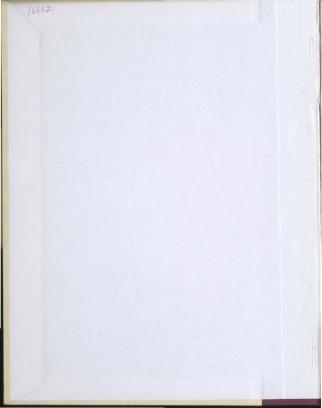
MARINE FISH HAEMATOZOA FROM NEWFOUNDLAND WATERS

CENTRE FOR NEWFOUNDLAND STUDIES

TOTAL OF 10 PAGES ONLY MAY BE XEROXED

(Without Author's Permission)

BERNARD KAI-FAI SO







MARINE FISH HAEMATOZOA FROM NEWFOUNDLAND WATERS

by

Bernard Kai-Fai So, B. Sc. (M.U.N.)

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science

Department of Biology

Memorial University of Newfoundland,

St. John's, Newfoundland, Canada.

March, 1971

CONTENTS

	Page
ABSTRACT	
ACKNOWLEDGEMENTS	
LIST OF TABLES	i
INTRODUCTION	1
MATERIALS AND METHODS	8
RESULTS AND DISCUSSION	16
Trypanosoma Gruby	16
Haemogregarina Danilewsky	27
Haemohormidium Henry	42
<u>Kudoa</u> Meglitsch	53
Intraerythrocytic inclusions of unknown aetiology	54
Unidentified artifacts	56
GENERAL DISCUSSION AND SUMMARY	58
REFERENCES	63
ILLUSTRATIONS	73

ABSTRACT

 $$\operatorname{\textsc{Of}}$$ 797 fish of 28 species, 159 of 12 species yielded blood parasites.

Eight species of protozoan parasites are reported - three each of $\underline{\text{Trypanosoma}}$ and $\underline{\text{Haemoqregarina}}$, and two of $\underline{\text{Haemohormidium}}$.

Trypanosoma rajae Laveran & Mesnil is recorded from Raja radiata. Undetermined trypanosomes are reported from Glyptocephalus cynoglossus and Gadus morhua respectively.

Haemogregarina myoxocephali Fantham et al. was found in Myoxocephalus octodecemspinosus. The occurrence of haemogregarine sporozoites (perhaps of this species) in the gut of a piscicolid leech (Malmiana nuda) from Myoxocephalus scorpius represents the first discovery of a potential vector of any fish haemogregarine. New hosts are listed for H. delagei Laveran & Mesnil and H. platessae Lebailly.

Haemohormidium terraenovae n. sp. is described from six hosts:- Ammodytes americanus, Urophycis tenuis, Melanogrammus aeglefinus, Limanda ferruginea, Glyptocephalus cynoglossus and Hippoglossoides platessoides; and Haemohormidium beckeri n.sp. from Myoxocephalus octodecemspinosus.

Intracrythrocytic inclusions of unknown origin were common in <u>Clupea harengus</u> and <u>Argentina silus</u>, and myxosporidans occurred as blood film contaminants in <u>Raja radiata</u> and <u>Gadus morhua</u> (in the latter case the parasite was referable to <u>Kudoa</u> sp.). Other artifacts reported were bacterial contaminants from <u>Squalus acanthias</u> and <u>Limanda ferruginea</u>.

ACKNOWLEDGEMENTS

It is pleasant to record the ready co-operation of Dr. W. Templeman, Director of the St. John's Biological Station of the Fisheries Research Board of Canada, in facilitating my two visits to the Grand Banks. I also had many occasions to be grateful for the helpfulness of Mr. Richard Wells, the Scientist-in-Charge on my two trips. his technicians, and the crew on board the research vessel, A. T. Cameron. The patience and quidance of my Supervisor, Dr. M. Laird is acknowledged with appreciation, as is the assistance of Dr. G. Bennett, Dr. R. A. Khan and Dr. Wm. Threlfall, also of Dr. F. A. Aldrich, Director of the Marine Sciences Research Laboratory at Logy Bay. The technical advice of Dr. M. S. Grewal, a visiting protozoologist from Panjab, India was most helpful too. I am also grateful to my fellow graduate student, Mr. Ray Côté, and Dr. C. D. Becker, Ecosystems Department, Pacific Northwest Laboratory, Battelle Memorial Institute, Richland, Washington, for their contributions to my work. Mr. R. Ficken provided invaluable photographic assistance. I am deeply indebted to Mrs. F. Lee, upon whose patience and perseverance much of the appearance of this thesis depends.

Lastly, I wish to express my sincere thanks for fellowship assistance from Memorial University and the Fisheries Research Board of Canada, and for research support from my Supervisor's operating grant from the National Research Council of Canada.

LIST OF TABLES

No.	Page
Table 1. Study material in present survey	11-14
Table 2. Haematozoa found in present survey	15
Table 3. Dimensions of various skate trypanosomes	17
Table 4. A preliminary list of skate trypanosomes	19
Table 5. Dimensions and other specific criteria of various flounder trypanosomes	21
Table 6. A preliminary list of haemogregarines from skates, rays and other elasmobranchs	30
Table 7. Previous records of Haemohormidium spp.	48-50

INTRODUCTION

The history of our knowledge of blood-inhabiting protozoans is intimately connected with their discovery in poikilothermic vertebrates. Numerous important "firsts" originated from the interest of the early investigators in parasites of fish, amphibians and chelonians. Thus, the first observation of a blood parasite was that of Valentin (1841), who saw trypanosomes in trout, Salmo fario (= Salmo trutta Linnaeus, 1758). In the following two years frog trypanosomes were recorded from Germany, Belgium and France. Mammalian trypanosomes were first recorded from European field mice and moles by Gros (1845), but another quarter of a century was to go by before Lewis (1878) adequately described related parasites from the blood of rats in India.

In North America, the first report of any blood parasite from marine fish was that of Mavor (1915). He recorded <u>Haemogregarina</u> sp. from the squirrel hake, <u>Urophycis chuss</u> (Walbaum), while searching for protozoan parasites in specimens from Passamaquoddy Bay. His material was collected at or near the mouth of the St. Croix river, St. Andrew's, New Brunswick. Several years later, Kudo (1922) found that of 50 fish representing 25 species from Woods Hole, Mass., only one winter skate (<u>Raja ocellata</u> Mitchill) was infected. This fish was scantily infected with haemoflagellates which

Kudo referred to Trypanosoma rajael Layeran & Mesnil, a parasite of European skates. Ellis (1930) subsequently examined blood smears from an unstated number of fish of nine species at St. Andrew's, N. B., without finding any haematozoa.

Following the examination of marine fish collected from St. Andrew's, N. B., Halifax, Nova Scotia, and Montreal, Fantham et al. (1942) described a number of blood parasites. These included a haemoflagellate from the yellow perch, Perca flavescens (Mitchill), which they designated as a new variety (canadensis) of Trypanosoma percae Brumpt, 1906. of the European perch, Perca fluviatilis. Two new species, T. myoxocephali and Haemogregarina myoxocephali, were described from the longhorn sculpin, Myoxocephalus octodecemspinosus2 (Mitchill) and H. urophysis3 from the white hake, Urophycis tenuis (Mitchill). These authors also identified H. aeglefini Henry from the Atlantic cod (Gadus callarias) = G. morhua L. caught off Labrador. Other undesignated haemogregarines were also discussed by Fantham et al. (1942). One of these, a haemogregarine

Spelt as T. raiae by Kudo.

²Spelt as M. octodecimspinosus by Fantham et al.

³Emended to H. urophycis by Laird and Bullock, 1969. Spelt as \underline{H} . $\underline{\text{aeglifini}}$ by Fantham et al. (see Laird and $\underline{\text{Bullock}}$, $\underline{\text{1969}}$).

closely allied to <u>H. platessae</u> Lebailly, was recorded from the winter flounder, <u>Pseudopleuronectes americanus</u> (Walbaum). Another, probably <u>H. bigemina</u>, was observed in the blood of the ocean pout, <u>Macrozoarces americanus</u> (Bloch & Schneider). Still another, which was described as a haemosporidian different from but probably allied to <u>H. bigemina</u>, was reported from the black sea bass, <u>Centropristes striatus</u> (L.). The last two parasites were found in fish from the Montreal market.

Ten years later, during the winter of 1952,
Bullock (1952, 1953) discovered a species of Cryptobia in
the blood of a young winter flounder taken through ice in
Greenland Bay, New Hampshire. In the summer of the
following year, Bullock surveyed the blood protozoans of
marine fish from southern New England. He (1958) reported
T. rajae from a little skate, Raja erinacea Mitchill, and
undesignated haemogregarines from summer flounder,
Paralichthys dentatus (L.), northern puffer, Sphaeroides
maculatus (Bloch & Schneider), and black sea bass,
Centropristes striatus. The summer flounder parasite was
identified by Laird & Bullock (1969) as Haemogregarina
platessae. In C. striatus, the white blood cells held
division stages of a parasite recognized as Haemogregarina
bigemina by Laird & Bullock (1969). Bullock (1958) also

encountered a myxosporidan in the blood of <u>Pseudopleuronectes</u>
<u>americanus</u> and a mummichog, <u>Fundulus</u> <u>heteroclitus</u> (L.).

This parasite was afterwards identified as <u>Kudoa</u> sp. by
Laird & Bullock (1969).

In the late 1950's, Laird made an extensive study of haematozoa in the Bay of Fundy. His results were published together with Bullock's earlier ones from Woods Hole, Mass. (Laird & Bullock, 1969). Their joint account included various records of haematozoa additional to those already given by Bullock (1952, 1953, 1958). Haemogregarina delagei Laveran & Mesnil was reported from spiny dogfish (Squalus acanthias L.) and the skates Raja erinacea Mitchill and R. radiata Donovan, the latter also proving positive for Trypanosoma rajae.

Haemogregarina aeglefini was reported from three species of gadid fish, Melanogrammus aeglefinus (L.), Pollachius virens (L.) and Urophycis tenuis (Mitchill). This parasite is not known from hosts outside the family Gadidae. Laird & Bullock (1969) relegated H. urophycis to synonymy with H. aeglefini, and also considered the organism seen by Mavor (1915) to be referable to this species. Laird & Bullock (1969) obtained fresh records of Haemogregarina myoxocephali from the longhorn sculpin, and described H. mavori.as new from Macrozoarces americanus.

They also extended the host range of H. platessae to Pseudopleuronectes americanus, and noted undesignated haemogregarines in a rock gunnel, Pholis gunnellus (L.) and a seasnail Liparis atlanticus (Jordan & Evermann). A particularly interesting finding of Laird & Bullock was that of the babesioid Haemohormidium sp. from the sea raven, Hemitripterus americanus (Gmelin), and the American plaice, Hippoglossoides platessoides (Fabricius). These authors designated as Piscine Erythrocytic Necrosis an apparent viral disease causing destruction of the red cell nucleus in four fish representing three genera (one Gadus morhua, one Liparis atlanticus and two Myoxocephalus octodecemspinosus). Cryptobia bullocki Strout (1965) was found in a number of hosts.

From the mid-fifties to the mid-sixties, in warmer waters to the south, Saunders made extensive surveys for haematozoa of marine fish. All in all, she recorded six species of haemogregarines and one of <u>Trypanosoma</u>. In particular, she found <u>Haemogregarina</u> in numerous hosts in Florida (Saunders 1955, 1958a, 1964), Bahamas (Saunders, 1958b), and Puerto Rico (Saunders, 1966). In Florida, she described <u>H. achiri</u> from the hogchoker, <u>Achirus fasciatus</u> Lacépède (Saunders, 1955). An undesignated haemogregarine recalling the European <u>H. polypartita</u> Neumann was observed in the blood of the spotted sea trout, <u>Cynoscion nebulosus</u> (Cuvier) (Saunders, 1954). Two other haemogregarines were

also reported from the same area. H. mugili Carini
(for which the first record had been obtained from Brazilian
waters by Carini, 1932) was found in a striped mullet,
Mugil cephalus L., and H. brevoortiae Saunders, 1954 was
described from three menhaden, Brevoortia tyrannus (Latrobe).
Elsewhere in the Bahamas, Saunders (1958b) described a new
haemogregarine, H. dasyatis from the southern stingray,
Dasyatis americana (Hildebrand & Schroeder). The same
author also described a flagellate, Trypanosoma balistes
from the common trigger fish, Balistes capriscus Gmelin,
and a great barracuda, Sphyraena barracuda (Walbaum), in
the Florida Keys (Saunders, 1958a, 1959).

Little work has been done in the northern and western regions of the continent. Laird (1961a) described H. irkalukpiki from the arctic char, Salvelinus alpinus (L.) in southern Ungava Bay, Quebec. Later in the same year the same author (1961b) reported H. bigemina from the padded sculpin, Artedius fenestralis Jordan & Gilbert, at Nanaimo, Vancouver Island. Other fish blood parasites reported from North America are from freshwater hosts and need not concern us here.

The above paragraphs, then, sum up the major work on the haemotozoa of North American marine fish. Most of the information presently available concerns the Atlantic

coast of the mainland. In Newfoundland, at the threshold of one of the world's major sea fisheries and at the easterly limit of the continent, no previous work had been undertaken on marine fish haemotozoa. The purpose of the present survey was threefold -

- To describe the fish haemotozoa found in Newfoundland waters, if any.
- 2. To compare them with those described from other areas.
- To search for vectors of any blood parasites discovered.

MATERIALS AND METHODS

Blood films of fish were made on the Grand Banks of Newfoundland aboard the "A. T. Cameron" of the St. John's Biological Station, Fisheries Research Board of Canada during November, 1967 and May, 1968. The specimens were obtained by otter traw1, from depths ranging from 25 to 150 fathoms. Additional fish were collected by handline and throw net along the eastern coast of Conception Bay at St. Phillips and Portugal Cove, also on the open Atlantic coast of the Avalon Peninsula at Middle Cove. All three localities are within a few kilometers of St. John's.

Blood was always obtained from live fish. The area posterior to the isthmus was first slit open with a sharp pair of scissors or scalpel, exposing the pericardial cavity. Pericardial fluid often proved abundant, particularly in elasmobranchs. Care was taken to sponge this away, and then to wipe dry the surface of the heart before making the incision, to avoid contaminating the smears. After first allowing a brief free flow from the cut, the corner of a clean slide was touched against the welling blood. The end of another clean slide was then used to make a thin smear in the standard fashion.

Immediately after air-drying, the blood smears were fixed in absolute methyl alcohol. They were always brought back to the laboratory for further processing. Giemsa's stain was used, in a 1 : 40 dilution in distilled water buffered to a slight alkalinity (pH between 7.2 - 7.4 was found most satisfactory). Smears were stained for 45 minutes, then washed in running tap water for three minutes and allowed to dry. The most satisfactory staining results were obtained by turning the fixed smears face down on racks in a shallow tray which was then filled with the staining solution until it touched the lower surface of the slides. This prevented the deposition of stained particles on the smears. Good results were also obtained by overnight staining with Giemsa (1: 80 dilution) in an incubator at 37°C. All preparations were left uncovered, and searched for parasites under a 8x ocular and 40x or 100x oil immersion objective for at least 15 minutes. Whenever time allowed the whole area of each blood smear was carefully examined, in view of the lightness of many trypanosome infections in fish.

While collecting at sea it proved quite impracticable to examine fresh drops of blood for moving trypanosomes, the rough waters of the Grand Banks not being conducive to sustained high power microscopy.

Drawings were made with the aid of a Zeiss drawing apparatus. A 12.5x ocular was substituted for the 8x used in searching for parasites, and a magnification of 1250x was obtained. The photographs were taken with a Zeiss Photomicroscope I at a magnification of 1400x.

All measurements were made from camera lucida outline drawings, the results (in millimeters) being converted into microns with the assistance of a stage micrometer.

The length of trypanosomes was measured by tracing out the course of the midline on the sketch with a piece of thread, as described by Minchin (1909a).

Table 1 lists the fish examined, and indicates those found to be parasitized. Table 2 classifies these parasites. It should be noted that the common and scientific names used for North American fishes are those listed by the American Fisheries Society (Bailey, 1960).

Table 1. Study material in present survey

Scientific name	Common name	Number examined &	positive	Locality
CHONDRICHTHYES				
Squaliformes				
Squalidae (dogfish sharks)				
Squalus acanthias L.	Spiny dogfish	24	1	G.B. (Grand Banks)
Rajiformes				
Rajidae (skates)				
Raja radiata Donovan	Thorny skate	87	65	G.B.
Raja senta Garman	Smooth skate	2	2	G.B.
OSTEICHTHYES				
Clupeiformes				
Clupeidae (herrings)				
Clupea harengus harengus L.	Atlantic herring	34	0	G.B.
Osmeridae (smelts)				
Mallotus villosus (Müller)	Capelin	17	0	Middle Cove
Argentinidae (argentines)				
Argentina silus Ascanius	Atlantic argentine	39	0	G.B.

Table 1. Study material in present survey (contd.)

Scientific name	Common Name	Number examined &	positive	Locality
Anguilliformes				
Anguillidae (freshwater eels)				
Anguilla rostrata (LeSueur)	American eel	2	0	G.B.
Gadiformes				
Gadidae (codfishes and hakes)				123 Grand Banks
Gadus morhua L.	Atlantic cod	180	4	57 Portugal Cove, C.B.
Melanogrammus aeglefinus (L.)	Haddock	11	3	G.B.
Micromesistius poutassou	Blue whiting	5	0	G.B.
(Risso) ⁵				
Urophycis chesteri	Longfin hake	4	0	G.B.
(Goode & Bean) 6				
Urophycis tenuis (Mitchill)	White hake	40	4	G.B.
Macrouridae (grenadiers)				
Nezumia bairdi	Marlin-spike	2	0	G.B.
(Goode & Bean)				
Perciformes				
Labridae (wrasses)				
Tautogolabrus adspersus	Cunner	26	0	St. Phillips.C.
(Walbaum)				

Table 1. Study material in present survey (contd.)

Scientific name	Common name	Number examined	& positive	Locality
Scorpaenidae (scorpionfishes				
and rockfishes)				
Sebastes marinus (L.)	Redfish	121	0	G.B.
Cottidae (sculpins)				
Hemitripterus americanus	Sea raven	26	0	G.B.
(Gmelin)				
Myoxocephalus octodecems-	Longhorn sculpin	12	8	G.B.
pinosus (Mitchill)				
Myoxocephalus scorpius (L.)	Shorthorn sculpin	4	0	St. Phillips,
Cyclopteridae (lumpfishes				С. В.
and snailfishes)				
Cyclopterus lumpus L.	Lumpfish	1	0	G.B.
Ammodytidae (sand lances)				
Ammodytes americanus DeKay	American sand lance	23	19	G.B.
Anarhichadidae (wolffishes)				
Anarhichas lupus L.	Atlantic wolffish	1	0	G.B.
Anarhichas minor Olafsen	Spotted wolffish	1	0	G.B.
Pleuronectiformes				
Bothidae (lefteye flounders)				
Scophthalmus aquosus	Windowpane	4	2	G.B.
(Mitchill)				

Table 1. Study material in present survey (concl.)

Scientific name	Common name	Number	examined	& positive	Locality
Pleuronectidae (righteye					
flounders)					
Glyptocephalus cynoglossus	Witch flounder		19	10	G.B.
(L.)					
Hippoglossoides platessoides	American plaice		60	28	G.B.
(Fabricius)					
Hippoglossus hippoglossus	Atlantic halibut		2	0	G.B.
(L.)					
Limanda ferruginea (Storer)	Yellowtail flounder		47	13	G.B.
Lophiiformes					
Lophiidae (goosefishes)					
Lophius americanus	Goosefish		3	0	G.B.
Valenciennes					
	5	Totals:	797	159	

⁵Not listed by Bailey (1960).

⁶Listed as Phycis chesteri Goode & Bean by Bailey (1960). (With these exceptions all scientific and common names are in accordance with the usage of the American Fisheries Society's "A list of common and scientific names of fishes from the United States and Canada")

Table 2. Haematozoa found in present survey

Fish host Number 6	examined	Protozoan parasites						
		Trypanosoma	Haemogregarina	Haemohormidium	Others			
qualus acanthias L.	24							
aja radiata Donovan	87	1	65		1			
aja senta Garman	2		2		1			
adus morhua L.	180	1			3			
elanogrammus aeglefinus (L.)	11			3				
rophycis tenuis (Mitchill)	40			4				
yoxocephalus octodecems- pinosus (Mitchill)	12		4	5				
mmodytes americanus DeKay	23			19				
cophthalmus aquosus (Mitchill)	4		2					
lyptocephalus cynoglossus (L.	.) 19	1	7	3				
ippoglossoides platessoides (Fabricius)	60			28				
imanda ferruginea (Storer)	47			13	1			

RESULTS AND DISCUSSION

SARCOMASTIGOPHORA: ZOOMASTIGOPHOREA

Trypanosoma Gruby

Trypanosoma rajae Laveran & Mesnil, 1902 (Figs. 1, 2, 98)

Eighty-seven thorny skates (Raja radiata) and two smooth skates (R. senta) were caught on the Grand Banks of Newfoundland. Only a single example of the former species sampled in May, 1968, was found to be parasitized by trypanosomes during the present study. The infection was light, only one parasite being found in the entire blood smear.

MORPHOLOGICAL ACCOUNT

Synopsis

Length of free flagellum	10.8	μ
Length of body	59.5	μ
Total length	70.2	μ
Width of body at centre of nucleus	8.0	μ
Greatest width of undulating membrane	3.8	μ
Length of nucleus	7.0	μ
Width of nucleus	7.0	μ
Distance of kinetoplast from posterior extremity	3.5	H

The flagellate is quite tightly coiled (Figs. 2, 98), as is often the case with the larger fish and amphibian trypanosomes. Its broad membrane is well developed, 13 deep folds being evident around the outer margin. A relatively short free flagellum originates from the pointed anterior

end, and the posterior extremity is very blunt. Six distinct myonemes run longitudinally. The large, rounded nucleus occupies almost the entire breadth of the body, and exhibits scattered chromatic granules. The kinetoplast is small and compact, and is located very close to the posterior extremity.

Table 3. Dimensions of various skate trypanosomes

Species	Total length	Length of free flagellum	Body width with membrane	Reference
T. rajae Laveran & Mesnil,1902	35-80 µ	6-20 μ	3-13 µ	Laveran & Mesnil (1902b); Kudo (1922)
T. giganteum Neumann, 190	125-130 μ	25-30 μ	14 μ	Neumann (1909)
T. variabile Neumann, 190	30-85 μ 9	10-15 μ	-	Neumann (1909)
T. gargantua Laird, 1950	66 -1 32 μ	No free flagellum	5-17.5 μ	Laird (1950)

Among the trypanosomes so far known from skates, the organism described herein most closely resembles T. rajae Laveran & Mesnil, 1902, and T. variabile Neumann, 1909. Minchin & Woodcock (1910) believed that T. variabile is conspecific with T. rajae, which shows marked polymorphism. AND THE RESIDENCE OF THE PROPERTY OF THE PROPE

Laird & Bullock (1969) have recorded <u>T</u>. <u>rajae</u> from a thorny skate (<u>R</u>. <u>radiata</u>) from the Bay of Fundy, N. B. Large trypanosomes of fish are often very polymorphic, and additional material of the present flagellate would certainly reopen the matter of its specific identity. However, there is nothing in the size and appearance of the one example available to justify its separation from <u>T</u>. <u>rajae</u> of European skates. Although this species has already been reported from North American Atlantic waters (Kudo, 1922; Bullock, 1958; Laird & Bullock, 1969), the present record is the most northerly to date and the first from the Grand Banks.

Many of the skates examined, including the example positive for <u>T. rajae</u> also harboured haemogregarines, which will be discussed later.

Table 4. A preliminary list of skate trypanosomes

Trypanosome	Host	Locality	Reference
Trypanosoma rajae Laveran & Mesnil, 1902	(R. mosaica) = R. undulata	Europe	Laveran & Mesnil,1902b
	R. macrorhynchus		
	R. clavata		
	(R. punctata) = R. asterías	"	
	R. batis	England	Coles, 1914
	R. ocellata	N. America	Kudo, 1922
	Raja sp.	Europe	Minchin & Woodcock,1910
n .	Raja sp.	Shetlands	Henry, 1913
	R. erinacea	Woods Hole (N. America)	Bullock,1958
	R. erinacea R. radiata	N. America (Woods Hole & St. Andrew's)	
Trypanosoma giganteum Neumann, 1909	R. oxyrhynchus	Europe	Neumann,1909
Trypanosoma variabile Neumann, 1909	(R. punctata) = R. asterias	Europe	Neumann,1909
Trypanosoma sp.	R. capensis	S. Africa	Fantham, 1919
Trypanosoma marplatensis Bacigalupo & Plaze, 1948	A South American ray	S. America	Bacigalupo & Plaza, 1948
Trypanosoma gargantua Laird, 1950	R. nasuta	New Zealand	Laird, 1950

Trypanosoma sp. (Figs. 3, 4, 99)

Of 19 specimens of Glyptocephalus cynoglossus

THE PROPERTY OF THE PROPERTY O

(witch flounder) caught on the Grand Banks of Newfoundland in May, 1968, one proved to be parasitized by the trypanosome described herein. Once again, only one parasite was found in the thin smear. 7

MORPHOLOGICAL ACCOUNT

Synopsis

Length of free flagellum	6.5 µ
Length of body	59.2 μ
Total length	65.8 µ
Width of body at centre of nucleus	3.2 µ
Greatest width of undulating membrane	1.5 µ
Length of nucleus	4.8 µ
Width of nucleus	3.2 µ
Distance of kinetoplast from posterior extremity	3.0 µ

The body of the trypanosome is very much coiled. There is a well defined undulating membrane showing five folds (Figs. 4, 99). The anterior end is pointed, while the posterior one is somewhat blunt. Its vacuolated

Nith regard to the "lightness" of many fish trypanosome infections, chronic infections can involve so few trypanosomes being in the circulation at the time of smearing that many apparently negative fish are in fact parasitized. "No parasites found" is thus a better diagnosis than "negative".

cytoplasm stains dark blue with Giemsa. The oval, pinkstaining nucleus shows tiny chromatic granules. It occupies the entire width of the body and is situated some 70% of the total body length from the anterior extremity. The free portion of the flagellum accounts for 10% of the overall length of the trypanosome. A small and compact kinetoplast, staining dark red, is located 3.0 μ from the posterior end.

Table 5. Dimensions and other specific criteria of various flounder trypanosomes

		otal engtl				Body um width		References
<u>T</u> .	soleae Laveran & Mesnil, 1901	40	μ	8	μ	-	pointed	Laveran & Mesnil,1907
Ţ.	platessae Lebailly, 1904	52	μ	12	μ	3-3.5 μ	very attenuated	
T.	flesi Lebailly, 1904	55	μ	10	μ	5 μ	pointed	n
T.	limandae Brumpt & Lebailly, 1904	45	μ	20	μ	2-2.5 μ	very attenuated	
T.	caulopsettae Laird,	100	μ	18	μ	5-6 μ	very attenuated	Laird, 1950

The single trypanosome now described differs radically from these other species in having a bluntly rounded posterior end and a relatively shorter free flagellum. Nevertheless, while it is not readily assignable to any of the known trypanosomes from fish, polymorphism in these parasites is such that I do not propose to follow the example of some earlier authors in assigning a new specific name without having a reasonable range of examples before me. The situation is quite different from that encountered in connexion with the preceding species. Although only one trypanosome was located then too, it fell well within the range for a well-characterized species. Therefore, with the observation that the organism under discussion may prove to merit description as new when further material becomes available, it is simply designated for present purposes as Trypanosoma sp.

Trypanosoma sp. (Figs. 5, 6, 7, 100)

One hundred and eighty specimens (123 from the Grand Banks; 57 from Portugal Cove, Conception Bay) of Gadus morhua (the Atlantic cod) were examined. Only one (from Portugal Cove) caught in summer of 1969, yielded trypanosomes. The infection was quite heavy, 15 flagellates being seen on the thin smear. A myxosporidan was also found amongst the other cod smears from Portugal Cove (see p. 53).

MORPHOLOGICAL ACCOUNT

Synopsis (10 examples measured)

	Range	Average
Length of free flagellum	7.0-7.5 µ	7.3 u
Length of body	62.5-81.5 µ	73.6 µ
Total length	69.5-88.5 µ	80.8 µ
Width of pody at centre of nucleus	2.8-3.5 µ	3.0 µ
Greatest width of undulating membrane		1.0 µ
Length of nucleus	4.6-5.5 µ	4.8 µ
Width of nucleus	2.8-3.5 µ	3.0 µ
Distance of kinetoplast from posterior extremity	7.0-13. 5 μ	10.8 µ

The flagellate is long, slender, and tapered at both ends, most of the examples showing a well-extended body (Figs. 6, 100), with a few coiled examples (Fig. 7). It has alveolar, rather granular cytoplasm, exhibiting many discrete vacuoles. The majority of the latter are anteriorly positioned. All but the posterior portion of the cytoplasm, which stains whitish-blue, appears sky blue with Giemsa. The oval nucleus stains a light pinkish-purple. Every example seen shows a prominent karyosome. The nucleus is situated 38% of the total body length from the root of the very short free flagellum, the length of which averages 7.3 µ and is only one-tenth that of the body proper. It originates from a basal granule just anterior to the nearly oval, red-staining kinetoplast, which is located some 11 µ (14% of the total body length) from the posterior extremity. The undulating membrane is not evident in most of the examples. In suitably stained specimens, two or three myonemes (Figs. 6, 7, 100) are seen as dark streaks in the cytoplasm and across the nucleus.

Henry (1913) recorded $\underline{\mathbf{T}}$. aeglefini from "Gadus aeglefinus" - the haddock, <u>Melanogrammus</u> aeglefinus (L.) - without giving a recognizable description of his parasite. Nikitin (1927) described $\underline{\mathbf{T}}$. <u>murmanensis</u> from four of 15 Gadus callarias (= $\underline{\mathbf{G}}$. <u>morhua</u>). Staining dark blue with Giemsa, this species has a long, serpent-like body, measuring 50 - 60 μ . The cytoplasm exhibits a number of vacuoles, and the undulating membrane is not well developed. The kinetoplast is located not far from the posterior extremity, and the anterior extremity is said to be rounded. The violetstaining oval nucleus is situated at the middle of the body.

The present flagellate is somewhat longer than T. murmanensis as described by Nikitin (1927). It further seems to differ from the latter in tapering anteriorly; and in that the nucleus is located in the anterior half of the body (38% of the body length from the root of the free flagellum). Since piscine trypanosomes are often highly polymorphic, it is conceivable that T. murmanensis and the species under discussion are in fact identical. However, further material from both sources will be needed to settle this point.

Laird (1950) described <u>T</u>. <u>coelorhynchi</u> from <u>Coelorhynchus australis</u> (Richardson), the javelin fish, and <u>Physiculus bachus</u> (Forster), the red cod, in New Zealand waters. <u>T</u>. <u>coelorhynchi</u> shows a considerable degree of polymorphism. It has small, intermediate and large forms, the intermediate one being the commonest. Small and large forms comparable with those of the New Zealand trypanosome were not present in my material. A comparison of the intermediate form of <u>T</u>. <u>coelorhynchi</u> with the present species follows:-

	T. coelorhynchus	T. bacalaoi
Length of free flagellum	11.0 ^µ (av.)	7.3 µ (av.)
Length of body	55.8 µ (av.)	73.6 µ (av.)
Width of body at centre of nucleus	3.2 µ (av.)	3.0 µ (av.)
Position of nucleus	38% of total body length from root of free flagellum	38%
Shape of nucleus	Reniform to circular	Oval
Granules in cytoplasm	Absent	Present
Vacuoles in cytoplasm	Numerous	Numerous
General appearance of flagellate	Curled into a tight spiral	Well-extended

The present flagellate thus resembles the intermediate form of <u>T</u>. <u>coelorhynchi</u> in the following respects: body width, location of the nucleus, presence of numerous vacuoles in the cytoplasm and staining reaction with Giemsa. However, it has an appreciably shorter flagellum, and a decidedly longer

body, than the intermediate form of the New Zealand species. The presence of granules in the cytoplasm, the oval nucleus, the internal location of the kinetoplast and particularly the generally extended rather than coiled appearance of the trypanosome under discussion show it to be distinct from T. coelorhynchi.

In the absence of a large range of specimens it is felt inadvisable to describe the Newfoundland cod trypanosome as new. Neither can it be identified as

T. murmanensis on the basis of existing information. It is therefore only determined to the generic level at this time.

The example illustrated in Fig. 6 is ringed on the slide, which has been deposited in the collection of the U. S. National Museum (catalogue number USNM Helm. Coll. 71800).

Mr. Ray Côté, a fellow graduate student, first noticed this trypanosome of <u>Gadus morhua</u>, and furnished the material from Portugal Cove.

SPOROZOA: TELOSPOREA

Haemogregarina Danilewsky

<u>Haemogregarina</u> <u>delagei</u> Laveran & Mesnil, 1902 (Figs. 8-16, 101-103)

This elongate, sausage-shaped haemogregarine, varies in shape from straight (Fig. 9) to crescentic (Figs. 10-12, 14-16, 101). One end is usually somewhat broader than the other. It was present in erythrocytes of most (65/87) of the thorny skates (R. radiata) examined, the only one sampled in November, 1967 and 64/86 of those taken in May, 1968, besides two smooth skates (R. senta), one from each of the two collecting dates. Although 73% of the skates were parasitized, infections were usually so light that only 1 - 5 haemogregarines could be detected in 15 - 20 minutes of observation under a high dry objective (oil being applied to the slide). Heavier infections were only seen in about a dozen instances where 10 - 15 haemogregarines were recorded in the same period of microscopic examination.

Morphological Account

Pifty intraerythrocytic haemogregarines were measured, and found to range from 9.5 - 19.0 μ (av. 12.8 μ) in length, and 2.2 - 4.5 μ (av. 3.5 μ) in breadth.

The ovoid to irregular nucleus, which stains reddish purple with Giemsa, is usually centrally positioned in the larger examples. In smaller ones, it is sometimes subterminal. It measures 3.2 - 9.2 μ (av. 5.0 μ) by 1.0 - 3.0 μ (av. 2.4 μ), the chromatin granules numbering from 12 - 16 when individually distinguishable (Figs. 12, 16). However, these granules usually appear as an undifferentiated mass (Figs. 11, 13, 14, 15, 103). The alveolar cytoplasm, staining from light to dark blue, sometimes exhibits dark-staining granules (Figs. 9, 10, 14).

Fifty normal erythrocytes of R. radiata, measured 23.2 - 37.8 μ (av. 32.0 μ) by 14.5 - 20.0 μ (av. 17.2 μ). Fifty parasitized ones measured 24.5 - 36.0 μ (av. 31.2 μ) by 13.8 - 21.0 μ (av. 17.6 μ). The nuclei of the former ranged from 8.5 - 16.0 μ (av. 10.4 μ) by 7.0 - 9.2 μ (av. 7.8 μ); those of the latter, from 8.5 - 11.0 μ (av. 9.6 μ) by 6.5 - 8.8 μ (av. 7.5 μ). The parasite thus causes neither appreciable hypertrophy nor distortion of the host cells. Nor does it cause any obvious harm to the host cell nucleus.

Simple division of the parasite into three or four was detected on three occasions. In one instance, one of these products had become separated from the others (Fig. 13). These small haemogregarines measured 4.0 - 5.0 μ (av. 4.4 μ) by 1.0 - 1.5 μ (av. 1.5 μ), all having a subterminal nucleus.

Free haemogregarines were seen twice. They measured 15.0 μ by 4.5 μ , appearing slightly larger, more clearly defined and darker staining than intraerythrocytic ones (Figs. 15, 102). Double invasion in which two parasites at different stages of development occur within the same erythrocyte was observed twice only (Fig. 14). Fig. 103 illustrates an instance of double invasion in which the haemogregarines are of similar size and morphology.

In R. senta, the parasites found measured from 8.0 - 10.5 μ (av. 9.2 μ) in length by 2.8 - 4.0 μ (av. 3.2 μ) in breadth. The nucleus measured 4.0 - 4.5 μ (av. 4.3 μ) by 1.5 - 3.0 μ (av. 2.1 μ). Infections were again very light, yielding only four or five parasites in 15 - 20 minutes of searching. A single free haemogregarine (measuring 9.5 μ by 3.0 μ) was observed. Double invasion of the host erythrocyte was not recorded for this host.

None of the haemogregarines from either host showed polar caps.

Table 6. A preliminary list of haemogregarines from :

A. Skates

Parasite	Host	Locality	Reference
Haemogregarina delagei	Raja radiata	Europe	Laveran & Mesnil,1902a
	R. erinacea	St. Andrews, N. B.	Laird & Bullock,1969
	R. radiata	St. Andrews, N. B.	
B. Rays			
H. dasyatis Saunders, 1958	Dasyatis americana	Bahamas	Saunders, 1958
H. lobianci Kohl- Yakimoff & Yakimoff, 1915	Torpedo marmorata	Europe	Kohl- Yakimoff & Yakimoff,1915
H. torpedinis Neumann, 1909	Torpedo ocellaris	Europe	Neumann,1909

C. Other elasmobranchs

_	Parasite	Host	Locality	Reference
н.	carchariasi	Carcharias sp.	Australia	Laveran,1908
<u>н</u> .	delagei	Squalus acanth		
<u>н</u> .	<u>hemiscylli</u> i	Hemiscyllium ocellatum	Australia	Mackerras & Mackerras, 1961

 \underline{H} . carchariasi Laveran (1908) was described from an Australian shark of the genus <u>Carcharias</u>. It measures 20 - 27 μ by 7 - 10 μ , and is thus altogether larger than the parasite under discussion. Besides, it causes radical elongation of the host cell. Normal host erythrocytes averaged some 26 μ in length, those containing \underline{H} . <u>Carchariasi</u> attaining as much as 34 μ .

H. delagei averages 13 μ by 2 μ , according to Laveran & Mesnil (1902a). Its nucleus consists of a mass of chromatin granules, and there are discrete volutin granules in the cytoplasm. No examples with a polar cap were seen. Double invasion was recorded. Laird & Bullock (1969) found the same parasite in a single dogfish and two species of skates. Their haemogregarines averaged 11.0 by 2.4 μ , the nucleus measuring some 3.0 by 1.9 μ . The latter structure exhibited up to 16 chromatin granules. Free examples (av. 11.3 by 3.7 μ) and double invasion of the host erythrocyte were observed. Polar caps were evident in some of the examples illustrated.

Saunders (1958b) described <u>H</u>. <u>dasyatis</u> from the southern stingray, <u>Dasyatis</u> <u>americana</u>, in the Bahamas. The maximum size of the mature gametocyte (13.0 by 3.8 µ), and the absence of polar caps in Giemsa-stained examples agreed with the organism under discussion. However, red cells apparently never held two parasites.

Another species, <u>H</u>. <u>hemiscyllii</u> Mackerras & Mackerras, 1961 occurs in the Australian dogfish, <u>Hemiscyllium ocellatum</u>. This haemogregarine is, however, slightly larger than the one under discussion. Some examples range up to 16 - 19 µ by 5 - 8 µ, and exhibit bluntly or smoothly rounded extremities.

Haemogregarines have been described from two members of the family Torpedinidae (electric rays).

H. lobianci was described from Torpedo marmorata by Kohl-Yakimoff & Yakimoff (1915), and H. torpedinis from Torpedo ocellaris by Neumann (1909). The former species was said to have a "centrosoma" at one end. H. torpedinis, the mature gametocytes of which attain at least 18 by 4.5 µ, exhibits polar caps according to Neumann (1909).

The present organism is identified as <u>H</u>. <u>delagei</u> - it is considered that the brevity of the original description, and the parasite's occurrence in the type host (<u>R</u>. <u>radiata</u>) argue in favour of the general resemblances outlined outweighing minor dissimilarities. <u>R</u>. <u>senta</u> is a new host record.

One of the 65 R. $\underline{\text{radiata}}$ found positive harboured both Trypanosoma rajae and H. $\underline{\text{delagei}}$.

Haemogregarina platessae Lebailly, 1904 (Figs. 17-22, 105)

A rather large haemogregarine, occupying the whole length of the host cell and half of its volume, was found in seven witch flounders (Glyptocephalus cynoglossus) and a single windowpane (Scophthalmus aquosus) caught in May, 1968. The body is slightly curved. Most of the examples have one of the extremities broadly rounded, the other tapering (Figs. 18, 19, 105) and sometimes pointed (Figs. 21, 22). All infections were light, only one or two parasites being encountered per host in 20 minutes of microscopic examination.

The organism measures 8.0 - 10.5 μ (av. 9.2 μ) in length, and 2.0 - 3.2 μ (av. 2.8 μ) in breadth. The large, oval nucleus, taking up the whole width of the parasite, occupies one-third to more than one-half of the body (Figs. 18, 19, 21). This structure, containing numerous chromatin granules, measures 2.8 - 4.8 μ (av. 3.5 μ) by 1.2 - 2.5 μ (av. 1.8 μ). It is sometimes centrally located (Fig. 19), but is usually somewhat closer to the broader extremity (Figs. 18, 105). The alveolar cytoplasm, staining light blue, frequently exhibits one or more terminal to subterminal vacuoles (Figs. 18, 19). Polar caps are usually lacking in examples from witch flounders, although not in those from Scophthalmus aguosus (Fig. 21).

Parasitized erythrocytes measured 8.8 - 13.0 μ (av. 11.0 μ) by 6.2 - 8.2 μ (av. 7.5 μ), as compared with the 9.8 - 12.5 μ (av. 10.6 μ) by 7.0 - 8.0 μ (av. 7.6 μ) of normal ones. The nuclei of the former ranged from 3.8 - 5.2 μ (av. 4.3 μ) by 2.5 - 3.0 μ (av. 2.7 μ), those of the latter from 3.8 - 4.8 μ (av. 4.5 μ) by 2.5 - 3.5 μ (av. 3.0 μ). Nuclei of parasitized cells seemed to be slightly reduced in size, and were often markedly displaced as well (Figs. 18, 19). Free haemogregarines were not observed. Neither were doubly invaded erythrocytes. Leucocytes were not seen to be parasitized.

A large and small form of the parasite were seen in <u>Scophthalmus aquosus</u>. The larger measured 9.5 by 1.5 μ , its nucleus (6.0 by 1.5 μ) being at the tapered extremity. A polar cap was clearly evident (Fig. 21). The smaller measured 3.5 by 1.0 μ , its round nucleus measuring 1.0 by 0.8 μ (Fig. 22). Infection was once again light, only one of the four examples of <u>S. aquosus</u> being parasitized.

Laveran & Mesnil (1901) first reported the occurrence of haemogregarines (their <u>H</u>. <u>simondi</u>) from a flatfish, the European <u>Solea vulgaris</u> (= <u>Solea solea (L.)</u>). This organism is a member of the <u>bigemina</u> group, undergoing schizogony in circulating erythrocytes. It is therefore quite different from the species under discussion.

Lebailly (1904) briefly described three species of haemogregarines from flatfish off the coast of France. Unfortunately, he furnished no illustrations. One of these three, H. platessae, was found in a plaice, Platessa vulgaris (= Pleuronectes platessae L.). The other two organismsin question were H. flesi Lebailly, 1904, found in Pleuronectes flesus (= Flesus vulgaris) and H. laternae Lebailly, 1904, from Platophrys laternae. H. platessae was described as measuring 9 by 2 u (examples of smaller dimensions were also found), its body generally being crescentic, although only slightly curved. One extremity is rounded, the other bluntly pointed (and in larger examples, conspicuously vacuolated). The nucleus extends the whole width of the body and rather more than a third of its length, being usually located near the broad end of the body. This description suits the present organism very well, justifying its identification as H. platessae. Glyptocephalus cynoglossus and Scophthalmus aquosus are new host records.

The second statement of the second se

H. platessae, or a form closely allied thereto, was first reported from North America by Fantham et al. (1942) from the winter flounder, <u>Pseudopleuronectes americanus</u>. However, no further information was given. Laird & Bullock (1969) described the same species from this host and another, <u>Paralichthys dentatus</u>, in North American waters (Woods Hole, Mass., and St. Andrews, N. B.).

Except for the smaller size of their haemogregarines (av. 7.7 by 1.4 µ), there is nothing in their description at variance with the present parasite.

Saunders (1955) reported another haemogregarine (<u>H</u>. <u>achiri</u>) from an American flatfish, the hog choker (<u>Achirus fasciatus</u>). Free merozoites and early invasive stages were observed. On the basis of an analysis of available data, Laird & Bullock (1969) suggested that <u>H</u>. <u>achiri</u> may be a synonym of <u>H</u>. <u>platessae</u>.

One example of <u>Glyptoglossus cynoglossus</u>, from which haemogregarines were not recorded, was parasitized by <u>Trypanosoma</u> sp. (p. 20). Three other <u>G. cynoglossus</u> harboured babesioids, which will be discussed later (p. 43). It should be noted that of these three examples, only one was infected with H. platessae.

Haemogregarina myoxocephali Fantham, Porter & Richardson, 1942. (Figs. 23-25, 104)

Another haemogregarine was found in the blood of the longhorn sculpin, Myoxocephalus octodecemspinosus. Four of 12 caught on the Grand Banks in May, 1968 were infected, none of them harbouring any ectoparasites. The same host is sometimes infected by babesioids (see p. 46).

All infections were light, not more than one haemogregarine being found per host in 20 minutes' examination of the smear. The parasite is sausage-like (Figs. 24, 25, 104) and sometimes slightly crescentic (Fig. 25). Both extremities may be smoothly rounded, one of them being slightly broader than the other (Figs. 24, 25). Overall dimensions of those examined were 9.2 - 10.0 μ (av. 9.5 μ) by 1.8 - 2.8 μ (av. 2.3 μ). The finely granular and alveolar cytoplasm stains light blue, the irregularly-shaped and deeply-staining nucleus exhibiting up to nine discrete chromatin granules (Fig. 25). It varies from 2.8 - 4.0 μ (av. 3.3 μ) by 1.2 - 1.8 μ (av. 1.5 μ) and is always displaced towards one pole.

Normal erythrocytes of the host measured 12.2 - 13.0 μ (av. 12.7 μ) by 8.0 - 10.8 μ (av. 9.4 μ), their nuclei varying from 4.5 to 5.2 μ (av. 4.9 μ) by 3.2 to 4.2 μ (av. 3.8 μ). Parasitized examples measured from 12.2 to 17.0 μ (av. 13.7 μ) by 7.5 to 8.5 μ (av. 7.5 μ), their nuclei ranging from 4.5 to 5.2 μ (av. 4.8 μ) by 2.5 to 3.5 μ (av. 2.9 μ). Thus parasitized erythrocytes are appreciably longer and narrower than normal, their nuclei being slightly reduced in size. Fifty percent of the parasitized red cells showed marked nuclear displacement (Figs. 24, 104). A polar cap was evident at the broader end of all the examples

seen. No free haemogregarines were found in any of the hosts, and double invasions were not observed.

The first haemogregarine ever found in a sculpin, H. cotti, was reported from Europe by Brumpt & Lebailly (1904), the host being Cottus <u>bubalis</u> Euphrasen. It was described as being a little more thick-set than H. <u>callionymi</u> Brumpt & Lebailly, (1904), which it otherwise closely resembles. The latter species has a curved body measuring 12 by 2.5 µ, its nucleus being somewhat closer to the more rounded extremity.

Fantham, Porter & Richardson (1942) described \underline{H} . $\underline{myoxocephali}$ from a longhorn sculpin, \underline{M} . $\underline{octodecemspinosus}$, from Eastern Canada. This organism measures 5.5 to 9.2 μ by 2.2 to 2.8 μ . Its nucleus ranges from 1.5 to 4.5 μ by 1.5 to 2.8 μ . Laird & Bullock (1969) rediscovered this parasite in the type host, from adjacent waters. Their overall measurements were 4.9 - 9.6 μ (av. 7.9 μ) by 1.6 - 3.7 μ (av. 2.4 μ), the nucleus ranging from 1.8 - 4.1 μ (av. 2.7 μ) by 1.2 - 2.7 μ (av. 1.7 μ) and exhibiting 8 - 14 discrete chromatin granules. Polar caps were present in most of their examples. These dimensions are close to those of the haemogregarine under discussion, which further resembles \underline{H} . $\underline{myoxocephali}$ in its general morphology and effect upon the host cell, and is therefore identified accordingly.

Laird & Bullock (1969) made the additional suggestion that <u>H</u>. <u>myoxocephali</u> might well in fact prove conspecific with <u>H</u>. <u>cotti</u> Brumpt & Lebailly, 1904, once sufficient data and illustrations of the latter species become available. Indeed, many of the older species of haemotozoa are so inadequately characterized as to preclude certain recognition from new material. Redescription of these older European species is urgently called for.

Possible Vectors of Fish Haemogregarines

Despite the many species of piscine haemogregarines described over the past 70 years, the method of transmission from one fish to another remains unknown. While there have been suggestions that leeches and/or parasitic copepods may serve as intermediate hosts, no proof has yet been brought forward.

Reichenow (1910) was the first to establish the transmission of a haemogregarine (H. stepanowi Danilewsky of the European water tortoise, Emys orbicularis (= Cistudo europea) by the leech, Placobdella catenigera). H. stepanowi supposedly begins its life cycle as a zygote (= ookinete) in the gut of the leech, P. catenigera. The zygote divides three times, forming eight sporozoites. When an infected leech takes blood from the turtle, E. orbicularis, the sporozoites pass to the vertebrate host and invade red

blood cells. Schizogony occurs there, leading to the production of merozoites, which are liberated from the host cells to infect further erythrocytes. Eventually, some of the merozoites which enter these host cells develop into gametocytes. If the red cells in question are ingested by a leech, the gametocytes are liberated. They then mature to gametes, zygote formation duly taking place.

The above life cycle of H. stepanowi was soon confirmed (Robertson, 1910) for H. nicoriae Castellani & Willey, 1904. This haemogregarine of the Ceylon lake tortoise, Nicoria trijuga, is transmitted by the leech, Ozobranchus shipleyi. Shortly after Reichenow and Robertson published their findings, Wenyon sectioned leeches obtained from Sternothaerus adansonii. Erythrocytes from this Sudanese water tortoise held looped haemogregarines considered possibly referable to H. Stepanowi. The intestine of the leech, which Wenyon (1926) sectioned yielded haemogregarine life-history stages in every way comparable to this species. Many other haemogregarines of cold-blooded aquatic vertebrates will probably prove to have a similar life-history.

Piscicolid leeches (Malmiana nuda Richardson, 1970)
were found on five shorthorn sulpins, Myoxocephalus scorpius,
in November, 1969, maintained in tanks at the Marine Sciences

Research Laboratory, Logy Bay. Unfortunately it was impossible to obtain blood films from these fish, which were being kept alive for other studies in the laboratory. Upon examination of their gut smears, 80% of the leeches (8/10) showed polymorphic sporozoites, of oval to elongate form (Figs. 26-29, 106). Twenty were measured, and found to range from 5.0 - 15.0 µ by 2.0 - 3.2 µ, the irregular nucleus measuring from 1.8 - 3.0 µ by 1.2 - 2.5 µ. Their alveolar cytoplasm was stained very pale blue.

The presence of such sporozoites in leach gut smears combined with that of haemogregarines in the erythrocytes of closely related sculpins, strongly suggests that Malmiana nuda transmits Haemogregarina myoxocephali. Experimental proof will of course be necessary to test this hypothesis. Nevertheless, the present evidence represents the first actual clue towards elucidation of the transmission of any fish haemogregarines.

SARCOMASTIGOPHORA: PIROPLASMEA

Haemohormidium Henry

Haemohormidium terraenovae n.sp. (Figs. 30-71, 107-113)

Many of the fish examined were found to be parasitized by babesioids of the genus <u>Haemohormidium</u> Henry, 1910 (Wenyon, 1926; Laird & Bullock, 1969). Six hosts were involved (Table 2), three of them being very frequently infected - Ammodytes americanus (19/23), <u>Hippoglossoides</u> Platessoides (28/60) and <u>Limanda ferruginea</u> (13/47). A few fish individually exhibited quite heavy infections. These were the exceptions, though, most hosts being only lightly infected (some 10 RBC per 10,000 parasitized). From one to six amoeboid babesioids may occur in an individual RBC, the central zone of which assumes a paler stain than the surrounding host cell cytoplasm. Indeed, many examples take up very little stain at all. The embayed periphery stains faint blue to dark blue with Giemsa.

Although approximately 80% of the Ammodytes americanus sampled in November, 1967, were positive, individual infections were light except in four cases. In these, as many as 10% of the red cells were parasitized. The majority of the RBC harboured only one trophozoite, but cells with two or three have also been encountered (Figs. 30-47). Overall measurement gave a

range of 1.0 - 3.2 μ by 0.3 - 1.0 μ . Small chromatic granules and particles often accompany these amoeboid bodies, the presence of which does not visibly affect the host cells.

Only 10% (4/40) of the <u>Urophycis tenuis</u> sampled in May, 1968, were found to harbour this babesioid. The infections were light, except in one host where some 10% of the RBC showed parasites. The organism measured 1.0 - 2.5 μ by 0.2 - 1.0 μ (Figs. 48-51, 108).

Three out of 11 Melanogrammus aeglefinus were parasitized, (3/6 sampled in November, 1967 were positive, but none of the five taken in May, 1968), one being heavily infected. The parasites measured 1.0 - 2.0 μ by 0.5 - 1.5 μ (Figs. 52-55, 110), and did not appear to affect the host erythrocytes.

Twenty-eight per cent of the <u>Limanda ferruginea</u> (13/47) were lightly infected, (3/8 sampled in November, 1967, and 10/39 taken in May, 1968), as many as six trophozoites being found in one erythrocyte (Fig. 61). The organism measured 1.2 - 3.2 µ by 0.4 - 2.2 µ (Figs. 56-61, 109), and caused no outward effect upon the host RBC.

Three out of 19 examples of <u>Glyptocephalus</u>
<u>cynoglossus</u> caught in May, 1968, were found to be parasitized
by this babesioid. Again the infections were light, but in this
case the nuclei of the host erythrocytes were usually markedly
displaced (Figs. 63-65). The babesioid measured 0.8-3.5 µ by

0.2 - 1.0 µ. It should be remembered that <u>G</u>. <u>cynoglossus</u> was also subject to infection by <u>Trypanosoma</u> sp. and <u>Haemogregarina</u> <u>platessae</u>. Of the three examples harbouring the babesioid, one was parasitized by H. platessae as well.

Some 50% (28/60) of <u>Hippoglossoides platessoides</u> proved positive (20/47 sampled in November, 1967, and 8/13 taken in May, 1968). In this host too, all the infections were very light. The overall measurements of the parasite were 0.5 - 4.0 µ by 0.1 - 2.5 µ. As in <u>G. cynoglosus</u>, the parasite was associated with marked displacement of the host cell nucleus (Figs. 68, 112). Furthermore, the host cells were sometimes distorted (Figs. 69, 70). It is possible, though, that such distortion might have come about during preparation of the smear. Horseshoe-shaped haemogregarines with a chromatic granule at either end and thought to be division stages were encountered in two of the American place (Figs. 71, 113).

The organism under discussion is clearly a babesioid. In both morphology and size it is in very close agreement with a haematozoan from British sculpins (Henry, 1913b). Henry had earlier (1910) proposed the name of <u>Haemohormidium cotti</u> for this organism, which was described as an irregularly round or oval body lying embedded in the protoplasm of the RBC, and measuring 2.0 - 4.5 µ by 1.0 - 3.0 µ. However, Henry (1913b) made no mention of the name that he had proposed only three years previously. He now apparently took the amoeboid bodies to be developmental stages of <u>Haemogregarina cotti</u> Brumpt & Lebailly, 1904. It remained

for Wenyon (1926) to validate <u>Haemohormidium</u> by publishing a recognizable account, although it can also be argued that the "indication" in Henry's (1910) paper was a sufficient validation under articles 11, 12 and 16 of the International Code of Zoological Nomenclature, 1961 (Mackerras & Mackerras, 1961; Laird & Bullock, 1969).

Laird & Bullock (1969) described as <u>Haemohormidium</u> sp. a morphologically similar organism from two <u>Hemitripterus</u> <u>americanus</u> and one <u>Hippoglossoides</u> <u>platessoides</u> (St. Andrew's, N. B.). These amoeboid bodies, as seen in a Giemsa-stained thin blood film, measured 2.2 - 4.8 µ by 0.8 - 3.2 µ.

The present organism resembles <u>Haemohormidium</u> in both size and morphology, and is therefore identified accordingly, all the host records except <u>Hippoglossoides</u> <u>platessoides</u> being new.

Neither <u>H. cotti</u> nor <u>Haemohormidium</u> sp. of Laird & Bullock (1969) are described in sufficient detail for new material to be assigned to them with any confidence. However, it is recognized that a full description of <u>H. cotti</u> from the type host and locality might well indicate the conspecificity of this species and mine (also that found by Laird & Bullock, which certainly seems to correspond closely with the Newfoundland material under discussion). In such an event, the earlier name would of course take

precedence over that proposed below. <u>H. aulopi</u> differs appreciably from the present parasite because of its larger size and general morphology - for example, its chromatin occurs as blocks at the periphery of the body (Mackerras & Mackerras, 1961).

For the reasons given, the Newfoundland babesioid is described as new. It is designated <u>Haemohormidium</u>

<u>terraenovae</u> n.sp., having the characters outlined herein, the specific name being derived from the Latin for "Newfoundland".

Syntypes have been deposited in the collection of the U. S. National Museum (cat. no. USNM Helm. Coll. 71802).

Haemohormidium beckeri n.sp. (Figs. 72-79, 114-116)

Five of 12 longhorn sculpins, Myoxocephalus octodecemspinosus caught in May, 1968, were found to harbour babesioids of quite another kind than that described above. All infections were quite light, some 10 red cells per 1,000 being parasitized.

Amoeboid stages measured 2.0 - 5.0 μ by 0.5 - 2.5 μ . They differed from <u>H</u>. <u>terraenovae</u> in attaining a larger size and in the fact that cruciform division stages were seen (Figs. 79, 116). Rarely encountered, the latter measure 2.0 μ in diameter. They are fully comparable with the equivalent stages characterizing the genus <u>Babesiosoma</u>

as first described by Jakowska and Nigrelli (1956).

Laird & Bullock (1969) relegated Babesiosoma Jakowska & Nigrelli, 1956 to synonymy with Haemohormidium Henry, 1910.

Levine (1971) has followed their lead, emending the description of the family Dactylosomatidae Jakowska & Nigrelli, 1955, as follows. - "In erythrocytes of cold-blooded vertebrates; schizogony generally present, with formation of 4 - 16 merozoites; vectors unknown."

He recognizes three genera - Dactylosoma Labb6, 1894,

Haemohormidium Henry, 1910, and Sauroplasma du Toit, 1938 - in this family. Dactylosoma forms more than four merozoites at schizogony, Haemohormidium only four; while Sauroplasma undergoes only binary fission or budding into two daughter cells, without schizogony.

Table 7. Previous records of Haemohormidium spp.

(adapted from Levine, 1971)

Parasite	Host	Locality	Reference
Haemohormidium aulopi (Mackerras	(Marine fish)		Mackerras &
& Mackerras, 1925)	Aulopus purpurissatus	Australia	Mackerras, 1961
Laird & Bullock, 1969			
Syn. <u>Haemogregarina aulopi</u> Mackerras & Mackerras, 1925	Parma microlepis		Laird & Bullock, 1969
			Levine, 1971
H. clariae (Haiba, 1962)	(Freshwater fish)	Egyptian	Levine, 1971
Levine, 1971	Clarias lazera	Nile	
Syn. Cytauxzoon clariae Haiba, 1962			
H. cotti Henry, 1910	(Marine fish)	U. K.	Henry, 1910
(TYPE SPECIES)	Cottus bubalis		
	C. scorpius		
H. guglielmi (Carpano, 1939)	(Turtle)	Europe	Levine, 1971
Levine, 1971	Testudo		
Syn. <u>Nuttallia guglielmi</u> Carpano, 1939	campanulata		

Table 7. Previous records of Haemohormidium spp. (concl.)

Parasite	Host	Locality	Reference
Haemohormidium sp.	(Marine fish)		Laird & Bullock,
Laird & Bullock, 1969	Hemitripterus americanus N. B. 1969 Hippoglossoides platessoides		1969
H. stableri (Schmittner & McGhee, 1961)	(Frog)	N. America	Schmittner &
Laird & Bullock, 1969	Rana pipiens pipiens		McGhee, 1961
Syn. <u>Babesiosoma stableri</u> Schmittner & McGhee, 1961			Laird & Bullock, 1969
			Levine, 1971
H. tetragonis (Becker & Katz, 1965) Laird & Bullock, 1969	(Freshwater fish) Catostomus sp.	N. America	Becker & Katz, 1965
Syn. <u>Babesiosoma tetragonis</u> Becker & Katz, 1965	- sucker		Laird & Bullock, 1969 Levine, 1971

The present babesioid differs from H. terraenovae n.sp. in having rosette-shaped or cruciform schizonts in circulating erythrocytes. However, the total lack of such cruciform stages in the large amount of material reported upon with respect to H. terraenovae n.sp. leads me to suggest that there might be at least two different groups of babesioids in marine fish. One of these, characterized by conspicuous oval, ellipsoid or amoeboid trophozoites but lacking division stages in circulating RBC, might bear close comparison with Sauroplasma (of which only the type species, S. thomasi du Toit, 1938, of a lizard, is yet known). The other, somewhat larger, has more conspicuous chromatin/cytoplasm differentiation and exhibits rare rosette-shaped or cruciform schizonts in the RBC. However, the very rarity of schizonts in the latter group (to which the present species belong) suggests that the examination of additional material of H. terraenovae might still reveal schizonts. The matter clearly cannot be settled at this time, and must await further study.

<u>Haemohormidium</u> <u>beckeri</u> n.sp. having the characters described herein, is delicated to Dr. C. D. Becker⁸ who has contributed much to the study of North American piscine

⁸ Dr. C. D. Becker, Ecosystems Dept., Pacific Northwest Laboratory, Battelle Memorial Institute, Richland, Washington.

haematozoa and who provided greatly appreciated advice during my visit to his laboratory in July, 1969. Syntypes have been deposited in the collection of the U. S. National Museum (cat. no. USNM Helm. Coll. 71801).

CNIDOSPORA: MYXOSPORIDA

Kudoa Meglitsch

(a) Kudoa sp. (Figs. 95, 96, 117)

Quadricapsulate chloromyxid myxosporidan spores distinctly quadrate as viewed from the anterior extremity (Fig. 95) were present in heart blood films from three Atlantic cod from Portugal Cove, Conception Bay. They measure 5.5 - 5.8 µ by 4.8 - 5.0 µ and closely resemble Kudoa clupeidae (Hahn) in both size and shape. They are only mentioned here as an addition to the already long list of organisms accidentally present in blood films, these histozoic parasites having presumably escaped from heart muscle slit during the smearing operation.

(b) Undetermined myxosporidan (Fig. 97)

A myxosporidan trophozoite measuring 20 μ by 21 μ was identified in a blood film from a thorny skate. The vegetative forms of myxosporidans in Giemsa-stained material show no features of taxonomic value, merely appearing as variously crumpled or creased sheets of purplish-staining protoplasm. However, it is noted that a form such as the present one could pass for the vegetative stage of a widespread myxosporidan known from the gall bladder of various European and eastern North American

elasmobranchs including <u>Raja</u> spp., <u>Chloromyxum</u> <u>leydigi</u>
Mingazzini (Kudo, 1920). It is suggested that if indeed
it is referable to the Chloromyxidae, though, a more likely
explanation of its presence in a heart blood smear would
be the one given above, namely, that it is a histozoic
<u>Kudoa</u> sp. adventitiously released into the blood at the
time of smearing.

Intracrythrocytic inclusions of unknown actiology (Figs. 82-94, 118, 119)

Upon examining 34 Atlantic herrings, (<u>Clupea</u>
<u>harengus</u>) and 40 Atlantic argentines (<u>Argentina silus</u>)

caught in May, 1968, all the erythrocytes showed the inclusions
illustrated.

In <u>C. harengus</u>, some RBC exhibited one or two dark blue-staining spots (0.5 μ diameter) in their cytoplasm (Fig. 82). Others showed many (up to 40 or 50) oval or rod-like bodies of smaller size. Staining uniformly light blue with Giemsa, these were sometimes scattered throughout the cytoplasm and sometimes quite regularly arranged around the nucleus (Figs. 85, 87, 118). The presence of these bodies was not associated with hypertrophy of the RBC, but in about 2% of the cells, the nucleus showed marked displacement (Fig. 84).

In A. silus, similar bodies were found in all erythrocytes. It was tempting to hypothesize a developmental sequence from the material studied.

However, a more critical assessment provided jarring notes. For example, some of the bodies stained uniformly pinkish, others blue. Also, the majority (in A. silus) were of irregular outline (Figs. 90, 92, 96), some of them being markedly elongated (Fig. 96). There was no definite pattern of arrangement around the nucleus as in the Atlantic herring.

The first few slides examined were merely thought to be contaminated by bacteria or some other extraneous microorganisms. It was also speculated that the fish concerned were dead when the films were made. However, the field notes showed that all fish were still alive at the time of smearing. Moreover, further slides subsequently showed precisely the same apparent infection, although other species of fish collected at the same time and treated in the same way had RBC that stained normally and were altogether devoid of such bodies.

At first sight, these bodies resemble <u>Grahamella</u> sp.
(Brumpt, 1911) - found in many small mammals - and

<u>Bartonella</u> sp. (Strong, Tuzzer, Brues, Sellards & Gastiaburu,

1915) - responsible for Oroya fever in South America - in

their shape and tiny size. Nevertheless, their staining reaction and incidence (all RBC exhibiting them) separates them from the two organisms mentioned. The possibility of their being rickettsiae was also considered.

Due to the extremely small size of these bodies $(0.5-1.5~\mu$ by $0.2-0.5~\mu$) and the shortcomings of the Giemsa method, it was not possible to make out any structural details. Because of their uniform incidence it is suspected that they might prove to be merely a '.ructural peculiarity of the RBC of clupeid fish. They are therefore, at this time, simply noted and referred to as intracrythrocytic inclusions of unknown origin.

Unidentified artifacts (Figs. 80, 81, 120, 121)

Bacteria-like entities were found in blood smears from one spiny dogfish and one yellowtail flounder. In both instances, only a single groupsof organisms was encountered. Their regular arrangement and uniform staining reaction with Giemsa, suggested that they were bacterial artifacts the presence of which was accidental and associated with the preparation of the smears.

In the spiny dogfish, these organisms appeared to be intracrythrocytic (Figs. 80, 120) and in the same plane of focus as the blood cell. In the yellowtail flounder, on the other hand, they were both intra- and extraerythrocytic and were rather obviously superimposed upon the smear (Figs. 81, 121).

GENERAL DISCUSSION AND SUMMARY

Three species of trypanosomes were recorded in this survey. Trypanosoma rajae has already been reported from North American waters in Raja radiata (Kudo, 1922; Bullock, 1958; Laird & Bullock, 1969). While Trypanosoma sp. from Glyptocephalus cynoglossus is the first record of trypanosome found in this host, and the single example found showed differences from trypanosomes previously described from flounders, determination of the species must obviously await additional material. The Atlantic cod, Gadus morhua, has once previously been found to harbour trypanosomes. The species in question, Trypanosoma murmanensis Nikitin, 1927, differs in detail from Trypanosoma sp. described herein from only one of 180 cod-fish examined. This record is felt to be of particular interest in view of the desirability of our gaining an exhaustive knowledge of the pathogens and parasites of a fish of such economic importance. Nothing whatsoever being known about the role of fish trypanosomes in causing disease in their hosts, it is submitted that this cod trypanosome might be thought of as a particularly appropriate candidate for intensive laboratory investigations. Such studies would of course demand early elucidation of the vector, and piscicolid leeches have been implicated in this connexion with respect to some other fish trypanosomes (Laveran & Mesnil, 1907).

Three known species of <u>Haemogregarina</u> were recorded. <u>H. delagei</u> was found in <u>Raja radiata</u> and <u>R. senta</u>, the latter being a new host for this parasite. <u>Glyptocephalus cynoglossus</u> and <u>Scophthalmus</u> <u>aquosus</u> are new host records for <u>H. platessae</u>. <u>H. myoxocephali</u> was recorded from the type host, <u>Myoxocephalus</u> <u>octodecemspinosus</u>.

Free sporozoites in every way comparable with those described from hirudinid vectors of tortoise haemogregarines by earlier workers, were found in gut smears of piscicolid leeches (Malmiana nuda) attached to Myoxocephalus scorpius. This, together with the fact that so close a relative to M. octodecemspinosus might well be expected to harbour the same species of haemogregarine, strongly suggests that this leech is in fact a vector of H. myoxocephali. Although a number of investigators have devoted considerable efforts to searching for vectors of piscine haemogregarines since the first description of these parasites 70 years ago, this is the first evidence (circumstantial though it is) ever obtained to suggest that the cycle is the same as that elucidated for H. stepanowi and H. nicoriae in the early part of the century (Reichenow, 1910; Robertson, 1910).

Haemohormidium terraenovae n.sp. was recorded from six hosts:- Ammodytes americanus, Hippoglossoides platessoides,

Limanda ferruginea, Urophycis tenuis, Melanogrammus aeglefinus, and Glyptocephalus cynoglossus. While this babesioid might eventually prove conspecific with H. cotti Henry (1910) and Haemohormidium sp. Laird & Bullock (1969) more detailed descriptions of the latter organisms are prerequisite to final clarification. The common occurrence of this parasite in several species of fish on the Grand Banks of Newfoundland, and its rather high incidence in individual hosts, are of very considerable interest. Aside from the fact that the record constitutes only the fifth for babesioids from marine fish anywhere in the world, the facts that this group of haematozoa are responsible for serious diseases of some mammals (and have recently been confirmed to be parasites of man), and that the type locality is one of the world's major fishing grounds, demand follow-up investigations.

A second babesioid, <u>Haemohormidium beckeri</u> n.sp. was recorded from <u>M. octodecemspinosus</u>. While cruciform and/or rosette-shaped schizonts characterize <u>H. beckeri</u> n.sp., they have yet to be reported from <u>H. terraenovae</u> n.sp.. The possibility of future separation at the subgeneric level must therefore be kept in mind. All in all, the combination of a good range of unusually interesting haematozoan material, the proximity of a major fishery of great economic importance to several countries and the

facilities of the Marine Sciences Research Laboratory at Logy Bay make Memorial University of Newfoundland a singularly appropriate centre for the field and laboratory studies that will be necessary for experimental elucidation of the problems mentioned above.

Some of the negative hosts in the present project had been found to be hosts for other haematozoans by earlier workers. Thus, Squalus acanthias is known to harbour Haemogregarina delagei off the North American mainland (Laird & Bullock, 1969), where the same authors found Melanogrammus aeglefinus and Urophycis tenuis to be parasitized by H. aeglefini; Anarhichas lupus by H. anarhichadis Henry, 1912 (spelling emended from anarrhichadis by Fantham et al., 1942); and Hemitripterus americanus by Haemohormidium sp. The reasons for different incidences of marine fish haematozoan as between different localities may be basically ecological (for example, the type of substrate influencing the incidence of potential vectors such as leeches, which thrive on hosts frequenting sandy and muddy bottoms but not on stony ones). Sample size is of obvious importance too (e.g. my single record of a species of Trypanosoma from 180 cod), and again, the extremely low incidence of presumably chronic infection characteristic of most fish haematozoa must inevitably lead to some infections being overlooked.

Intraerythrocytic inclusions of unknown origin were seen in all the herrings and argentines examined. The prevalence of these bodies and their indeterminate nature suggested that they might simply represent staining artifacts in normal red cells the cytoplasmic structure of which responds to Giemsa in an unusual way. Bacterial artifacts were noted in slides from a spiny dogfish and a yellowtail flounder, and chloromyxid myxosporidan protozoans from three cod and a thorny skate. The two latter parasites may well have been histozoic organisms accidentally introduced into the blood in the act of slitting the heart wall.

REFERENCES

- Bacigalupo, J., and N. De La Plaza. 1948*. Presencia de tripanosomes en las rayas de Mar del Plata. <u>Trypanosoma</u> marplatensis, n.sp. Rev. Soc. Arc. Biol. 24: 269-274.
- Bailey, Reeve M. (chairman). 1960. A list of common and scientific names of fishes from the United States and Canada. 2nd ed. Spec. Publ. No. 2. Am. Fish. Soc., Wash. D. C.
- Becker, C. D. 1970. Haematozoa of fishes, with emphasis on North American records. pp. 82-100 in "A symposium on diseases of fishes and shellfishes", Spec. Publ. No. 5, Amer. Fish.
- Becker, C. D., and M. Katz. 1965. <u>Babesiosoma tetragonis</u>
 n.sp. (Sporozoa: Dactylosomidae) from a
 California teleost. J. Protozool. <u>12</u>:
 180-193.
- Brumpt, E., 1906. Sur quelques espéces nouvelles de trypanosomes parasites des poissons d'eau douce; leur mode d'évolution. C. R. Soc. Biol. 60: 169.
- Brumpt, E., 1911. Note sur le parasite des hématies de la taupe: <u>Grahamella talpae</u>n.g.n.sp. Bull. Soc. Path. exot., <u>4</u>: 514.

- Brumpt, E., and C. Lebailly. 1904. Description de quelques nouvelles espéces de trypanosomes et d'hémogrégarines parasites des téléostéens marins. Compt. Rend. Acad. Sci., Paris, 139: 613-615.
- Bullock, W. L. 1952. The occurrence of a species of

 Cryptobia (Protomonadina) in the blood of a
 marine fish. J. Parasitol. 38: 26.
- Bullock, W. L. 1953. An interesting blood parasite of a

 New Hampshire fish. Proc. New Hampshire

 Acad. Sci. (1952 and 1953) 2: 7-8.
- Bullock, W. L. 1958. The blood protozoa of the marine fish of Southern New England. J. Parasitol. 44: 24-25.
- Carini, A. 1932*. Sobre uma hemogregarina de um peixe do mar do Brasil. 7th Reun. Soc. Argentina Patol. Reg del Norte, <u>2</u>: 920-921.
- Coles, A. C. 1914. Blood parasites found in mammals, birds and fishes in England. Parasitology 7: 17-61.
- Dollfus, R. P. 1953. Encyclopédie Biologique. XLIII.

 Parasites animaux de la MORAE ATLANTO
 ARCTIQUE (<u>Gadus callarias</u> L. = <u>G</u>. <u>morhua</u> L.).

 p. 17-32.

- Ellis, Marjorie E. 1930. Investigations on the protozoan fish parasites of the St. Andrew's Region. Proc. Trans. Nova Scotian Inst. Sci. (1929-1930) 17: 268-275.
- Fantham, H. B. 1919. Some parasitic protozoa found in South African fishes and amphibians -I. S. Afr. J. Sci. 15: 337.
- Fantham, H. B., Annie Porter, and L. R. Richardson. 1942.

 Some haemotozoa observed in vertebrates in

 Eastern Canada. Parasitology 34: 199-226.
- Frost, W. E., and M. E. Brown. 1967. The Trout. Collins.
- Gros, G. 1845. (Bull. Soc. Nat. Moscow, p. 424: not seen in original, reference derived from Laveran & Mesnil, 1907, q.v.).
- Henry, H. 1910. On the haemoprotozoa of British sea-fish.

 J. Path. Bact. 14: 463-465.
- Henry, H. 1912. <u>Haemogregarina anarrhichadis</u> from

 <u>Anarrhichas lupus</u>, the catfish. Parasitology
 5: 190-196.
- Henry, H. 1913a. A summary of the blood parasites of

 British sea-fish. J. Path. Bact. 18: 218-223.
- Henry, H. 1913b. An intracorpuscular parasite in the

 blood of <u>Cottus bubalis</u> and <u>Cottus scorpius</u>.

 J. Path. Bact. <u>18</u>: 224-227.

- Hoare, C. A. 1930. On a new <u>Dactylosoma</u> occurring in fish of Victoria Nyanza. Ann. Trop. Med. Parasitol. 24: 241-248.
- Honigberg, B. M., et al. 1964. A revised classification of the phylum protozoa. J. Protozool. 11: 7-20.
- Jakowska, S., and R. F. Nigrelli. 1956. <u>Babesiosoma</u> gen. nov. and other babesioids in erythrocytes of cold-blooded vertebrates. Ann. N. Y. Acad. Sci. 64: 112-127.
- Kohl-Yakimoff, N., and W. L. Yakimoff. 1915*. Hamogregarinen der Seefische. Zentr. Bakteriol. Parasitnek. Abt. I. Orig. 76: 134-146.
- Kudo, R. 1923. Skate trypanosome from Woods Hole. J. Parasitol. 9: 179-180.
- Kudo, R. 1920. Studies on Myxosporidia. Illinois Biol. Monogr. 5, (3 & 4), 265 pp.
- Laird, M. 1951. Studies on the trypanosomes of New Zealand fish. Proc. Zool. Soc. Lond. 121: 285-309.
- Laird, M. 1952. New haemogregarines from New Zealand marine fishes. Trans. roy. Soc. New Zealand 79: 589-600.
- Laird, M. 1953. The protozoa of New Zealand intertidal zone fishes. Trans. roy. Soc. New Zealand 81: 79-143.

- Laird, M. 1961a. Parasites from Northern Canada. II.

 Haematozoa of fishes. Can. J. Zool.

 39: 541-548.
- Laird, M. 1961b. Trichodinids and other parasitic protozoa from the intertidal zone at Nanaimo, Vancouver Island. Can. J. 2001. 39: 833-844.
- Laird, M., and W. L. Bullock. 1969. Marine fish haematozoa from New Brunswick and New England. J. Fish. Res. Bd. Canada. 26: 1075-1102.
- Laveran, A. 1908. Sur une hémogrégarine, un trypanosome et un spirille, trouvés dans le sang d'un requin. Bull. Soc. Path. exot. 1: 148-150.
- Laveran, A., and F. Mesnil. 1901. Deux espèces nouvelles d'hémogrégarines des poissons. Compt. Rend. Acad. Sci., Paris. <u>133</u>: 572-577.
- Laveran, A., and F. Mesnil. 1902a. Sur les hématozoaires des poissons marins. Compt. Rend. Acad. Sci., Paris. 135: 567.
- Laveran, A. and F. Mesnil. 1902b. Des trypanosomes des poissons. Arch. Protistenk. $\underline{1}$: 475.
- Laveran, A., and F. Mesnil. 1907. Trypanosomes and

 Trypanosomiases. (Transl. and enlarged by

 D. Nabarro]. W. T. Keener, Chicago. 538 pp.

- Laveran, A., and F. Mesnil. 1912. Trypanosomes et

 Trypanosomiases. Deuxième ed. Masson,
 Editeurs, Paris. 999 p.
- Lebailly, C. 1904. Sur quelques hémoflagelles des téléostéens marins. Compt. Rend. Acad. Sci., Paris. 139: 576-577.
- Levine, N. D. 1971. Taxonomy of the piroplasms. Trans.

 Amer. Micr. Soc. 90: 2-33.
- Lewis, T. R. 1878. The microscopic organisms found in the blood of man and animals, and their relation to disease. Ann. Rep. San. Commissioner Govt. India, Calcutta, 1877, XIV. 157.
- Lewis, T. R. 1879. Flagellated organisms in the blood of healthy rats. Quart. J. micr. Sci. 19: 109.
- Mackerras, I. M., and M. J. Mackerras. 1925. The haematozoa of Australian marine teleostei. Proc. Linn. Soc. N. S. Wales 50: 359-366.
- Mackerras, M. J., and I. M. Mackerras. 1961. The haematozoa of Australian frogs and fish. Aust. J. Zool. 9: 123-139.
- Mavor, J. W. 1915. Studies on the sporozoa of the fishes of the St Andrew's Region. Govt. Canada, Marine fish. Sessional Paper 39b: 25-38.

- Minchin, E. A. 1909a. The structure of <u>Trypanosoma lewisi</u> in relation to microscopical technique. Quart. J. micr. Sci. 53: 755.
- Minchin, E. A., and H. M. Woodcock. 1910. Observations on certain blood parasites of fishes occurring at Rovigno. Quart. J. micr. Sci. 55: 113-154.
- Misra, K. K., D. P. Haldar, and M. M. Chakravarty. 1969.

 <u>Babesiosoma ophicephali</u> n.sp. from the
 freshwater teleost <u>Ophicephalus</u> <u>punctatus</u>

 Bloch. J. Protozool. 16: 446-449.
- Neumann, R. O. 1909. Studien über protozoische Parasiten im Blut von Meeresfischen. Z. Hyg. Infektionskrankh. 64: 1-112.
- Nigrelli, R. F. 1929a. <u>Dactylosoma jahni</u> sp. nov., a sporozoan parasite of the erythrocytes and erythroplastids of the newt (<u>Triturus</u> viridescens). J. Parasitol. <u>16</u>: 102.
- Nikitin, S. A. 1927. Matériaux sur des parasites du sang chez des vertébrés nordiques.

 Russian Journ. Tropical Medicine. Moscou, t.V, 1927, No. 6, p. 350-356. (Reference from Dollfus, R. P. 1953).

 (Original in Russian).

- Reichenow, E. 1910.* Haemogregarina stepanowi. Die
 Entwicklungsgeschichte einer Hamogregarine.
 Arch. Protistenk 20: 251.
- Richardson, L. R. 1970. A new marine piscicolid leech from Newfoundland placed provisionally in the genus <u>Malmiana</u>. Can. J. Zool. 48: 841-845.
- Robertson, M. 1910. Studies on Ceylon Haematozoa.

 No. II Notes on the life-cycle of

 Haemogregarina nicoriae, Cast. and Willey.

 Quart. Jour. micr. Sci. 55: 741.
- Saunders, D. C. 1954. A new haemogregarine reported from the spotted squeteague, <u>Cynoscion nebulosus</u>, in Florida. J. Parasitol.
 40: 699-700.
- Saunders, D. C. 1955. The occurrence of <u>Haemogregarina</u>

 <u>bigemina</u> Laveran & Mesnil and <u>H. achiri</u>n.sp.

 in marine fish from Florida.

 J. Parasitol. 41: 171-176.
- Saunders, D. C. 1958a. Blood parasites of the marine fishes of the Florida Keys. Year Book Am. Phil. Sco.: 261-266.

- Saunders, D. C. 1958b. The occurrence of <u>Haemogregarina</u>

 <u>bigemina</u> Laveran and Mesnil and <u>H. dasyatis</u>

 n.sp. in marine fish from Bimini, Bahamas,

 B. W. I. Trans. Amer. micr. Soc. 77: 404-412.
- Saunders, D. C. 1959. Trypanosoma balistes n.sp. from

 Balistes capriscus Gmelin, the common

 triggerfish, from the Florida Keys. J.

 Parasitol. 45: 623-626.
- Saunders, D. C. 1960. A survey of the blood parasites in the fishes of the Red Sea. Trans. Amer. micr. Soc. 79: 239-252.
- Saunders, D. C. 1964. Blood parasites of marine fish of
 Southwest Florida, including a new
 haemogregarine from the menhaden, <u>Brevoortia</u>
 tyrannus (Latrobe). Trans. Amer. micr. Soc.
 83: 218-225.
- Saunders, D. C. 1966. A survey of the blood parasites of the marine fishes of Puerto Rico. Trans. Amer. micr. Soc. 85: 193-199.
- Schmittner, S. M., and R. B. McGhee. 1961. The intraerythrocytic development of <u>Babesiosoma</u> stableri n.sp. in <u>Rana pipiens pipiens</u>. J. Protozool.

 8: 381-386.

- Strong, R. P., E. E. Tyzzer, C. T. Brues, A. W. Sellards, and J. E. Gastiaburu. 1915. Report of First Expedition to South America, 1913. Harvard School of Tropical Medicine.
- Valentin 1841*. Über ein Entozoon im Blute von <u>Salmo</u>
 fario. Arch. Anat. Phys. Wiss. Med.: 435.
- Wenyon, C. M. 1926. Protozoology, Vol. II. Baillière, Tindall and Cox, London. P. ix + 779 - 1563.

^{*} Papers not seen in original.

Fig. 1. Erythrocyte, Raja radiata.

Fig. 2. Trypanosoma rajae, from R. radiata.

Fig. 3. Erythrocyte, Glyptocephalus cynoglossus.

Fig. 4. Trypanosoma sp., from G. cynoglossus.

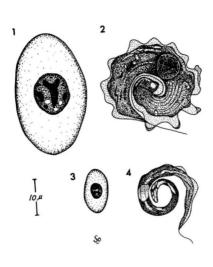


Fig. 5. Erythrocyte, <u>Gadus morhua</u>.

Figs. 6-7. <u>Trypanosoma</u> sp. from <u>G. morhua</u>.

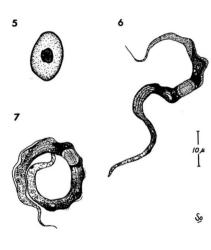


Fig. 8. Erythrocyte, Raja radiata.

Figs. 9-11. Haemogregarina delagei, R. radiata.

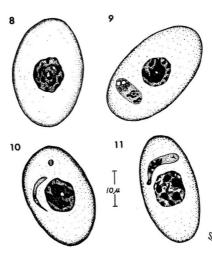


Fig. 12. <u>Haemogregarina</u> <u>delagei</u>, <u>Raja</u> <u>radiata</u>.

Fig. 13. Dividing <u>H. delagei</u>, from <u>R. radiata</u>.

Fig. 14. <u>H. delagei</u> from <u>R. radiata</u>, showing double invasion of host erythrocyte.

Fig. 15. Free vermicule of <u>H</u>. <u>delagei</u>, from <u>R</u>. <u>radiata</u>.

Fig. 16. H. delagei from Raja senta.

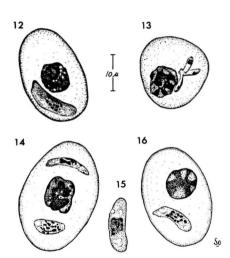




Fig. 17. Erythrocyte, Glyptocephalus cynoglossus.

Figs. 18-19. <u>Haemogregarina platessae</u>, from <u>G. cynoglossus</u>.

Fig. 20. Erythrocyte, Scophthalmus aquosus.

Figs. 21-22. H. platessae, from S. aquosus.
Fig. 23. Erythrocyte, Myoxocephalus

octodecemspinosus.

Figs. 24-25. H. myoxocephali, from $\underline{\overline{M}}$. octodecemspinosus.

Figs. 26-29. Sporozoites from gut of the leech, Malmiana nuda.

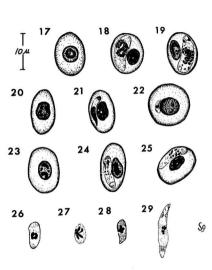


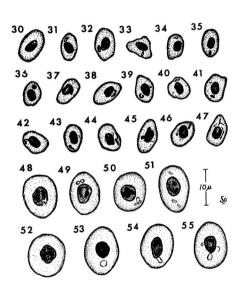
Fig. 30. Erythrocyte, Ammodytes americanus.

Figs. 31-47. Haemohormidium terraenovae n.sp. from A. americanus.

Fig. 48. Erythrocyte, <u>Urophycis</u> tenuis.

Figs. 49-51. H. terraenovae n.sp., from U. tenuis.

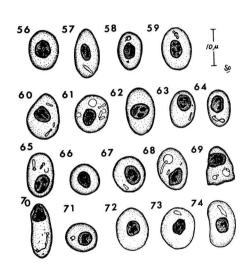
Figs. 53-55. H. terraenovae n.sp., from M. aeglefinus.



- Fig. 56. Erythrocyte, <u>Limanda ferruginea</u>.
 Figs. 57-61. Haemohormidium terraenovae n.sp.,
- from L. ferruginea.

 Fig. 62. Erythrocyte, Glyptocephalus cynoglossus.
- Figs. 63-65. Erythrocyte, Glyptocephalus cynoglossus.

 Figs. 63-65. H. terraenovae n.sp., from G. cynoglossus.
- Fig. 66. Erythrocyte, Hippoglossoides platessoides.
- Figs. 67-71. H. terraenovae n.sp., from platessoides.
- Fig. 72. Erythrocyte, Myoxocephalus octodecemspinosus.
- Figs. 73-74. <u>Haemohormidium beckeri</u> n.sp., from <u>M</u>. <u>octodecemspinosus</u>.



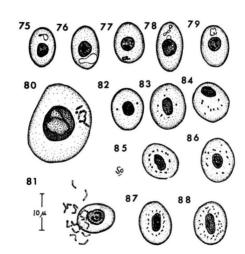
Figs. 75-79. <u>Haemohormidium beckeri n.sp., from Myoxocephalus octodecemspinosus.</u>

Fig. 80. Bacterial contaminants, from Squalus acanthias.

-

Fig. 81. Bacterial contaminants, from Limanda ferruginea.

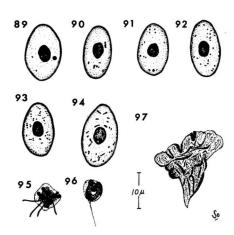
Figs. 82-88. Intracrythrocytic inclusions of unknown actiology, from Clupea harengus.



Figs. 89-94. Intraerythrocytic inclusions of unknown aetiology, from <u>Argentina</u> silus.

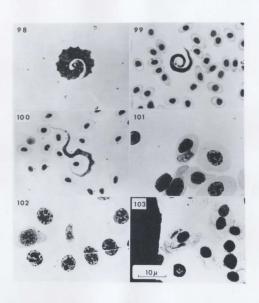
Figs. 95-96. Kudoa sp., as a contaminant in blood of Gadus morhua.

Fig. 97. Vegetative stage of unidentified myxosporidan present as contaminant in blood of <u>Raja</u> <u>radiata</u>.



- Fig. 98. Trypanosoma rajae, from Raja radiata.

 Fig. 99. Trypanosoma sp., from Glyptocephalus cynoglossus.
- Fig. 100. Trypanosoma sp., from Gadus morhua.
- Fig. 101. Haemogregarina delagei, from R. radiata.
- Fig. 102. H. <u>delagei</u>, from <u>R</u>. <u>radiata</u>, showing free vermicule.
- Fig. 103. H. delagei, from Raja senta, showing double invasion of host erythrocyte.



Myoxocephalus octodecemspinos	us.
Fig. 105. Haemogregarina platessae, from Glyptocephalus cynoglossus.	m
Fig. 106. Free sporozoite from gut smea piscicolid leech, Malmiana nu	
Fig. 107. Haemohormidium terraenovae n. from Ammodytes americanus.	sp.,
Fig. 108. <u>Haemohormidium terraenovae</u> n. from <u>Urophycis</u> tenuis.	sp.,
Fig. 109. Haemohormidium terraenovae n. from Limanda ferruginea.	sp.,



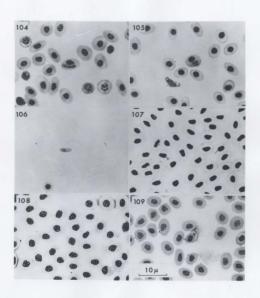
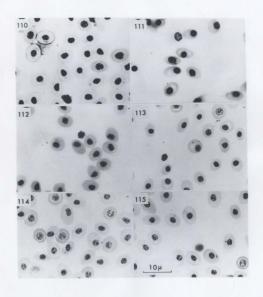


Fig. 110. Haemohormidium terraenovae n.sp., from Melanogrammus aeglefinus.

Fig. 111. Haemohormidium terraenovae n.sp., from Glyptocephalus cynoglossus.

Figs. 112-113. Haemohormidium terraenovae n.sp., from <u>Hippoglossoides platessoides</u>.

Figs. 114-115. Haemohormidium beckeri n.sp., from Myoxocephalus octodecemspinosus.



- Haemohormidium beckeri n.sp., from Fig. 116. Myoxocephalus octodecemspinosus, showing rosette schizont.
- Fig. 117. Kudoa sp., from Gadus morhua.
- Intraerythrocytic inclusions of Fig. 118. unknown aetiology, form Clupea harengus.
- Intracrythrocytic inclusions of Fig. 119. unknown aetiology, from Argentina silus. Bacterial contaminants, from Fig. 120.
 - Squalus acanthias. Fig. 121.
 - Bacterial contaminants, from Limanda ferruginea.

