 1964, Canada Mercer, 1965, Canada

<u>Dinobothrium</u> (sensu lato)

Linton, 1897, U.S.A. Frost and Thompson, 1932, Newfoundland Squires, 1957, Canada Aldrich, 1964, Canada Mercer, 1965, Canada

<u>Illex</u> <u>illecebrosus</u> <u>coindeti</u> (Verany, 1859):

Phyllobothrium sp.

Euzet, 1959, France

Phyllobothrium tumidum

Legendre, 1939, France (vide. Dollfus, 1964)

Dinobothrium sp.

Legendre, 1939, France (vide. Dollfus, 1964)

Table I (continued)

Ommastrephes sloani pacificus (Steenstrup, 1880)

Phyllobothrium loliginis

Giuart, 1933, France

Scolex polymorphus

Yamaguti, 1934, Japan

Nybelinia sp.

Yamaguti, 1934, Japan

Todaropsis eblanae (R. Ball, 1841)

Phyllobothrium sp.

Dollfus, 1936, France Legendre, 1933, France (vide. Dollfus, 1964)

Dinobothrium sp.

Legendre, 1933, France (vide. Dollfus, 1964)

Todarodes sagittatus (Lamark, 1799)

Phyllobothrium dohrni

Dollfus, 1936, Naples

Phyllobothrium loliginis

Guiart, 1933, France

Nybelinia sp.

Dollfus, 1958, France

Dosidicus gigas (d'Orbigny, 1835)

Phyllobothrium sp.

MacGinitie and MacGinitie, 1949, U.S.A. Riser, 1949, U.S.A.

Table I (continued)

Pelichnibothrium sp.

Riser, 1949, U.S.A.

Tetrarhynchus sp.

MacGinitie and MacGinitie, 1949, U.S.A.

Gonatus fabricii (Lichtenstein, 1818)

Nybelinia sp.

Riser, 1949, Canada

The fact that great numbers of squid are readily available in Newfoundland waters for approximately four months of the year and that little work has been done on the parasites of this species, led to the initiation of this study.

Larval forms of two genera of the Family Phyllobothriidae have been recovered from <u>Illex illecebrosus illecebrosus</u> by Frost and Thompson (1932), Squires (1957), Aldrich (1964), and Mercer (1965). The life cycle of these helminths is incompletely known and the species have never been positively identified. It was the purpose of this study to establish what helminth parasites were present in <u>I. i. illecebrosus</u>, their frequency of appearance, numbers per squid and the percentage of squid infected. Monthly and yearly variations in degree of infestation

and possible correlations between percentage of infestation and mantle length of the host were investigated as was the possibility of a relationship between parasite burden and sex and maturity of the squid.

The definitive host for tetraphyllideans is reported to be elasmobranchs, (Linton, 1922), where the mature parasites are found in the digestive tract. A study of the cestode parasites of dogfish (Squalus acanthias Linnaeus, 1755) and the thorny skate (Raja radiata Donovan, 1807) was initiated to locate adult tetraphyllideans, hoping to cast light upon the life cycle of the forms found to infest the short-finned squid.

Squires (1957), Dollfus (1958), and Mercer (1965) state that parasites are commonly found in the rectum, stomach and caecum of <u>I. i. illecebrosus</u>, and Squires (1957) pointed out that the parasites move freely between these regions. Dollfus (1958) also records tetraphyllidean cestodes as being recovered from the mantle cavity, esophagus, pancreas and liver of <u>Illex illecebrosus coindeti</u>. It was the purpose of the author to establish where cestodes are most commonly found in the host and to determine if movement between body organs was as common as had been stated.

BIOLOGY OF THE SQUID

In any parasitological study, the biology of the host organism must be considered, which in this work was the decaped cephalopod Illecebrosus (LeSueur).

Taxonomy

The Newfoundland short-finned squid was first described by LeSueur (1821) and was given the name Loligo illecebrosa¹. The present generic name was erected in 1880 by Steenstrup to include the common European form Illex coindeti. It is now believed that these two, Illex illecebrosus and Illex coindeti represent two subspecies of the species I. illecebrosus, namely, I. i. illecebrosus and I. i. coindeti, and it was Pfeffer who in 1912, proposed that this was true. The useage has been accepted and used by Bigelow (1926), Grimpe (1933), Adam (1952), Mangold-Wirz (1963), Mangold and Fioroni (1966), and others working with the genus.

A third subspecies is now assigned to the species, namely,

Illex illecebrosus argentinus. De Castellanos (1960) described a new

Ommastrephid from the southwest Atlantic, and called it Ommastrephes

argentinus. Subsequently, de Castellanos (1964) accepted and published
the opinion of G. L. Voss that O. argentinus is, in fact, a third

subspecies of I. illecebrosus. Zuev (1966) although recognizing three

 $^{^1}$ According to Voss (1962) the holotype which was for years at the Academy of Natural Sciences of Philadelphia, is no longer existent.

"geographical races" of I. i. illecebrosus was apparently unaware of the work of de Castellanos in this regard.

The species here considered may be classified as follows:

PHYLUM: Mollusca

CLASS: Cephalopoda

SUBCLASS: Coleoidea

ORDER:

Decapoda (Teuthoidea)

SUBORDER: Ommastrephidae

FAMILY:

Illicinae

GENUS:

Illex

SPECIES

I. illecebrosus

General Description

The body of the short-finned squid (Plate 1) is oblong in shape with ten arms surrounding the mouth or buccal cavity. Eight of the arms, called the sessile arms, are of nearly equal length and are equipped with toothed, stalked suckers along their length. The remaining two arms, or tentacular arms, are longer than the other eight and bear two rows of toothed, stalked suckers only on the terminal enlargements, or "clubs". The body of squids of the Family Ommastrephidae are streamlined for speed, with a triangular fin at the functional posterior end of the mantle. The apex of the fin is terminal, the fin joining the body approximately one-third of the mantle length toward the functional anterior end.

Plate 1. The short-finned squid Illex illecebrosus illecebrosus. The smallest and largest squid obtained during the 1967 sampling period. The smaller squid shown measured 120 mm. in mantle length.



Distribution

Verrill (1881) reported that the range for <u>I. i. illecebrosus</u> was from Cumberland Sound, Nova Scotia, to Cape Hattaras, North Carolina. Grimpe (1933) enlarged this range to include Newfoundland, Greenland, Iceland and the Faroes. With respect to its southern-most distribution, it has been reported from waters of a depth of 105 fathoms in the Gulf of Mexico and from off Cuba (Voss, 1954, 1955, 1956a, 1956b). The delimitation of the two subspecies <u>I. i. illecebrosus</u> and <u>I. i. argentinus</u> in the western Atlantic has not been investigated, but it is known that <u>I. i. argentinus</u> at least ranges as far north as 36° S latitude (de Castellanos, 1964).

Illex i. illecebrosus ranges nearly the entire length of the coast of eastern North America, and is abundant in the coastal waters of Newfoundland in sufficient quantities to support a long-term fishery. In fact, the fishery for the short-finned squid in Newfoundland is the largest for any species of cephalopod outside the Orient, such as their numbers (Aldrich, 1968). The fishery is indeed a long-term one (Cartwright, 1792). This is evident from his statement in his diary for the entry of Friday, 27 May 1785,

"... they are caught in great numbers in the harbours of Newfoundland; and multitudes run on shore in high water, where they are left by the tide, specially if a fire be made on the beach. They are used in Newfoundland for bait to catch codfish, and are excellent for the purpose."

In Newfoundland waters, the squid usually move inshore from the Grand Banks in late July and remain in embayments until late October. However, they have been taken from inshore waters as early as June and as late as November as was the case in 1967. Most of the squid taken commercially, are caught from heads of bays around the coast of the Province, with Holyrood Arm, Conception Bay, being one of the first places where large numbers of squid appear. As the migration season progresses, populations may be found as far north as White Bay at the base of the Great Northern Peninsula, and as far west as Francois, on the south coast of the Island (Frost and Thompson, 1932; Squires, 1957). As was noted above, these seasonal migrations seem to originate from the Grand Banks and it is not clear why the squid come into the inshore waters (Aldrich, 1964).

Internal Anatomy

No.

We shall consider here a brief description of those organs and organ systems reported to be the site of metazoan parasite fauna of decapod cephalopods.

The morphology of the short-finned squid has not been intensively studied and so we must rely in many cases on descriptions of other closely allied species, as decapod cephalopods are basically similar with respect to their general internal anatomy. Full accounts of the general morphology and histology of the digestive system of Loligo pealeii, the common Atlantic squid can be found in Williams (1909)

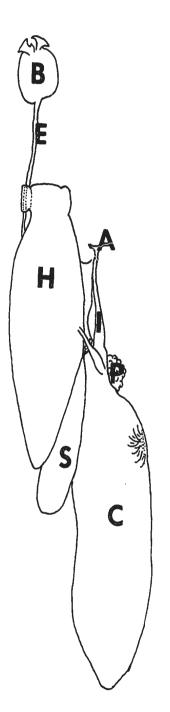
and his descriptions, along with those of Bidder (1950) on <u>Loligo</u>

<u>vulgaris</u> and <u>Loligo forbesi</u>, and Fields (1965) on <u>Loligo opalescens</u>,

can be applied to most species.

In general the digestive system (Figure 1) is composed of a buccal mass containing the mandibles (beaks), radula, and salivary glands. Attached to the buccal mass and passing towards the posterior is the muscular esophagus. The esophagus is overlain by the hepatopancreas and joins the stomach, a muscular sac lined with a chitinous cuticle. Adjoining the stomach is, perhaps, the most complex organ of the digestive system, the caecum. The caecum is composed of two portions, the spiral section and an elongate, blind sac. Detailed descriptions of these can be found in Bidder (1950) and Williams (1909). Into the caecum pass the secretions of the hepatopancreas, a large organ commonly referred to as two separate entities, the "liver" and the "pancreas". In actuality, it is one organ composed of two regions, an hepatic portion and a pancreatic portion. Both produce enzymes which pass into the caecum, those of the pancreatic portion being produced continually while those of the hepatic portion are produced only when digestion is taking place. Undigested materials are passed along a tapering intestine into the rectum which opens into the mantle cavity behind the hyponome, or funnel.

Overlying the pancreas is the renal organ, an organ of excretion which is the site of infestation by mesozoan parasites in some species of cephalopods. The organ is extremely fragile and decomposes



- A. Anus
- B. Buccal Mass
- C. Caecum
- E. Esophagus
- H. Hepatic Part of Hepatopancreas
- I. Intestine
- P. Pancreatic Part of Hepatopancreas
- S. Stomach

Figure 1. Diagram of generalized decapod alimentary system (modified from Bidder, 1950).

rapidly upon the death of the animal.

Food

The food of <u>I. i. illecebrosus</u> has been the subject of intensive study by students of Dr. Aldrich since 1962, but very little of this work has been published save that of Mercer (1965). Earlier, Squires (1957) reported upon the food of this species, as will be discussed later. De Castellanos (1964) presented the following food cycle for <u>I. i. argentinus</u>, and it is believed that this cycle can be applied to <u>I. i. illecebrosus</u>.

zooplankton

† †

phytoplankton planktonic fish

† targer fish squid

† + +

carnivorous fish

Life History

Very little is known of the life cycle of the short-finned squid as neither the mating behaviour, egg capsules nor early juvenile stages have been described. Aldrich et. al. (1968) have, however, described fertilization and early cleavage in this species. The reproduction of Ommastrephes sloani pacificus (=Todarodes pacificus), an ommastrephid from the western Pacific is known and has been described

by Hamabe (1962, 1963), Okutani (1965), and Choe (1966). It is probable that reproduction in $\underline{I.\ i.\ illecebrosus}$ is similar to that of the above species, as both are oceanic squid of the same Family. Aldrich et. al. (1968) have shown that there are great similarities between the two species in their early ontogeny.

MATERIALS AND METHODS

Random samples of the squid <u>I. i. illecebrosus</u> were obtained from five areas around the coast of Newfoundland from July to September 1966, and July to November 1967. The samples came from Holyrood, Conception Bay; Chapel Arm, Trinity Bay; Freshwater, Freshwater Bay; Cuckold's Cove, near St. John's; and Summerville, Bonavista Bay. Two other samples were obtained from bait-squid freezing plants located at Bonavista, Bonavista Bay, and Fortune, Fortune Bay, (Figure 2). All squid were caught with either a Neyle patent jigger (Plate 2), or a Japanese mechanized jigger (Plate 3) in relatively shallow water.

Immediately upon capture, the squid were placed in a styrofoam container with crushed ice and then transported to the Laboratory where they were kept chilled until they could be examined. A number of other methods of preserving the squid until examination were employed in the early stages of this study. However, these were rejected in favour of the one outlined above. The reasons for this and other methods employed temporarily, will be discussed later in this work. The squid supplied by the bait depots were shipped to the Laboratory in frozen forty-pound lots.

The standard (mantle) length was taken using a measuring board marked in centimeters (Plate 4). The posterior end of the mantle was placed against the head of the board and the mantle length was measured



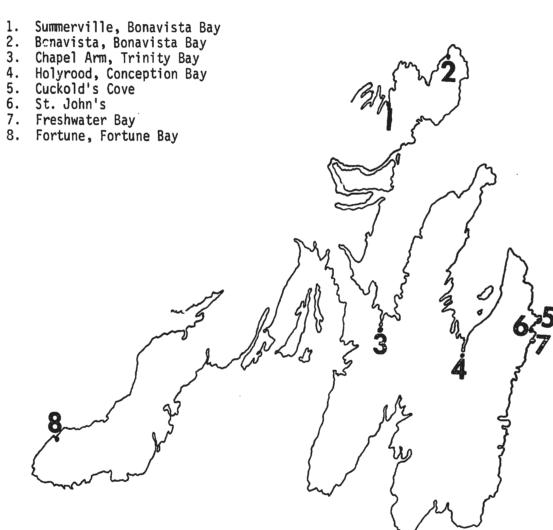


Figure 2. Map of Avalon Peninsula and adjacent portions of Newfoundland showing sites of collection stations for Illex illecebrosus illecebrosus.

Plate 2. The Neyle Patent hand jigger. Total length 8.5 cms.



Plate 3. The Japanese mechanized jigger.

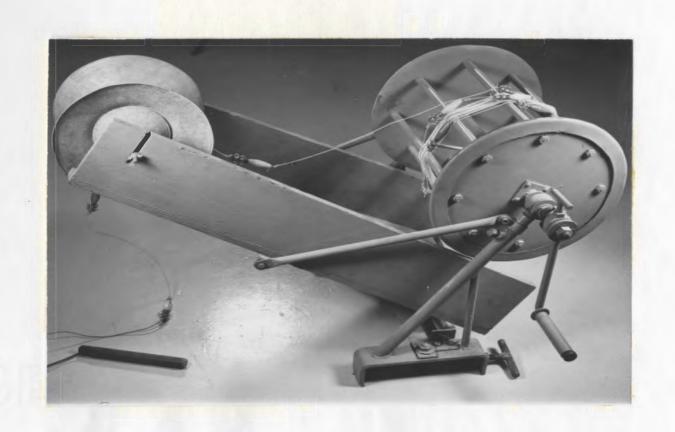
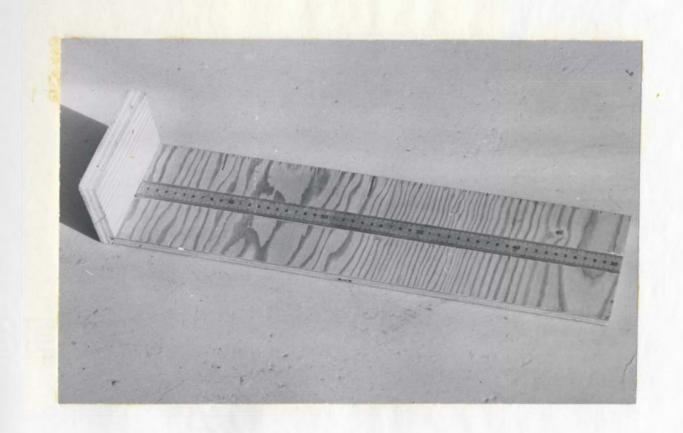


Plate 4. Measuring board used for determining the mantle length of the squid examined.





to the anterior-most protrubrance of the functional dorsal surface of the mantle, this being the standard length as defined by Haefner (1964). Because of the pliable nature of the squid, all measurements were taken to the nearest millimeter. Whole wet weights (in grams) were taken of specimens obtained during 1967, using a Mettler heavy duty balance.

All squid were examined to determine their sex and state of maturity. The presence of spermatophores in the penis or spermatophoric gland was taken as the criterion for maturity in the males, while the presence of eggs in the oviduct was taken as the criterion in the females.

The squid were then examined for their helminth parasite burden according to a definite plan. The mantle was opened by making a median incision along its full length, thus exposing the mantle cavity and the internal organs. The hyponome, or funnel, was also cut ventrally exposing the hyponomal cavity for examination.

The following internal organs were examined for the presence of parasites:

- (a) hyponome
- (b) mantle cavity
- (c) digestive gland, or hepatopancreas (hepatic and pancreatic portions)
- (d) esophagus
- (e) stomach
- (f) caecum

- (g) rectum
- (h) nephridium

All these organs, save the esophagus, are shown in Plate 5. Figure 1 shows the relative position of the esophagus.

The nephridia were examined in all cases where decomposition of tissue was not extreme. Squashes were made of each nephridium and then examined under a high power (400x) Wild M-20 microscope to detect any metazoan parasites present.

Both cestodes and nematodes were recovered from the squid, and were treated in the following manner.

Cestodes: The number of parasites from each body organ of the squid were counted, the parasites then being placed in a petri dish containing a 1 percent aqueous solution of ethyl carbamate (Urethane)¹, which was allowed to stand at room temperature for approximately two hours or until the parasites became relaxed. Relaxation was considered complete when the scolex became fully expanded exposing the four bothridia. The parasites were then transferred to a 70 percent solution of ethyl alcohol and held for further examination. The cestodes were stained when necessary for identification, in Borax carmine¹ or Acid carmine¹, dehydrated and cleared. The helminths were then examined under a low power (20x) dissecting microscope. Representative specimens were then mounted in Permount¹, closely examined under a low power (60x) compound microscope and drawn. The slides were then retained for future reference.

¹See Appendix I

Plate 5. Photograph of the internal organs of the short-finned squid. Appended to this plate is a keyed diagram indicating the organs examined for their parasite burden.



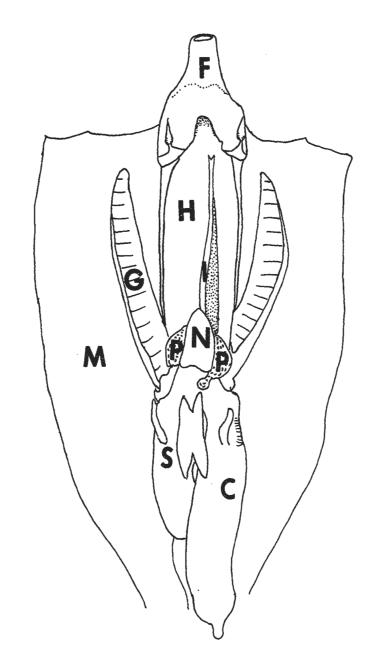


Diagram of internal organs examined for parasite burden.

- F. Funnel or hyponome
- H. Hepatic part of hepatopancreas
- P. Pancreatic part of hepatopancreas
- N. Nephridium or renal organ
- G. Ctenidium or gill
- M. Mantle or pallium
- S. Stomach
- C. Caecum
- I. Intestine

Some cestodes were fixed in Bouins solution¹, embedded in parafin wax at 62° C., sectioned at 10° , and mounted on 3x1 inch glass slides. The sections were then stained with Mallory's Triple Stain¹, dehydrated, cleared and mounted in Permount¹. These slides were prepared to determine how the parasites were attached to the body organs and to note whether any cysts present were secreted by the host as a reaction against the parasite, or whether the cyst was secreted by the parasite, or whether both organisms contributed to the cyst wall.

Nematodes: Parasitic nematodes were placed, immediately upon removal from the squid, in hot lacto-phenol¹ for fixing and clearing and then mounted in glycerine jelly for examination and identification.

Dogfish and Skates

In addition to squid, approximately 100 dogfish (<u>Squalus acanthias</u>) and 100 skates (<u>Raja radiata</u>) were examined for metazoan parasites. The specimens examined were secured from trawls made by Mr. Ray Riche, a St. John's commercial fisherman.

Both species of elasmobranchs were measured in centimeters from the tip of the snout to the last vertebra in the tail. External surfaces and gills were examined for ectoparasites. Internal organs were exposed by making an incision in the ventral body wall, and the liver, stomach and spiral caecum of both species were examined for helminth parasites. In all cases, the stomach was separated from the spiral caecum

¹See Appendix I

and the gastric and caecal contents were washed separately through a 500μ sieve. The contents which remained in the sieve were again washed into a petri dish and closely examined. All parasites found, except nematodes, were preserved in 70 percent ethyl alcohol, while nematodes were immediately fixed and cleared in hot lacto-phenol.

In an effort to evaluate any relationship existing between parasite burden and squid length, sex and date of capture, various statistical analyses were applied to relevant data. Amongst these were frequency tests, x^2 contingency tests and homogeniety tests, all according to Simpson, et. al. (1960).

RESULTS

A total of 802 squid were examined for their helminth parasite burden. Cestode parasites of two Orders were recovered, namely,

Tetraphyllidea and Trypanorhyncha. Within the Tetraphyllidea, four genera of cestodes were recovered, while a single genus of the Trypanorhyncha was collected. One genus of the Family Heterocheilidae (Phylum Nematoda) was also found. Each of these groups is described below.

Order: Tetraphyllidea Carus, 1863

As a result of the study, it is apparent that more than one larval form of Phyllobothrium is capable of infesting I.j.jillecebrosus. To date, two species of Phyllobothriidae have been recovered. These are Phyllobothrium sp. and Pelichnibothrium speciosum Monticelli, 1889 and are described below.

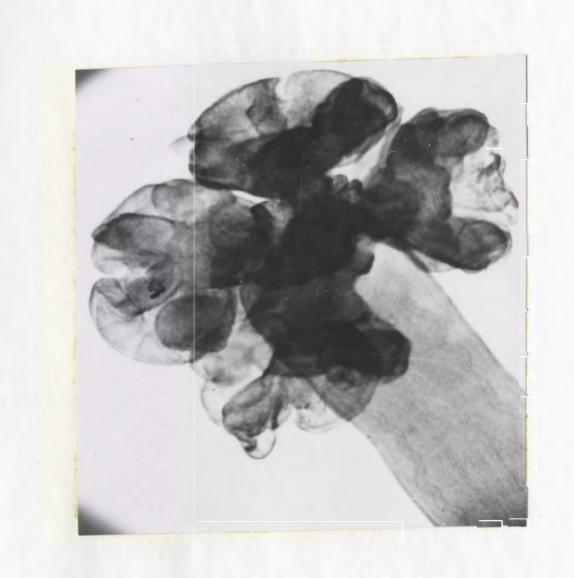
Genus: Phyllobothrium Van Beneden, 1858

Phyllobothrium sp. (Figure 3, Plate 6)

These are larval (plerocercoid) tetraphyllideans ranging in length from 10 mm. to 36 mm. They have four folded bothridia* characterized by undulating edges, each of the bothridia bearing an accessory sucker. The bothridia measure 1.308 mm. in length and 0.774 mm. in width with the accessory suckers measuring 0.324 mm. in diameter. There is also an anterior apical sucker measuring 0.462 mm. in diameter, and this structure combined with the four bothridia comprise the pars bothridialis. This

^{*}Riser (1949) refers to these structures as phyllidea, however, I prefer the term bothridia as used by Dollfus (1964).

Plate 6. Photograph of the scolex and part of the pars metabothridialis of a plerocercoid of Phyllobothrium sp. from the caecum of Illex illecebrosus illecebrosus.



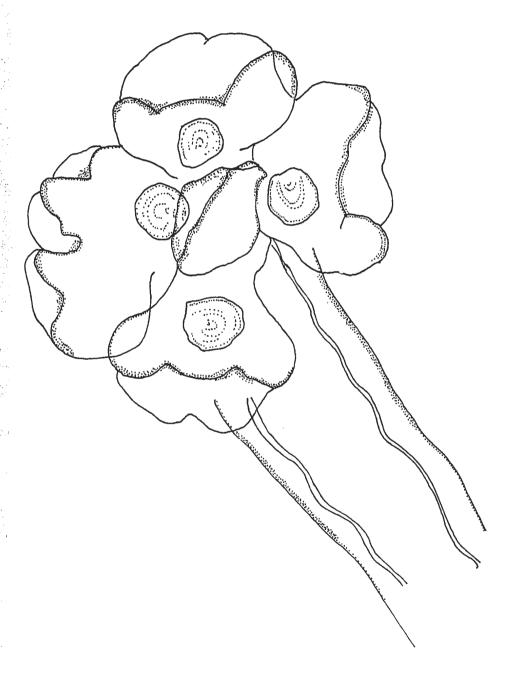


Figure 3. Scolex and portion of the pars metabothridialis of plerocercoid of $\underline{Phyllobothrium}$ sp. from caecum of \underline{Illex} $\underline{illecebrosus}$ $\underline{illecebrosus}$.

region is followed next posteriorally by the pars metabothridialis, or the region of growth which may or may not appear to be segmented. The latter region is opaque and pale yellow in colour in living specimens. The pars metabothridialis is followed by the pars prostica or larval tail, which represents a translucent region generally longer than the preceding one. Seen through the cuticle of both the pars metabothridialis and pars prostica are convoluted excretory vessels.

This form of larval <u>Phyllobothrium</u> sp. was found to occur in 99.37 percent of the squid infested with plerocercoids of this genus.

Another, apparently younger, form of the above plerocercoid, was found in which the bothridia were contracted giving it a somewhat different appearance (Figure 4, Plate 7). The bothridia of this specimen measured 1.068 mm. in length and 1.056 mm. in width. The apical sucker was clearly visible and measured 0.264 mm. in length and 0.192 mm. in width. Only a single specimen of this "type" was found and the differences in appearance were probably due to fixation.

Genus: Pelichnibothrium

Pelichnibothrium speciosum Monticelli, 1889 (Figure 5, Plate 8)

Nine specimens of this species were found in a single squid in

1967. The scolex of this species is large with four muscular bothridia,

each bearing an accessory sucker measuring from 0.23 - 0.25 mm. in diameter.

A functional apical sucker is present measuring 0.21 - 0.22 mm. in diameter.

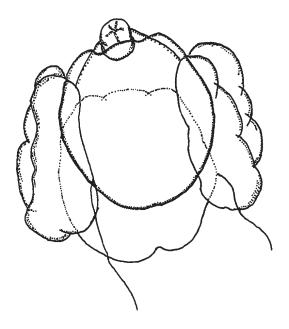


Figure 4. Scolex of atypical specimen of plerocercoid of Phyllobothrium sp. from caecum of Illex illecebrosus illecebrosus.

Plate 7. Photograph of an atypical plerocercoid of Phyllobothrium sp. from the caecum of Illex illecebrosus illecebrosus.



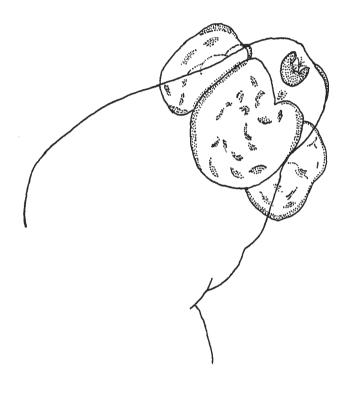


Figure 5. Scolex and portion of pars metabothridialis of a plerocercoid of Pelichnibothrium speciosum, from the caecum of $\underline{\text{Illex illecebrosus}}$ $\underline{\text{illecebrosus}}$.

Plate 8. Photograph of the scolex and portion of the pars bothridialis of Pelichnibothrium speciosum from the caecum of Illex illecebrosus illecebrosus.



The total length of the parasite shown in Plate 8 was approximately 4.0 - 4.2 mm. with a width of 1.30 - 1.35 mm. The body is divided into the classical three regions, the pars both ridialis, pars metaboth ridialis and pars prostica.

Genus: Scolex

Scolex polymorphus Rudolphi, 1810 (Figure 6, Plate 9)

A single specimen of a third larval type was found and has been designated <u>Scolex</u> polymorphus Rudolphi, 1810.

The total length of the specimen as figured measured 3.5 mm., and was 0.65 mm. in width. The bothridia are biloculate and measured 0.40 mm. by 0.30 mm. There were no accessory suckers present on the bothridia, and neither was there an apical sucker. No internal structures could be seen through the cuticle.

Statistical Analysis of Data on the Genus Phyllobothrium

Of the squid examined during the 1966 sampling period, 157 (40.25 percent) were infested with plerocercoids of <u>Phyllobothrium</u> spp. while in 1967, 161 (39.03 percent) were found to be parasitized. The distribution of plerocercoids in the squid examined is indicated in Table II.

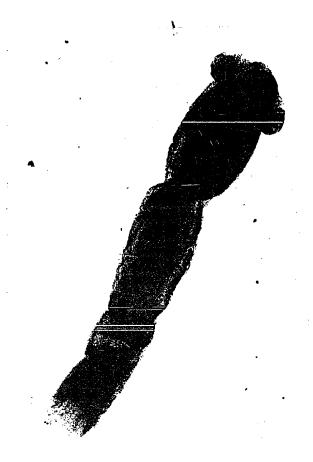
Plerocercoids of <u>Phyllobothrium</u> found in the caecum were normally attached to the caecal walls and caecal leaflets. This was true, whether or not specimens of <u>Phyllobothrium</u> spp. were found in the other sites indicated in Table II.



Figure 6. Scolex and portion of the pars metabothridialis of specimen designated as $\frac{\text{Scolex polymorphus}}{\text{from the caecum of }}$

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Plate 9. Photograph of the larval form <u>Scolex</u> polymorphus from the caecum of <u>Illex</u> illecebrosus illecebrosus.



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Table II. Distribution of plerocercoid larvae of Phyllobothrium spp. in I. i. illecebrosus, 1966 and 1967.

Site of Infestation	Number of squid infected	
	1966	1967
Mantle cavity	2	13
Esophagus	0	0
Hyponome	0	9
Kidney	0	0
Stomach	5	0
Pancreatic portion of hepatopancreas	0	6
Caecum	157	161
Intestine	4	11

With respect to numbers of parasites of the genus <u>Phyllobothrium</u>, infestation ranged from a single specimen in 32.07 percent of the infested squid, to a maximum of 41 plerocercoids in .31 percent of the squid. The average number of <u>Phyllobothrium</u> found per squid over the two-year period was 6.61. However, the average number per squid in 1966 samples was 2.96 and the average for 1967 samples was 10.23, this difference being statistically significant.

The numerical distribution of plerocercoids of $\underline{Phyllobothrium}$ spp.

in squid is found in Table III.

Table III. Numerical distribution of plerocercoids of Phyllobothrium spp. in infected squid \underline{I} . i. illecebrosus, $\underline{1966}$ and $\underline{1967}$.

Number of Parasites	Number of sq	uid infected
	1966	1967
1 - 4	131	48
5 - 8	13	28
9 - 12	8	25
13 - 16	3	27
17 - 20	0	12
21 - 24	1	9
25 - 28	1	7
29 - 32	0	3
33 - 36	0	1
37 - 41	0	1

In 1966, 84.07 percent of all squid parasitized were infested with one to four plerocercoids of Phyllobothrium, while in 1967, 29.81 percent of the infested squid were so parasitized. In 1967, many more squid were found to be infested with higher numbers of plerocercoids than

was the case in 1966. In 1966, no squid was found to harbour more than 28 plerocercoids, while the highest number of plerocercoids of Phyllobothrium found in any squid in 1967 was 41.

It was found that there is no significant difference in degree of infestation in either male or female squid throughout the 1966 sampling period as can be seen from Tables IV and V.

Infestation in males during 1966 was high on the first sampling date, however, as only one male squid was examined, it cannot be stated that this represents a true picture of the level of parasites at this sampling time in the whole squid population. Throughout the season the degree of infestation varied and seemed to reach a number of peak periods of infestation, as for example, during the month of August when infestation rose from 15 percent to 61 percent and then tapered off to 16 percent in late September. Much the same pattern is evident in female squid sampled during 1966, although the overall percentage of infested squid is generally higher.

In 1967, it was found that there was a significant difference in the degree of infestation in both sexes as the season progressed (Table VI, and VII).

In the male squid examined in 1967, infestation early in the sampling period was high and then dropped to 0.0 percent in late September. This was followed by a rise in percentage of squid infected and then there was another period when no parasites were found. A third, but smaller peak

Table IV. Degree of infestation with plerocercoids of <u>Phyllobothrium</u> spp. in male squid, <u>I. i. illecebrosus</u>, 1966.

Date	Number of Squid Infected	Total Sampled	Percent Infected
July 27	1	1	100
August 2	3	11	27.2
August 10	7	17	41.1
August 15	0	3	0
August 16	4	26	15.2
August 18	4	11	36.3
August 22	16	26	61.5
August 29	6	12	50.0
September 13	6	15	40.0
September 20	7	22	31.8
September 22	8 .	21	38.0
September 28	1	6	16.0
September 26	0	0	0.0
September 30	1	6	16.0

P = .100 - .250

Table V. Degree of infestation with plerocercoids of <u>Phyllobothrium</u> spp. in female squid, \underline{I} . \underline{i} , \underline{i} illecebrosus, 1966.

Date	Number of Squid infected	Total Sampled	Percent Infected
July 27	9	13	69.2
August 2	3	10	30.0
August 10	13	23	56.5
August 15	3	4	75.0
August 16	18	34	52.9
August 18	6	9	66.6
August 22	17	30	56.6
August 29	11	22	50.0
September 13	6	16	37.5
September 20	3	9	33.3
September 22	6	17	35.2
September 26	3	6	50.0
September 28	5	13	38.4
September 30	1	3	33.3
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P = .250 - .500

Table VI. Degree of infestation with plerocercoids of Phyllobothrium spp. in male squid, $\underline{I.\ i.\ illecebrosus}$, 1967.

Date	Number of Squid Infected	Total Sampled	Percent Infected
July 25	10	11	90.9
July 27	11	13	84.46
August 1	21	23	91.30
August 8	11	16	68.75
August 29	9	20	45.50
September 22	0	8	0.00
October 2	4	11	36.36
October 5	3	14	21.42
October 8	0	4	0.00
October 17	5	26	19.23
October 27	1	9	11.11
October 28	0	10	0.00
November 16	4	26	15.38

Table VII. Degree of infestation with plerocercoids of Phyllobothrium spp. in female squid, $\underline{\text{I. I.}}$ illecebrosus 1967.

Date	Number of Squid Infected	Total Sampled	Percent Infected
July 25	17	17	100:00
July 27	10	13	76.92
August 1	8	8	100.00
August 8	4	6	66.66
August 29	8	19	42.10
September 22	0	10	0.00
October 2	2	28	7.14
October 5	7	17	41.17
October 8	3	6	50.00
October 17	14	42	33.33
October 27	0	13	0.00
October 28	0	10	0.00
November 16	6	23	26.08

in infestation was seen in late October. The same pattern could be followed in female squid during that year, but only two peaks were evident.

On comparing the degree of infestation with increasing mantle length, it was found that there was no significant change in degree of infestation during 1966 as the percentage of squid infested ranged from 11.11 percent in squid with mantle length 140-159 mm. and rose to 40.65 percent in squid measuring 200-219 mm. in mantle length (Table VIII). Although 100.00 percent of squid with mantle length 300-319 mm. were infested, only one specimen of this size was examined, and the results cannot be construed to indicate that all squid of this mantle length would be infested.

During 1967, it was found that the degree of infestation was significantly different in squid of different mantle lengths (Table IX).

Infestation with plerocercoids of <u>Phyllobothrium</u> did not occur in squid of a mantle length less than 180 mm., and reached a peak in squid with a mantle length of from 200-219 mm. and then there followed a decrease in percentage infestation. In general the percentage of squid infested decreased with increasing mantle length during 1967. Although 75.0 percent of the largest squid were parasitized, this sample was once again small.

It was found that there was no significant difference in the number of squid parasitized by plerocercoids of $\underline{Phyllobothrium}$ spp. when the data for 1966 and 1967 were compared, (Table X).

Table VIII. Percent infestation with plerocercoids of <u>Phyllobothrium spp.</u> with increasing mantle length in $\underline{I.}$ $\underline{i.}$ $\underline{illecebrosus}$, 1966.

Mantle Length (mm.)	Number Infected	Total Sampled	Percent Infected
140-159	1	9	11.11
160-179	3	14	21.42
180-199	10	41	24.39
200-219	50	123	40.65
220-239	48	120	40.00
240-259	22	63	34.92
260-279	4	13	30.76
280-299	2	6	33.33
300-319	1	1	100.00

P = .500 - .250

Table IX. Percent infestation with plerocercoids of Phyllobothrium spp. with increasing mantle length in \underline{I} . \underline{i} . $\underline{illecebrosus}$ 1967.

Mantle Length (mm.)	Number Infected	Total Sampled	Percent Infected
140-159	0	0	0.00
160-179	0	5	0.00
180-199	15	33	45.45
200-219	70	102	68.62
220-239	29	69	42.02
240-259	20	98	20.40
260-279	16	68	23.52
280-299	9	33	27.27
300-319	3	4	75.00

Table X. Annual variation in number of squid, <u>I. i. illecebrosus</u> parasitized by plerocercoids of <u>Phyllobothrium</u> spp., 1966 and 1967.

1966	1967
168	164
222	248
43.07	39.80
390	412
	168 222 43.07

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Genus: Dinobothrium Linton, 1922

<u>Dinobothrium plicitum</u> Linton, 1922 (Figure 7, Plate 10)

A single species of the genus <u>Dinobothrium</u> was found in the course of this study. It was identified as <u>Dinobothrium plicitum</u> Linton, 1922.

This identification is based on the presence of "hook-like" projections in the angles of the bothridia, as first described by Linton, and believed by Dollfus (1964) to be species diagnostic.

These are plerocercoids with very large bothridia constituting approximately one-third of the total body length. The plerocercoid in Plate 10 measured 2.60 mm. in length and 0.46 mm. in width. The bothridia measured 0.93 mm. by 0.49 mm. and terminated in the recurved "hook-like" processes mentioned above. Each bothridium is supplied with an accessory sucker, measuring 0.13 mm. in diameter. The bothridia are muscular but thin at the edges with no foldings. No internal structures can be seen through the cuticle.

Statistical Analysis of Data on the Genus Dinobothrium

Of the squid examined, it was found that 150 (18.7 percent) were infested with <u>D. plicitum</u>. This represents a total of 61 (15.54 percent) of the squid parasitized in 1966 and 89 (21.6 percent) in 1967. The sites of infestation by <u>D. plicitum</u> are shown in Table XI. The plerocercoids were all very small, with an average length of 2 mm.

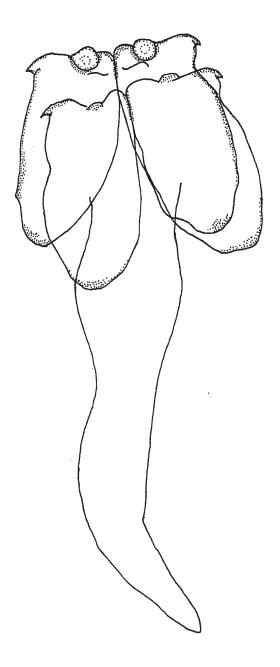


Figure 7. Plerocercoid of <u>Dinobothrium plicitum</u> removed from cyst in wall of intestine of <u>Illex illecebrosus</u> illecebrosus.

Plate 10. Photograph of a plerocercoid of $\frac{Dinobothrium}{plicitum}$ removed from a cyst in the intestine of $\frac{Illex}{hooks}$ illecebrosus illecebrosus. Note the

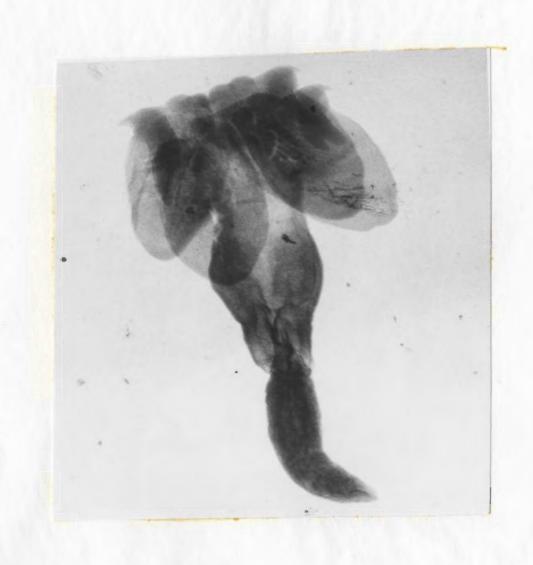


Table XI. Distribution of plerocercoids of <u>D. plicitum</u> in <u>I. i. illecebrosus</u> in 1966 and 1967.

Site of Infestation	Number of Squid Infected 1966 1967			
	Number Infected	Percent Infected	Number Infected	Percent Infected
Mantle Cavity	0	0.0	0	0.0
Esophagus	13	21.3	0	0.0
Hyponome	0	0.0	0	0.0
Kidney	1	1.6	0	0.0
Stomach	12	19.6	1	1.1
Pancreatic Portion of Hepatopancreas	0	0.0	0	0.0
Caecum	54	88.5	80	89.8
Intestine	38	62.2	51	51.6
Mesenteries	13	21.3	2	2.2

The degree of infestation in the squid ranged from a single plerocercoid to more than 100 in a heavily infested individual. In many cases, it was not possible to count the plerocercoids accurately as they were too numerous and encysted close together, making it impossible to discern one plerocercoid from another. In these cases, an estimation of the number of plerocercoids present was made.

The caecum was the site of heaviest infestation with 88.5 percent of squid infested in that region in 1966 and 89.8 percent in 1967. The site of next heaviest infestation was the intestine with 62.2 percent infestations in 1966 and 51.6 percent in 1967. Of note is the difference in percentage of squid infected in the esophagus, stomach and mesenteries during 1966 and 1967.

During the two year sampling period, 54.2 percent of the squid were infested in more than one body organ or area.

The incidence of multiple infestation was analysed with respect to the number of sites and combination of sites infested in individual squid. A total of 38 or 58.4 percent of the total number of infested squid in 1966 were found to have multiple infestations with D. plicitum. Multiple infestations with D. plicitum in 1967 were found in 50 percent of the squid examined, but the sites differed quite considerably from those infested in 1966 (Table XII).

The combination of sites infested by $\underline{\text{D. plicitum}}$ in 1966 ranged from two in 52.6 percent of the parasitized squid, to five sites

Table XII. Sites involved in multiple infestation by \underline{D} . plicitum in individual squid, \underline{I} . \underline{i} . illecebrosus, 1966 and 1967

Sites Infected	Number of Individu	nal Squid Infected
Intestine - Caecum	17	43
Intestine - Kidney Mesentry	1	
Intestine - Mesenteries		1
Esophagus - Mesenteries	1	
Caecum - Mesenteries	1	1
Intestine - Caecum - Mesenteries	4	
Intestine - Caecum - Stomach	5	
Intestine - Caecum - Esophagus	2	,
Caecum - Esophagus - Stomach	1	
Intestine - Caecum - Esophagus - St	comach 1	
Intestine - Caecum - Esophagus - Me	esenteries 1	
Intestine - Caecum - Esophagus - Stomach - Mesenteries	4	
TOTAL	38	45

in 10.5 percent of the squid. This was not the case in 1967 when no squid were infested in more than two sites.

The plerocercoids were normally found encysted in the intestinal or caecal walls (Plates 11, 12, 13, 14). Specimens found in other sites were also encysted.

There appeared to be a host reaction to the presence of the parasites resulting in an hypertrophy of the surrounding host tissue. In addition, a very thin, non-cellular layer was seen inside the mass of hypertrophied cells which formed the cyst and enclosed the parasite. This layer may have been secreted by either the parasite or the host.

It was possible in many cases to observeeliving plerocercoids and it was seen that they were mobile within the cyst wall.

Degree of infestation with $\underline{\text{D. plicitum}}$ was found to increase significantly with increasing mantle length during 1966 (Table XIII).

From the data presented in Table XIII, it can be seen that infestation was low in smaller squid (7.3 percent in mantle length 180-199 mm.), and rose to a maximum in the larger squid (100 percent in mantle length 280-299 mm.). It was found that the differences in degree of infestation with increasing mantle length were statistically significant.

The results pertaining to the incidence of infestation with increasing mantle length for the 1967 sampling period are presented in Table XIV.

Plate 11. Photograph of a transverse section through the intestine of <u>Illex illecebrosus</u> illecebrosus showing encysted plerocercoids of <u>Dinobothrium plicitum</u> in the wall.



Plate 12. Photograph of a longitudinal section through a plerocercoid of <u>Dinobothrium plicitum</u> in the wall of the intestine of <u>Illex</u> illecebrosus illecebrosus.

は 1000 mm 1



Plate 13. Photograph of a transverse section of an encysted plerocercoid of <u>Dinobothrium plicitum</u> in the wall of the <u>intestine</u> of <u>Illex illecebrosus</u> illecebrosus.



Plate 14. Photograph of a transverse section through a cyst of <u>Dinobothrium plicitum</u> in the wall of the intestine of <u>Illex illecebrosus</u> illecebrosus.

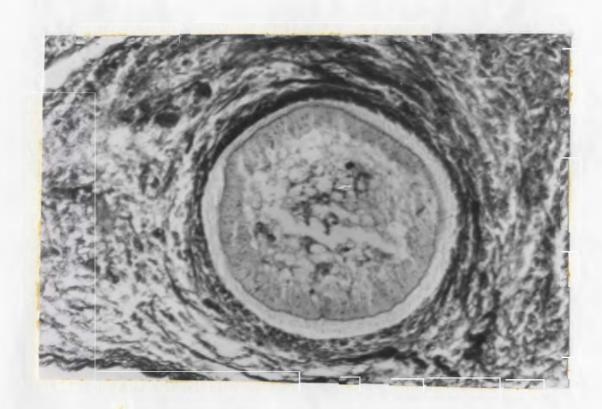


Table XIII. Percentage infection with <u>D. plicitum</u> with increasing mantle length in <u>I. i. illecebrosus</u>, 1966.

Mantle Length	Number Infected	Total Sampled	Percent Infected
140-159	0	9	0
160-179	0	14	0
180-199	3	41	7.3
200-219	13	123	10.5
220-239	21	120	17.5
240-259	18	63	28.5
260-279	7	13	53.8
280-299	3	3	100.0
300-319	0	1	0

P> .005

Table XIV. Percent infection with $\underline{\text{D. plicitum}}$ with increasing mantle length in $\underline{\text{I. i. illecebrosus}}$, 1967.

Mantle Length	Number Infected	Total Sampled	Percent Infected
140-159	0	0	0
160-179	0	5	0
180-199	6	33	18.1
200-219	23	102	22.4
220-239	16	69	23.1
240-259	22	98	22.4
260-279	15	68	22.0
280-299	7	33	21.5
300-319	1	4	25.0

P = .10 - .05

Here it was found that there was no significant difference in degree of infestation with increasing mantle length.

During 1967, no squid which measured less than 180 mm. in mantle length were parasitized with <u>D. plicitum</u>, as was the case in 1966. Infestation throughout the season ranged from 18.1 percent in squid measuring 180-199 mm.in mantle length to 25.0 percent in the largest squid obtained during the sampling period.

On comparing the data obtained in 1966 and 1967, it was found that there was no statistically significant difference in the degree of infestation with plerocercoids of \underline{D} . $\underline{plicitum}$ in squid between these two years (Table XV).

Table XV. Annual variation in number of squid, $\underline{I.\ i.}$ illecebrosus parasitized by plerocercoids of $\underline{D.\ plicitum}$, $\underline{1966}$ and $\underline{1967}$.

	1966	1967	
Number of squid infected	61	90	
Number of squid not infected	329	322	
Percent infected	15.64	21.84	
Total Examined	390	412	

P = .100 - .050

On comparing the degree of infestation between male and female squid in both 1966 and 1967, it was found that any differences in the degree of infestation were not statistically significant as can be seen from the data presented in Table XVI.

Table XVI. Number of male and female squid, <u>I. i. illecebrosus</u>, infested with plerocercoids of <u>D. plicitum</u> in 1966 and 1967.

	1966		196	<u>57</u>
	Male	Female	Male	Female
Number infected	31	30	41	48
Total Examined	179	209	191	164
	p = .	750500	P = .	.900750

There was a significant difference in the degree of infestation in both male and female squid in 1966 (Table XVII and XVIII) and female squid in 1967 (Table XX), as the season progressed. However, there was no significant difference in this respect in male squid during 1967 (Table XIX). During 1966, no squid were found to be infested with plerocercoids of <u>D. plicitum</u> until September 13th of that year. After that date infestation in male squid rose from 30.0 percent to 47.6 percent on September 22nd, after which infestation dropped to zero, and then began to rise once more. In female squid sampled over the same

Table XVII. Degree of infestation with plerocercoids of $\underline{\text{D.}}$ plicitum in male squid, $\underline{\text{I.}}$ i. illecebrosus, 1966.

Date	Number of Squid Infected	Total Sampled	Percent Infected
July 27	0	1	0.0
August 2	0	11	0.0
August 10	0	17	0.0
August 15	0	3	0.0
August 16	0	26	0.0
August 18	0	11	0.0
August 22	0	26	0.0
August 29	0	12	0.0
September 13	5	15	30.0
September 20	9	22	40.9
September 22	10	21	47.6
September 26	0	0	0.0
September 28	1	6	16.6
September 30	6	6	100.0

Table XVIII. Degree of infestation with \underline{D} . $\underline{plicitum}$ in female squid, \underline{I} . \underline{i} . $\underline{illecebrosus}$, $\underline{1966}$.

Date	Number of Squid Infected	Total Sample	Percent Infected
July 27	0	13	0.0
August 2	0	10	0.0
August 10	0	23	0.0
August 15	0	4	0.0
August 16	0	34	0.0
August 18	0	9	0.0
August 22	0	30	0.0
August 29	0	22	0.0
September 13	4	16	25.0
September 20	4	9	44.4
September 22	9	17	52.9
September 26	3	6	50.0
September 28	7	13	53.8
September 30	3	3	100.0

P > .005

Table XIX. Degree of infestation with plerocercoids of \underline{D} . $\underline{plicitum}$ in male squid, \underline{I} . \underline{i} . $\underline{illecebrosus}$, 1967.

Date	Number of Squid Infected	Total Sampled	Percent Infected	
			:	
July 25	2	11	18.1	
July 27	3	13	23.0	
August 1	9	23	39.1	
August 8	2	16	12.5	
August 29	6	20	30.0	
September 22	1	8	12.5	
October 2	0	11	0.0	
October 5	2	14	14.2	
October 8	0	4	0.0	
October 17	7	26	26.9	
October 27	0	9	0.0	
October 28	2	10	20.0	
November 30	7	26	26.9	

P = .500

Table XX. Degree of infestation with $\underline{\text{D. plicitum}}$ in female squid, $\underline{\text{I. i. illecebrosus}}$, 1967.

Date	Number of Squid Infected	Total Sampled	Percent Infected	
July 25	9	17	52.9	
July 27	0	13	0.0	
August 1	1	8	13.7	
August 8	4	6	66.6	
August 29	6	19	31.5	
September 22	0	10	0.0	
October 2	4	28	17.8	
October 5	7	17	41.1	
October 8	2	6	33.3	
October 17	8	42	19.0	
October 27	2	13	15.4	
October 28	0	10	0.0	
November 16	5	23	21.7	

P = .025 - .010

period, infestation was first encountered at 25.0 percent and continued to rise throughout the remainder of the sampling period, showing no tendency to decrease as the season progressed. In 1967, however, squid were found to be infested with plerocercoids of <u>D. plicitum</u> quite early in the sampling season. In male squid examined during that year, infestation varied throughout the season, resulting in a number of peaks, e.g. during October (Table XIX) infestation varied from 0.0 percent on October 2 to 14.0 percent a few days later, and dropped again to 0.0 only to rise to 26.0 percent during the middle of the month. Overall variation was not significant, however. In female squid examined during the same period, infestation showed definite peaks, with one occurring in August, one in mid-October, and another in November. Infestation was found to be low in July, September and late October.

Order: Trypanorhyncha Diesing, 1863

Genus: Nybelinia Poche, 1926 (Figure 8, Plate 15)

<u>Nybelinia</u> sp.

A single specimen of a trypanorhynch was recovered from a squid examined during 1967. This was a post-larval form of the genus Nybelinia Poche, 1926. This constitutes the first record of any specimen of the Order Trypanorhyncha from Illex illecebrosus illecebrosus.

The specimen recovered was a post-larva having four, smooth-edged bothridia. The total length of the specimen was 1.75 mm. and it was 0.93 mm. in width. Four probosces were present and bore hooks (Figure 9),

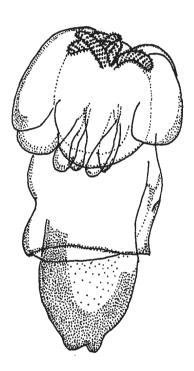


Figure 8. Post-larval form of Nybelinia sp. from the caecum of Illex illecebrosus illecebrosus.

Plate 15. Photograph of the post-larva of Nybelinia sp. from the caecum of Illex illecebrosus illecebrosus.



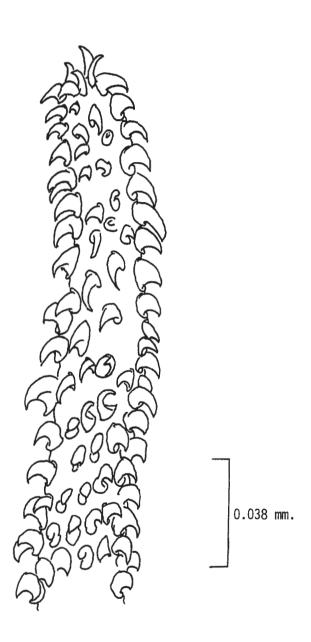


Figure 9. Portion of a proboscis of Nybelinia sp. from caecum of $\underline{\text{Illex illecebrosus}}$ illecebrosus.

which had an average measurement of 9.6 μ , the four proboscis bulbs being 0.28 mm. in length and 0.12 mm. in width. The bothridia measured 0.62 mm. in length and were 0.36 mm. in width.

Statistical Analysis of Combined Data on Cestode Parasites

Of the total number of squid examined during 1966 and 1967, 400 (49.8 percent) were parasitized with plerocercoids of tetraphyllidean cestodes.

In addition to the previous statistical analyses, analysis of data was carried out including all genera of cestodes found.

It was found that monthly variations (Table XXI) between July 1966 and 1967, and September 1966 and 1967 were statistically significantly different when tested by the x^2 method. However, any difference in August of 1966 and 1967 was not significant. As no samples were taken after September 30, 1966, comparisons beyond that date were not possible, even though squid were sampled through November in 1967.

Although the differences in degree of infestation were significantly different between July and September 1966 and 1967, on comparing the annual variations between these two years, no significant difference could be found. Similarly, on comparing monthly variations in degree of infestation for any year, no significant difference was evident between July, August and September, 1966. This was not the case

Table XXI. Monthly variation in rate of infestation by cestode parasites in <u>I. i. illecebrosus</u>, July - September, 1966 and 1967.

	July		August		September	
	1966	1967	1966	1967	1966	1967
Number of squid infected	10	52	111	74	76	1
Number of squid not infected	5	2	130	26	58	17
Total Sampled	15	54	241	100	134	18
	р :	050	P :	> .500	P >	.005

during 1967 when differences in degree of infestation between monthly samples were found to be highly significant (Table XXII).

As can be seen from the data presented in Table XXII, the months of peak infestation by cestodes were July and August. Only one sample of squid was obtained in September, and only one squid of this sample was parasitized.

On comparing degree of infestation by cestode parasites in female squid (Table XXIII), it was found that as the 1966 season progressed, there was no significant variation in degree of infestation. This was

Table XXII. Monthly variation in rate of infestation by cestode parasites in $\underline{I.\ i.\ illecebrosus}$, July-November, 1967.

	July	August	September	October	November	
Number of squid infested	52	74	1	71	19	
Number of squid not infested	2	26	17	130	30	
Total Sample	54	100	18	205	49	
		P > .005				

Table XXIII. Seasonal variation in degree of infestation by plerocercoids of cestodes, in female squid $\underline{I.\ i.}$ illecebrosus, 1966.

Date	Number of Squid infected	Total Sampled	Percent Infected
July 25	9	13	69.2
August 2	3	10	30.0
August 10	13	23	56.6
August 15	3	4	75.0
August 16	18	34	52.9
August 18	6	9	66.6
August 22	17	30	56.6
August 29	11	22	50.0
September 13	8	16	50.0
September 20	5	9	55.5
September 22	11	17	64.7
September 26	8	13	61.5
September 28	5	6	83.3
September 30	3	3	100.0

not the case in 1967 (Table XXIV), when a peak of infestation was seen in July and early August. This was followed by a sudden decrease in the percentage of female squid infected in September. The percentage of infestation then gradually rose to 66.6 percent in early October before decreasing again in late October and early November.

It was found that there was a significant variation in degree of infestation in male squid with cestodes as both the 1966 and 1967 sampling season progressed (Table XXV and XXVI). Infestation early in the season was high and decreased in early August. It then rose during August and was maintained through September until late in the month when the percentage of squid infested decreased.

During 1967, infestation was again high early in the season but was quite low in the single sample examined during September. Infestation then rose to 75.0 percent in early October before decreasing once again. By late October and early November, the percentage of male squid infested again showed an upward trend.

It was found that throughout the 1966 sampling period, there was no significant difference in the degree of infestation with cestode plerocercoids when compared with increasing mantle length (Table XXVII).

Only 11.11 percent of the squid with a mantle length of 140-159 mm. were infested, the degree of infestation increasing to 76.92 percent in squid of mantle length 260-279 mm. Only one squid with a mantle length

Table XXIV. Seasonal variation in degree of infestation by plerocercoids of cestodes, female squid <u>I.i.</u> i<u>llecebrosus</u>, 1967.

Date	Number of Squid Infected	Total Sampled	Percent Infected	
July 25	17	17	100.0	
July 27	12	13	92.3	
August 1	8	8	100.0	
August 8	6	6	100.0	
August 29	10	19	52.6	
September 22	0	10	0.0	
October 2	6	28	21.4	
October 5	10	17	58.8	
October 8	4	6	66.6	
October 17	16	42	38.0	
October 27	3	13	23.0	
October 28	1	10	1.0	
November 16	9	23	39.1	

P > .005



Table XXV. Seasonal variation in degree of infestation by plerocercoids of cestodes in male squid, \underline{I} . \underline{I} . \underline{I} . \underline{I} .

Date	Number of Squid Infected	Total Sampled	Percent Infected
July 25	1	1	100.0
August 2	3	11	27.2
August 10	7	17	41.1
August 15	0	3	0.0
August 16	4	26	13.3
August 18	4	11	36.3
August 22	16	26	61.5
August 29	6	12	50.0
September 13	7	15	46.6
September 20	10	22	45.4
September 22	12	21	57.1
September 26	1	6	16.6
September 28	0	0	0.0
September 30	6	6	100.0
	P = .0	10025	

Table XXVI. Seasonal variation in degree of infestation by plerocercoids of cestodes in male squid, $\underline{I.}$ $\underline{i.}$ $\underline{illecebrosus}$ 1967.

Date	Number of Squid Infected	Total Sampled	Percent Infected
July 25	11	11	100.0
July 27	12	13	92.3
August 1	23	23	100.0
August 8	12	16	75.0
August 29	11	20	55.0
September 22	1	8	12.5
October 2	3	11	27.2
October 5	4	14	28.5
October 8	3	4	75.0
October 17	8	26	30.7
October 27	1	9	11.1
October 28	5	10	50.0
November 16	10	26	38.4

P > .005

Table XXVII. Degree of infestation by cestode plerocercoids with increasing mantle length in \underline{I} . \underline{i} . \underline{i} . \underline{i} .

Mantle Length Intervals (mm.)	Number of Squid Infected	Total Sampled	Percent Infected
140-159	1	9	11.11
160-179	4	14	28.57
180-199	13	41	31.70
200-219	58	123	47.15
220-239	61	120	50.85
240-259	32	63	50.79
260-279	10	13	76.92
280-299	4	6	66.66
300-319	1	1	100.00

P = .500 - .250



of 300-319 mm. was examined and the fact that the squid was infested does not mean that all squid of this size will be infested.

This was not found to be the case in 1967 when it was found that the difference in degree of infestation with increasing mantle length was statistically significant (Table XXVIII).

No squid were infected which measured less than 180 mm. in length. Infestation was high (77.45 percent) in squid of mantle length 200-219 mm., and then decreased as mantle length increased. However, all squid with a mantle length of 300-319 mm. were found to be parasitized, although it must be remembered that this sample was very small and the figure obtained probably does not express the true picture of parasitism in squid of this size range.

Nematodes

Cestodes were not the only parasitic forms found in the squid examined. Thirteen (1.6 percent of the 802 squid examined) were found to be infested with larval nematodes of the genus <u>Contracaecum</u>, Railliet and Henry, 1912. Three squid were found to be so infested in 1966 while ten were infested in 1967. Sites of infestation with these parasites are indicated in Table XXIX.

More specifically, a single specimen was embedded on the surface of one of the branchial hearts; others were found embedded on the internal surface of the mantle, both at the anterior end, and

Table XXVIII. Degree of infestation by cestode plerocercoids with increasing mantle length in $\underline{I.}$ $\underline{i.}$ $\underline{illecebrosus}$, 1967

Mantle Length Intervals (mm.)	Number of Squid Infected	Total Sampled	Percent Infected
140-159	0	0	0.00
160-179	0	5	0.00
180-199	17	33	51.51
200-219	79	102	77.45
220-239	38	69	55.07
240-259	39	98	30.61
260-279	24	68	35.29
280-299	12	33	36.36
300-319	4	4	100.00

P > .005



Table XXIX. Site of infestation by larvae of Contracaecum sp. in I. i. illecebrosus, 1966 and 1967.

	Hepatic Portion of Hepatopancreas	Caecum	Branchial Heart	Stomach	Vena Cava	Mantle
1966	0	2	0	0	0	1
1967	1	4	1	3*	1*	1

^{*}In one instance, a squid was infested in both stomach and vena cava.

the posterior end near the fin; others were found on the surface of the hepatic portion of the hepatopancreas as well as on the surface of the posterior vena cava, the external surface of the caecum, and on the external surface of the stomach.

Due to the small numbers of nematodes recovered, no statistical analysis of the data was made.

Dogfish and Skates

A total of 95 dogfish (<u>Squalus acanthias</u>) and 90 thorny skates (<u>Raja radiata</u>) were examined for cestode parasites. The dogfish ranged in size from 390 mm. to 765 mm. in length and from 1301 grams to 3180 grams in weight. The length of the skates ranged from 264 mm. to 680 mm. and weighed from 986 grams to more than 4620 grams.

Only three of the dogfish examined were found to be infested with cestode parasites, isolated proglottids being recovered from the spiral caecum of two and complete cestodes from another.

The cestodes recovered from the dogfish were identified as belonging to the Order Trypanorhyncha. (Plate 16)

 $\label{eq:contracaecum} In \ addition \ to \ cestodes, 26 \ dogfish \ were \ found \ to \ be \\ infested \ with \ nematodes \ of \ the \ genus \ \underline{Contracaecum}.$

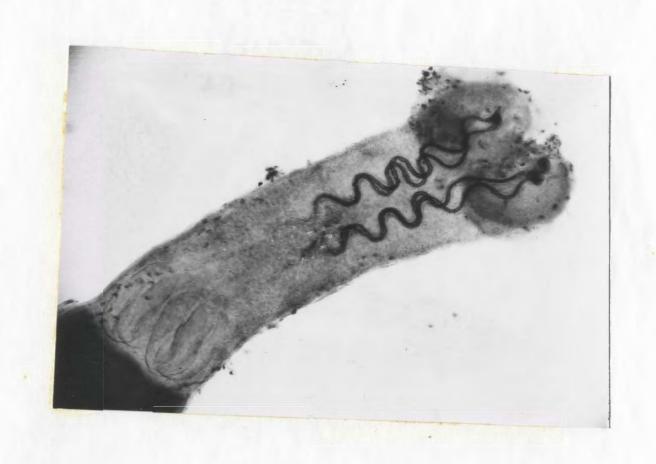
Of the 90 skates examined, two were found to be infested with cestode parasites. In both instances, whole worms were found in the spiral caecum, the helminths being identified as Trypanorhynchs.

Small numbers of larval nematodes of the genus <u>Contracaecum</u> were also found.



Plate 16. Photograph of a portion of an unidentified trypanorhynch from the spiral caecum of the spiny dogfish Squalus acanthias.

Dollfus identified this parasite as $\underline{\text{Gilquinia}}$ $\underline{\text{squali}}$ (0. Fabricius, 1794), personal communication.



DISCUSSION

Relatively little is known about parasites of decapod cephalopods. Perhaps the best known are the mesozoans which infest the renal organs of some cephalopods. Knowledge of them is due in large measure to the work of McConnaughey (1948) and yet, the life cycle of not a single species of dicyemid mesozoan is known.

Efforts have been made to suggest a correlation between infestation by parasites and mortality of cephalopods. Verrill (1881), in reporting on a mass mortality of giant squids of the genus Architeuthis in the Grand Banks area stated that the deaths were probably due to large infestations with parasites, yet he presented no data to substantiate his hypothesis. Too often, parasitism is confused with pathogenicity. As Lane (1960) pointed out, octopods are frequently infested with parasites, but as was observed by Robson (1929) "signs of disease are singularly rare", with only two specimens exhibiting diseased conditions from a sample of 400.

Numerous authors have reported the presence of cestode parasites in Ommastrephids (Table I). However, very little data concerning the host animal has been recorded in these works. The information that is given pertains generally to the locality where the host animal was caught. No indication is given of the age, size, sex, or numbers of decapods involved, and very seldom are numbers of parasites recorded.

The study of squid helminths is further complicated by the incomplete information concerning the parasites themselves. Little, if anything, is known about their life cycles, although many speculations regarding these have been postulated. The systematics of the genera, not to mention the species, are filled with synonomies.

Numerous records are available which state the locations in the host animal from which helminth parasites have been recovered.

More specifically, Guiart (1933) reported larval cestodes from the mantle of Ommastrephes (=Todarodes) sagittatus from the Mediterranean. Linton (1897) published a record of Phyllobothrium loliginis (first described in 1887 by J. Leidy as Taenia loliginis, then later designated as Tetrabothrium loliginis by Leidy (1890)), from the stomach of squid from the coast of Massachusetts. However, Linton does not state whether the squid was Illex illecebrosus or Loligo pealeii, both of which are inhabitants of that area.

free in the stomach, caecum and rectum of <u>I. illecebrosus</u>, stating that when living the plerocercoids moved freely between these organs. Dollfus (1958, 1964) reported plerocercoids of <u>Phyllobothrium tumidum</u> Linton, 1922 in <u>I. illecebrosus coindeti</u> from the mantle cavity, esophagus, stomach, pancreas and liver. Mercer (1965) duplicated much of Squires' work and agreed with his data, including observations with regard to both the composition of the parasite burden and the mobility of the parasites.

The validity of his observations with respect to collection sites is not doubted, but it is doubtful that the sites recorded are the actual 1 Dollfus corrects this and syas that Linton (1897) indicated the squid to be <u>Illex illecebrosus</u>. (Personal communication)

sites of infestation in nature.

As was stated earlier, several methods of preserving squid from time of capture to time of examination were employed. When squid were caught and placed in plastic bags and brought to the Laboratory for examination, parasites were found in the regions that were reported by Squires and others. However, whenever squid were placed in ice immediately upon capture, plerocercoids of Phyllobothrium were not found in any organ except the caecum. Commercially secured samples in 1967 from bait depots demonstrated this phenomenon well, as several hours elapsed between the time the squid were caught and the time they were frozen. These squid, when examined were found to have plerocercoids free in the mantle cavity, rectum and stomach. This serves to illustrate the fact that, if the squid are allowed to warm up after capture, the parasites will begin to move about in, and even leave, the host. Providing the squid are kept chilled, no such movement will occur, and parasites will be found where they would normally be found in nature. The earlier-held contention that larval Phyllobothriids exhibit a degree of freedom of movement between organs of decapod cephalopods can no longer be supported.

According to Riser (1949) the Tetraphyllidea can be divided into five types based on the configuration of their bothridia (phyllidea). Of the groups that he outlined, only one contains Phyllobothrids reported from squid, namely, Type 4. These forms have leaf-like bothridia which

bear accessory suckers and an apical sucker. Type 4 of Riser is further characterized by a pars prostica which is a parenchymatous caudal appendage that is lost in the transition to the adult form.

As can be seen from the information found in Table I, only two genera of the Family Phyllobothriidae have previously been recovered from I. i. illecebrosus, namely, Phyllobothrium and Dinobothrium.

The most recent records of these genera of helminths from the short-finned squid are those of Squires (1957), Aldrich (1964) and Mercer (1965). The specimens in the collections reported by these investigators have never been identified down to the species level because the extreme flexibility of the scolex so alters its configuration that identification based on this feature is not reliable. Moreover, species identification of cestodes is, in most cases, based on sexually mature individuals and only plerocercoid larvae have so far been found. It is doubtful if it is prudent to even attempt to assign tentative species identifications to such material. Riser (personal communication), himself a student of the Tetraphyllidea, cautions against any effort to tentatively assign any of the specimens to a definite species in the absence of definitive information. He insists that the only proper way to handle the material at the present time is to tentatively combine like material and refer to them as Type 1, Type 2, etc.

Numerous specimens of $\underline{Phyllobothrium}$ have been recovered from squids of the Family Ommastrephidae (Table I). Leidy (1887) recovered

a small larval cestode from Ommastrephes (=Illex) illecebrosa which he placed in the genus Taenia and published as Taenia loliginis. Three years later, Leidy (1890) reported what he considered to be the same larval form from O. (=I.) illecebrosa. On this occasion he changed the genus designation to Tetrabothrium referring to the larva as Tetrabothrium loliginis but he did not exclude the possibility of the larva belonging to the genus Phyllobothrium. Linton (1897) having found the same larval form in the stomach of O. illecebrosa from the coast of Massachusetts, published his record under the name Phyllobothrium loliginis. His illustrations of this species show that the larvae seen by both authors are probably identical. Linton later (1922) recorded the same species of helminth from Loligo pealeii, the common Atlantic squid. Dollfus (1929) illustrated and published a record of Phyllobothrium sp. from Loligo vulgaris.

Assuming their identifications to be correct, it is apparent that this helminth parasitizes both Ommastrephid and Loliginid decapods.

The plerocercoids recovered from $\underline{I.\ i.\ illecebrosus}$ in this study resemble that figured by Dollfus (1964, Figure 30, page 355), which he reports from $\underline{I.\ i.\ coindeti}$. The main difference between the helminth reported here and that of Dollfus is that the apical sucker in the plerocercoids from $\underline{I.\ i.\ illecebrosus}$ appears less well developed than it is in the form from $\underline{I.\ i.\ coindeti}$. This may be attributed to differences in method of preservation, for as stated earlier, Riser (1949) points out the extreme variability of scolices and their structures due to differing degrees and methods of preservation.

Not all the plerocercoids from <u>I. i. illecebrosus</u> were at the same stage of development. In the early parts of the sampling season in 1966, the plerocercoids were small (5.0 - 15.7 mm. in length) and showed no signs of strobilization until later in the sampling period when, in a small number of individuals, strobilization was evident. However, in 1967, strobilization was evident throughout the sampling period. This would seem to indicate that the length of time which the plerocercoid inhabits the host is of considerable duration. Furthermore, it appears that the contention held by Squires (1957) to the effect that the short-finned squid lives but one year to fifteen months is placed in serious doubt in that a longer life for the squid would better fit the observations pertaining to the development of the plerocercoids.

The first intermediate host for Phyllobothrium has been reported to be crustaceans, particularly euphausids on which it is postulated that the squid feed before they move into coastal waters (Mercer, 1965). Euzet (1959) introduced embryonated eggs of Phyllobothrium tumidum obtained from the smooth dogfish (Mustelus canis (Mitchill)) into the diet of the copepods Acartia clausi Giesbr. and Acartia discaudata Giesbr. and observed that the eggs developed into procercoid larvae. Dollfus (1964) reported unidentified tetraphyllidean larvae from the body cavity of another copepod Eucalanus pseudattenuatus obtained from plankton. He reported that the presence of these larvae caused parasitic castration and ovarian degeneration in their copepod hosts.

Embryonated eggs from gravid proglottids of Acanthobothrium

hispidum from the spiral valve of the ray Tetronarce californica (Riser,
1949) were introduced into the copepod Tigriopus fulvus where these eggs
developed into procercoids. From the records of experimental infestation
reported above, it seems clear that the intermediate host(s) for the
genus Phyllobothrium is/are species or planktonic crustacea. Therefore,
it is probably that the short-finned squid, in Newfoundland waters, obtains
procercoids of Phyllobothrium during that period when they feed on such
planktonic crustaceans as euphausids of the genera Thysanoessa and
Meganyctiphanes (Squires, 1957).

The definitive hosts for Phyllobothrium are elasmobranchs
(Joyeux and Baer, 1936). Linton (1922) reported adult Phyllobothrium from the white shark Carcharias (L.), and the mackerel shark, Isurus
dekayi Smith. In 1924 he collected Anthobothrium laciniatum from the sand shark Carcharhinus milberti (Müller and Henle) and the summer skate Raja
eglanteria Bosc. However, none of the 200 dogfish and skates examined in this study, were found to be infested with Phyllobothrium. In addition, none of the elasmobranchs examined contained remains of squid. Templeman (1944) examined over 1,000 specimens of the spiny dogfish, Squalus acanthias and found that only one percent of the specimens had fragments of squid in their stomachs. At best, the squid must be considered as an accidental or less favoured item in the diet of this species of elasmobranch:

Plerocercoids of <u>Phyllobothrium</u>, at the same or similar stages as those found in the squid, have been found encysted in the fat of a

porpoise (Van Beneden, 1870). Cowan (1967) reported encysted plerocercoids of Phyllobothrium from the pilot whale, which he said fed on squids. Cysticercoids of Phyllobothrium delphini (Bosc) were reported by Sergeant (1962) as being embedded in the blubber of the pothead whale, Globicephala melaena (Traill) from the Newfoundland area. It is well known that this species of whale is considered by some to feed exclusively on I. i. illecebrosus in the Newfoundland area (Sergeant, Recently Adlrich and Bradbury (1968) reported that this is not true, in that both the short-finned squid and the Arctic squid, Gonatus fabricii (Lichtenstein) are present in the diet of the pothead whale from Trinity Bay. Their data confirm that the former species far outnumber the latter. It may be assumed that upon consumption of the squid, the whale becomes infested with the plerocercoids here reported. If Sergeant's (1962) designation of the species as P. delphini is correct, then it is most probable that the species infesting the ommastrephid is P. delphini. It must not be overlooked, however, that the infestation of the whale may in fact, be via the introduction of larval cestodes from other sources, such as G. fabricii.

We shall next consider the species <u>Pelichnibothrium speciosum</u>. This species has not been previously recorded from <u>I. i. illecebrosus</u>. However, Riser (1949, 1956) reports this species as having been recorded from <u>Dosidicus gigas</u> taken at Corona del Mar, California, and from <u>Loligo opalescens</u> Berry. Although Riser (1956) reported small plerocercoids of <u>P. speciosum</u> in the above species of decapods, he went on to describe

in detail large (1,450 mm. in length) neotenic larvae from the lancet fish Alepisaurus ferox Lowe. He postulated that the parasite remained but a short time in this host.

Lancet fish (Family Alepisauridae) are deep sea fish, with A. ferox reported as occurring in the Atlantic "off the coast of Nova Scotia . . . and the Grand Banks" (Breder, 1948), occasionally entering more shallow coastal waters (Dollfus, 1967). It is conceivable that A. ferox feeds on I. i. illecebrosus prior to the migration of the squid into coastal embayments. Lane (1957) quotes a personal communication to him from G. L. Voss to the effect that "stripping this fish is one of the approved methods of collecting deep sea squids". It should be pointed out, however, that the diet of \underline{A} . \underline{ferox} is not restricted to deep sea squids alone, for in addition to numerous species of fish, surface-living squid are also eaten (G.E. Maul, fide Marshall, 1954). Rees and Maul (1956) published findings which state that as a total of 18 species of cephalopods were recovered from the stomachs of A. ferox, it may be concluded that cephalopods are a regular item in the diet of this fish. It may be in just this way that the plerocercoids are transferred to the definitive host, \underline{A} . \underline{ferox} . Certainly, no indication of strobilization was evident in the nine specimens reported here for the first time from I. i. illecebrosus.

According to Riser (1949) the definitive host being <u>A. ferox</u>, there may be no adult form of <u>P. speciosum</u>, in that it may rely on reproduction by the neotenic larval form. Yamaguti (1934) reported <u>P. speciosum</u> from the blue shark <u>Prionace glauca</u> (L.), and it is unclear

whether or not he is writing about an adult form. The blue shark occasionally occurs in the coastal waters of Newfoundland, and it may be that $\underline{I.\ i.\ illecebrosus}$ harbours the final larval stage in the life cycle of $\underline{P.\ speciosum}$, whether it is consumed by $\underline{A.\ ferox}$ or $\underline{P.\ glauca}$. It may be that $\underline{P.\ glauca}$ feeds directly upon $\underline{A.\ ferox}$, and the parasite is transmitted via this food source.

Since only one squid was found to be infested with \underline{P} . $\underline{Speciosum}$, it may be that the infestation of the short-finned squid was merely accidental, the squid not being a normal intermediate host. The voracious feeding habits of shoals of \underline{A} . \underline{ferox} would enable them to consume a large proportion of any school of squid they encountered, thereby greatly reducing the possibility of \underline{P} . $\underline{speciosum}$ -infected individuals being encountered inshore, and accounting for the low incidence of infestation with this species of helminth in the Newfoundland short-finned squid.

Scolex polymorphus is a species to which plerocercoid larvae of uncertain affinity have, in the past, been grouped. The single specimen encountered here for the first time in $\underline{I.\ i.\ illecebrosus}$ was unquestionably characterized by biloculate bothridia (Plate 9). On this basis, it can be considered as belonging to the "types" of $\underline{S.\ polymorphus}$ outlined by Dollfus (1964). It is not clearly any of the individuals he described or figured and on the basis of one specimen, little else can be said about this form at this time.

Another genus of the Family Phyllobothriidae that was present in the squid was <u>Dinobothrium</u>. The incidence of this genus in

Ommastrephids has been presented earlier. However, Squires (1957), Aldrich (1964) and Mercer (1965) all were content to leave their collections designated to the generic level. The genus is easily identified in that it is distinctive in having perhaps the largest holdfast for any tapeworm of its size (Wardle and McLeod, 1952).

There are several references to attempts to identify the species of <u>Dinobothrium</u> in <u>I. i. illecebrosus</u>. <u>Thysanocephalum crispum</u> Linton? (Linton, 1897) is perhaps the earliest found by Linton in <u>Ommastrephes illecebrosa</u> (=<u>I. illecebrosus</u>) from the Woods Hole, Massachusetts, area.

A second species, namely, <u>D. plicitum</u> was described by Linton (1922) from the basking shark, <u>Cetorhinus maximus</u> (Günner) at Menemsha Bight, Massachusetts. Sproston (1948) placed both of these species in synonomy with <u>D. septaria</u> Van Beneden. The systematics of this genus is little understood and is at best confusing. Dollfus (1936) and later (1964) stressed the importance of the characteristic feature of the scolex, namely, the roof-like ridge that connects the anterior ends of the bothridia pairs ending in curved hooks. These structures are readily evident in the present collections from <u>I. i. illecebrosus</u> (Plate 10), and bear great similarity to the *plateau apical* as illustrated by Dollfus (1964, Figure 33, page 358). It is proposed to accept Dollfus' contention that final identification of any larval <u>Dinobothrium</u> is, in the final analysis, dependent upon comparisons with Van Beneden's and Linton's

original type material and the designation <u>D. plicitum</u> is here temporarily adopted. As recently as 1955, Euzet has suggested a sweeping revision of the genus but leaves unresolved the individuals reported from decapod cephalopods.

Sproston (1948) concurs with the findings of Dollfus (1936) that cephalopods of the Family Ommastrephidae are undoubtedly the intermediate host for certain species of <u>Dinobothrium</u>. Specifically, she restricts this to <u>D. septaria</u> but the significance of this conclusion may only be realized when the taxonomy of the group is made clear.

Linton (1922) reported adults of <u>D. plicitum</u> as occurring in the white shark (<u>Carcharodon carcharias</u>) at Woods Hole, Massachusetts. Other species of <u>Dinobothrium</u> have been reported from a variety of sharks, including <u>D. spinosum</u> (Baylis, 1950) from the basking shark, <u>Cetorhinus maximus</u> (Günner) and <u>D. septaria</u> from a species of porbeagle Lamna cornubica (Gmelin) (Euzet, 1955).

 $\mbox{ Again, $\underline{\mbox{Dinobothrium}}$ was not found in any of the elasmobranchs examined in this study and the final host in this area is not known.}$

As was stated earlier, it was found that plerocercoids of \underline{D} .
Plicitum were normally encysted in the walls of the caecum and intestine of their host, \underline{I} . \underline{i} . \underline{i} illecebrosus. A thin structurless membrane was found which enclosed the parasite, while a mass of hypertrophied host tissue enclosed both the parasite and the membrane. No information is available concerning cyst formation by either the parasites or their cephalopod hosts. However, Sparks and Chew (1966), recovered plerocercoids

of the tetraphyllidean, <u>Echeneibothrium</u> sp. from the tissues of the littleneck clam, <u>Venerupis staminea</u>, and the results of histological sectioning of the parasites showed that they were encysted in the host tissue, the cyst being "a compact network of fine collagenous fibers and leucocystes". Dogier (1962) in referring to encysted forms in general, remarked that often the parasites are surrounded by a capsule of connective tissue.

The cysts surrounding \underline{D} . $\underline{plicitum}$, here reported, were most probably formed by the host, but as no histochemical staining procedures were carried out the exact nature of the cysts could not be determined.

One must speculate if Mercer's (1965) reference to a cyst of <u>Dinobothrium</u> measuring "less than two centimeters" is, in fact, a typographical error. He considers that this would be a "small" cyst, when in fact, cysts of such great size were never encountered in this study.

The final cestode parasite that was recovered from <u>I. i.</u> <u>illecebrosus</u> was a post-larval form of <u>Nybelinia</u> sp. (one specimen) of the Order Trypanorhyncha. This genus has not previously been recorded from this squid, however, it has been recovered from other cephalopods, including the Ommastrephids <u>Gonatus fabricii</u>, <u>Dosidicus gigas</u> and <u>Todarodes pacificus</u>.

This genus of cestodes has been described in detail by Dollfus (1966, 1967). Dollfus (1967) described and figured three forms of

However, the specimen taken from the short-finned squid Nybelinia. in this study does not correspond in many features to any of the previously described types. If the differences between the groups of Nybelinia figured by Dollfus (1967) and the single specimen here reported were confined to differences in total length, bothridia length, etc., they could be attributed to differences in age and methods of preservation. The most significant difference, however, involves hook sizes, in that those in the three groups described by Dollfus (1967) range from 12 μ to 13.33 μ (Form 1), 12 μ to 14 μ (Form 2) and 8 μ (Form 3) in size. The size of the hooks from the single specimen of Nybelinia was 9.6 μ placing it in an intermediate size class between Dollfus' Form 2 and Form 3. The hooks on the proboscis are similar in shape and distribution to the configuration shown for his Form 2 (Dollfus, 1967, Figure 16, page 165) and basically resemble in overall configuration the post-larva shown in his Figures 17 and 18 (page 165). In the specimen here reported, the retractable appendix is extended posteriorly. With respect to overall length, the specimen is twice as long as that stated for this form, but this may be a function of the "age" of the post larva in question, or method of fixation.

No conclusion may be drawn with respect to the specific identification of this single specimen but it agrees most closely with Form 2 of Dollfus (1967).

From the results presented earlier, it was seen that many variations occur in the parasite burden of $\underline{\text{I. i. illecebrosus}}$. Little

has been written on any relationship between parasite burden and the Newfoundland short-finned squid save that of Squires (1957) and Mercer (1965) who postulated that $\underline{I.\ i.\ illecebrosus}$ became infested with cestode parasites while they were in the offshore waters of the Grand Banks. Until it is certain that young squid feed in this area, this can only be assumed. In the preceding pages the species of parasites encountered in this study have been discussed in light of what is known concerning their life histories.

Planktonic crustaceans are probably the source of infestation in most instances and, it is not impossible that smaller teleosts such as the capelin, Mallotus villosus (Müller) occupy this position, as these fish also feed on crustaceans and in turn are fed upon by the squid. De Castellanos (1964) omitted this possibility when she outlined the food cycle of the closely related ommastrephid <u>I. i. coindeti</u>. It has further been shown how the cestodes found may be transmitted to definitive hosts, such as larger teleosts, numerous species of selachians and even cetaceans. Representatives of all these groups are found in Newfoundland coastal waters and the area of the Grand Banks.

With respect to <u>Phyllobothrium</u> sp. (page 105) it was pointed out that the older or more advanced plerocercoids exhibiting strobilization may constitute evidence of a second year in the life of the squid in Newfoundland waters. This is contrary to Squires' (1957, 1959) often quoted limit of 12 to 15 months for the life span of \underline{I} . \underline{i} . \underline{i} illecebrosus.

Too little is known about this squid to be able to use observed parasite populations as being indicative of essential facts in the life history of the host. Dogiel (1962) presents numerous examples pertaining to the shift in parasite fauna with host migration.

Lu (1968) reports a group of squid entering coastal embayments in the autumn months and these squid are small in that they are of the size range characteristic of squid that enter the same waters in late July. He, therefore, postulates the existence of two populations and also believes that the squid overwinter in the Newfoundland area. Squires (1957), Mercer (1965) and Templeman (1966) all infer a southerly spawning migration for the Newfoundland short-finned squid. The necessity for this is put into some question due to the recent discovery of maturing females in local waters and successful artificial fertilization of eggs, as well as the discovery of females bearing sperm bulbs (Aldrich, et al., 1968). Other evidence from this study of the parasites may further support Lu's basic contention.

In 1966, 84.07 percent of the squid were infested with low numbers (1-4) of specimens of Phyllobothrium sp. In 1967, this figure was only 29.81 percent and 1967 samples had the greatest number of individuals per squid. This could represent repeated infestation in 1966 squid over a longer period of time and these squid surviving into 1967. Certainly the total infestation was the same for the two years, 40 percent in 1966 and 39 percent in 1967.

Also with respect to <u>Phyllobothrium</u> sp. in 1966, the rate of infestation was homogeneous throughout the sampling period, while in 1967 the rate was not homogeneous, squid of mantle length 180 mm. and less being uninfested. In addition, nearly five times as many of the squid examined were less than 180 mm. in mantle length in 1966 than in 1967. These observations may further indicate that, not only larger squid, but representatives of a different year class were present in 1967 as opposed to 1966.

Mercer (1965) stated that there is an increase in infestation by plerocercoids of Dinobothrium (sensu lato) sp. with increasing mantle length and this is in agreement with the conclusion of Squires (1957). Likewise, Mercer (1965) stated that the same trend is evident in the case Specifically, he stated that of the incidence of Phyllobothrium sp. there is an increase in incidence of infestation throughout the whole range of mantle lengths sampled (130-300 mm.). Squires (1957) recorded the greatest incidence of Phyllobothrium sp. from smallest squid. He then went on to say that the plerocercoids "escape freely" from the squid, in other words, they leave the host before the host reaches a certain size. It is difficult to see how this can be so, for plerocercoids do not generally succeed in a free living existence. Furthermore, such a mode of transmission does not seem to suffice when one speculates on the matter of how the elasmobranch host would become infested with the plerocercoids from the surrounding medium.

The results of the present study support Squires (1957), however,

in his contention that Phyllobothrium is more frequently found in smaller squid, in this study mantle length 200-219 mm., for there is a decrease in infestation with Phyllobothrium sp. with an increase in mantle length. With regards to D. plicitum the present results show that in 1966 there was an increase in incidence of infestation with increasing mantle length amounting to agreement with both Squires (1957) and Mercer (1965) with regard to this species. However, no significant difference could be demonstrated with respect to data for 1967. This again may be due to older squid in the 1967 population.

Dinobothrium plicitum first appeared in squid samples on September13, 1966, while in 1967 it occurred much earlier in the sampling season, on July 25. This disparity cannot be easily explained. There is apparently no difference which can be attributed to sex, nor can this particular discrepancy be explained on the basis of mantle length. It may be due to unmeasured hydrographic conditions or to local populations of the first intermediate host being present or absent at the critical time in the life of the squid prior to sampling.

Tables II - XXIX in the text present analyses of data on individual species of cestodes, as well as combined data on the various genera of helminths found. There is little evidence of consistent trends, particularly with regard to monthly, annual, or sexual variations. One factor that complicates this is the scarcity of squid at certain times in the season. Daily trips were made for squid in the month of September, 1967, and it was not until September 22 that efforts met with success and,

as it turned out, only on that day were any animals obtained. It becomes apparent that it is difficult to compare parasitological data for the month of September because sampling occurred at weekly intervals. Aldrich, et al. (1968) have shown a marked lack of males in autumn samples, postulating their movement to offshore or deeper areas for reproductive reasons. The discrepancy of males in samples for such periods also influences the question of regular comparisons between sexes. From analysis of combined data, the burden carried by males is not homogeneous, while in the case of females the burden is homogeneous. The reasons for this are not known, but there may be some difference in food preferences between sexes throughout the sampling period.

As was presented earlier, 1.6 percent of the squid sampled in 1966 and 1967 were infested with larval nematodes. This is approximately twice the incidence (0.9 percent) reported by Mercer (1965) and agrees basically with the incidence (1.3 percent) reported by Squires (1957). The nematodes have been positively identified as belonging to the genus Contracaecum. Both Mercer and Squires leave the nematodes unidentified but Mercer intimates that the genera that are likely to employ a paratenic host are Porrocaecum and Anisakis, these being common fish nematodes in the Newfoundland area (Templeman, et al. 1957). Squires (1957) is probably correct in suggesting the capelin, Mallotus villosus is the infective agent, since the author has recovered larval Contracaecum sp. from M. villosus, collected immediately prior to the run of squid in 1966. Larval forms of Contracaecum similar to those from I. i. illecebrosus were found in both the spiny dogfish (Squalus acanthias) and the thorny skate (Raja radiata).

The parasitization of the short-finned squid, indeed of any decapod cephalopod, will be better understood when science knows more about such basic subjects as their life cycle, physiology, behaviour and biochemistry.

Further studies on the taxonomy of the Order Tetraphyllidea, and in particular on the Family Dinobothriidae, are needed before new records of parasites can be accurately evaluated. No life cycle of any tetraphyllidean is completely known. Further studies on these cycles are necessary as well as on the host-parasite relationship of this group, both with regard to the microenvironment and macroenvironment. A further study is intended with hopes of finding the definitive hosts of the larval cestodes so far found.

CONCLUSIONS

- 1. Five genera of cestode parasites were found in the short-finned squid. Three of these, <u>Pelichnibothrium speciosum</u> Monticelli, <u>Scolex polymorphus</u> Rudolphi, and <u>Nybelinia</u> sp. are new host records. The two remaining being <u>Phyllobothrium</u> and <u>Dinobothrium</u>, both of which have previously been described from <u>Illex illecebrosus</u> illecebrosus.
- 2. The specimens of <u>Dinobothrium</u> collected were identified to the species level (D. plicitum Linton) for the first time.
- 3. Contrary to conclusions drawn by other workers, the plerocercoids of Phyllobothrium do not wander freely about the body of the squid in nature but are restricted to the caecum.
- 4. The wandering of plerocercoids referred to above, is a function of rising temperature over the period between time of capture of the squid and time of preservation.
- 5. The greatest number of plerocercoids encountered were <u>Dinobothrium</u>

 <u>plicitum</u>. This species being found most frequently in the walls of the intestine and caecum of the host in cysts. The cyst appears to be of host origin.
- 6. Degree of infestation in 1966 and 1967 was similar (39% : 40%).

- 7. Contrary to earlier workers, infestation by cestodes need not be directly correlated with mantle length.
- 8. Any possible relations between sex of the squid and parasite burden are unclear.
- 9. Some of the parasitological data tends to give support to the theory that squid live for more than 12 to 15 months.
- 10. Nematodes of the genus <u>Contracaecum</u> were found in both the squid and selachians that were examined. Little new information was garnered with respect to the life histories of any of the parasites found in the short-finned squid.

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APPENDIX I

The following is a list of stains and chemicals used in the preparation of the helminths found during this study.

Borax Carmine (Grenacher's)

Borax ----- 10 gms.

Carmine ----- 8 gms.

H₂0 ----- 250 mls.

Boil together until the Borax and Carmine are dissolved. Let stand until cool, and filter. Add 250 ml. of 70% alcohol.

Acid Carmine (Semichon's)

Glacial Acetic Acid ----- 500 ml.

Distilled H₂0 ----- 500 ml.

Carmine, in escess

Combine, and heat to 95°C - 100°C for 15 minutes. Cool rapidly allowing the excess carmine to settle. Filter. Before using, dilute the stain with 2 to 3 parts of 70% alcohol.

Permount - Fisher Chemical Co.

A 60% solution of synthetic resin material.

Chemically stable - fast drying.

Bouin's Solution

Formalin (40%) ------ 20 cc.

Glacial Acetic Acid ----- 5 cc.

Picric Acid, Sat. aqueous ---- 75 cc.

Mallory's Triple Stain

First Solution: 1% Acid Fuchsin

Second Solution: 1% Phosphotungstic Acid

Third Solution:

 Water ---- 200 ml.

 Aniline Blue ---- 1 gm.

 Orange G ----- 4 gms.

 Oxalic Acid Crystals ---- 4 gms.

Lacto-phenol

 Melted Phenol Crystals ---- 100 ml.

 Lactic Acid Syrup ---- 100 ml.

 Glycerine ----- 200 ml.

 Distilled Water ---- 100 ml.

Use as a hot solution, heated in a water bath.



