1979


Ann L. Scarborough
Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_disstheses/3463

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in "sectioning" the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.

University Microfilms International
300 N. ZEEB ROAD, ANN ARBOR, MI 48106
18 BEDFORD ROW, LONDON WC1R 4EJ, ENGLAND
SCARBOROUGH, ANN L.

GLUGEA HERTWIGI (PROTOZOA: MICROSPORIDA) IN OSMERUS MORDAX (TELEOSTEI: OSMERIDAE): FIELD ECOLOGY, LABORATORY TRANSMISSION AND ULTRASTRUCTURAL STUDY OF PARASITE DEVELOPMENT AND THE HOST CELL-PARASITE INTERFACE

The Louisiana State University and Agricultural and Mechanical Col.

University Microfilms International
300 N. Zeeb Road, Ann Arbor, MI 48106
18 Bedford Row, London WC1R 4EF, England

PH.D. 1979
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ☑.

1. Glossy photographs ☑
2. Colored illustrations
3. Photographs with dark background ☑
4. Illustrations are poor copy
5. Print shows through as there is text on both sides of page
6. Indistinct, broken or small print on several pages throughout
7. Tightly bound copy with print lost in spine
8. Computer printout pages with indistinct print
9. Page(s) lacking when material received, and not available from school or author
10. Page(s) seem to be missing in numbering only as text follows
11. Poor carbon copy
12. Not original copy, several pages with blurred type
13. Appendix pages are poor copy
14. Original copy with light type
15. Curling and wrinkled pages
16. Other

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Zoology and Physiology

by

Ann L. Scarborough
B.A., University of California, San Diego, 1972
M.Sc., Louisiana State University, Baton Rouge, 1977
December 1979
EXAMINATION AND THESIS REPORT

Candidate: Ann L. Scarborough

Major Field: Zoology


Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

3 December, 1979
ACKNOWLEDGEMENTS

I wish to thank my major professor Dr. Earl Weidner for guidance and encouragement during all phases of this investigation. Additional appreciation to my committee members; Drs. Kenneth C. Corkum, William B. Stickle, J. Michael Fitzsimmons and Albert H. Meier of the Department of Zoology and Physiology and Dr. James A. Avault of the Department of Wildlife and Fisheries, for unlimited academic interchange.

Thanks are recorded to Mr. Charlie Wheeler of the National Marine Fisheries Service Aquarium, Woods Hole, Massachusetts; to Mr. Stephen Nepszy of the Canadian Ministry of Natural Resources, Lake Erie Fisheries Research Station, Wheatley, Ontario, and special appreciation to Mr. James Kennedy of the Massachusetts Fish and Wildlife Service, Buzzards Bay, Massachusetts.

Electron Microscopy facilities were supplied by Dr. Mort Maser of the Marine Biological Laboratory, Woods Hole, Massachusetts.

I dedicate this work to my father William E. Scarborough who started it all, and to my husband David L. Bull, who lived through it all.

This research was supported by NSF Doctoral Research Grant #DEB 77-08413.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>7</td>
</tr>
<tr>
<td>Adult Smelt</td>
<td>7</td>
</tr>
<tr>
<td>Transmission Experiments</td>
<td>7</td>
</tr>
<tr>
<td>Development of Xenomas</td>
<td>9</td>
</tr>
<tr>
<td>RESULTS</td>
<td>10</td>
</tr>
<tr>
<td>Adult Smelt</td>
<td>10</td>
</tr>
<tr>
<td>Parasite Transmission</td>
<td>10</td>
</tr>
<tr>
<td>Parasite Development</td>
<td>16</td>
</tr>
<tr>
<td>Light Microscopic Observations</td>
<td>16</td>
</tr>
<tr>
<td>Electron Microscopic Observations</td>
<td>16</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>54</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>77</td>
</tr>
<tr>
<td>VITA</td>
<td>89</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Experimental infection of laboratory-reared and collected smelt with <em>Glugea hertwigi</em> spores</td>
<td>11</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Young specimens of the smelt, <em>Osmerus mordax</em>, experimentally infected with <em>Glugea hertwigi</em></td>
</tr>
<tr>
<td>2.</td>
<td>Young smelt, 12 days after feeding on spore-carrying plankton</td>
</tr>
<tr>
<td>3.</td>
<td>Young smelt infected by both spore suspension and spore-carrying plankton</td>
</tr>
<tr>
<td>4.</td>
<td>Whole intestine 1 week after exposure to spore-carrying plankton</td>
</tr>
<tr>
<td>5.</td>
<td>Cross section of intestine 1 week after exposure to spore-carrying plankton</td>
</tr>
<tr>
<td>6.</td>
<td>Cross section of intestine from lightly infected smelt 2 weeks after feeding on a spore suspension</td>
</tr>
<tr>
<td>7.</td>
<td>Xenoma, 5 days after parasite infection</td>
</tr>
<tr>
<td>8.</td>
<td>Xenoma, 1 week after exposure to spore-carrying plankton</td>
</tr>
<tr>
<td>9.</td>
<td>Host-parasite interface, schizont, endoplasmic reticulum and mitochondria</td>
</tr>
<tr>
<td>10.</td>
<td>Host-parasite interface, schizont plasmodium and endoplasmic reticulum</td>
</tr>
<tr>
<td>11.</td>
<td>Host response to xenoma 14 days after parasite infection</td>
</tr>
<tr>
<td>12.</td>
<td>Host-parasite interface, schizonts and endoplasmic reticulum</td>
</tr>
<tr>
<td>13.</td>
<td>Cross section of schizonts</td>
</tr>
<tr>
<td>14.</td>
<td>Schizont plasmodium</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>15.</td>
<td>Asexual multiplication of schizont</td>
</tr>
<tr>
<td>16.</td>
<td>Plurinucleate plasmodium</td>
</tr>
<tr>
<td>17.</td>
<td>Onset of sporogony</td>
</tr>
<tr>
<td>18.</td>
<td>Division of plasmodium into primordial sporonts</td>
</tr>
<tr>
<td>19.</td>
<td>Early sporonts</td>
</tr>
<tr>
<td>20.</td>
<td>Sporonts during karyokinesis</td>
</tr>
<tr>
<td>21.</td>
<td>Early sporogonial plasmodium</td>
</tr>
<tr>
<td>22.</td>
<td>Sporogonial plasmodium</td>
</tr>
<tr>
<td>23.</td>
<td>Cross section of nuclei within sporogonial plasmodium</td>
</tr>
<tr>
<td>24.</td>
<td>Division of sporogonial plasmodium</td>
</tr>
<tr>
<td>25.</td>
<td>Cytokinesis of sporogonial plasmodium</td>
</tr>
<tr>
<td>26.</td>
<td>Stages of <em>G. hertwigi</em> development</td>
</tr>
<tr>
<td>27.</td>
<td>Young sporoblasts</td>
</tr>
<tr>
<td>28.</td>
<td>Sporogenesis</td>
</tr>
<tr>
<td>29.</td>
<td>Sporogenesis</td>
</tr>
<tr>
<td>30.</td>
<td><em>G. hertwigi</em> spores</td>
</tr>
</tbody>
</table>
ABSTRACT

Glugea hertwigi-induced microsporidiosis is a disease of the smelt Osmerus mordax. The yearly incidence of infection reaches over 50% in adult smelt and as high as 90% in juveniles. Primary infections localize as large intracellular colonies in submucosal cells of the digestive tract. Field observations indicate the ovaries of spawning females are a secondary site of infection. G. hertwigi was successfully transmitted to both laboratory reared and collected young smelt at 20° C by small filter-feeding vectors and by direct ingestion of spores. Infections transmitted by spore-carrying vectors numbered hundreds per animal, and were visible along the intestine one week after feeding. Large parasitized host-cells (xenomas) extended from the intestinal serosa and were easily recovered. G. hertwigi infections acquired by direct spore feeding numbered one or two per animal; these fish had the capacity to develop many new infections by feeding on spore-carrying vectors. This microscopic study revealed that G. hertwigi-induced xenomas are products of infected host cells which undergo extensive hypertrophy. After 1 week of growth, 20 to 50 μm xenomas
contained a few enlarged host nuclei and vegetative *G. hertwigi*; after 2 weeks, the xenomas measured 100 to 125 μm, exhibited multiple host nuclei and numerous *G. hertwigi* sporoblasts and spores. At 2 weeks, the host elaborated a capsule of collagen in opposing layers around the xenoma.

An extensive trabecular network (TBN) of membrane lamellae established continuity between *Glugea* and host cell components. The cisternae of the TBN contained condensed material in direct contact with parasite schizonts. Fine structure corresponds with the basic pattern revealed for other *Glugea*. Features of schizogony included a multilayered outer coat, multinucleate plasmodia and karyokinesis by an intranuclear centriolar plaque. Sporonts formed plasmodia within parasitophorous vacuoles. Sporoblasts were ovoid, contained a classical Golgi, endoplasmic reticulum and remained connected by a cytoplasmic bridge until sporogenesis. Sporogenesis organized the polar tube, polaroplast, endospore and exospore into a typical uninucleate microsporidan. Neither diplokaryon nor mitochondria were observed in any of the parasite developmental stages.
INTRODUCTION

Glugea hertwigi-induced microsporidiosis is a disease of the smelt, Osmerus mordax. *O. mordax* is an anadromous species that has been successfully introduced to temperate freshwater areas. Haley (1957) has provided some evidence which indicates *G. hertwigi* was in part responsible for the decline of the smelt fishery in the Atlantic. The incidence of *Glugea* infection reaches a seasonal peak of nearly 90% in juvenile Lake Erie smelt each summer and fall; Nepszy, Budd, and Dechtiar (1978) estimate vast economic losses in the smelt fishery in the Great Lakes each year due to mortality of infected juveniles. *G. hertwigi* cysts range from 0.4 to 5.0 mm in diameter; heavy smelt infections number over 200 xenomas per host (Legault and Delisle, 1967; Nepszy and Dechtiar, 1972).

*G. hertwigi* infections typically localize as parasite colonies in the submucosal layer of the intestine (Schrader, 1921). Mortality of the smelt host is believed to occur by starvation; in addition, infected fish have navigation problems, are more susceptible to predation, and less able to recover from environmental stress (Legault and Delisle, 1967; Nepszy and Dechtiar, 1972). Spawning
female smelt in Lake Erie characteristically have numerous G. hertwigi cysts in the ovaries as well as along the intestine. Chen and Power (1972) reported a 42% decrease in fecundity of Glugea infected females. The microsporidan Pleistophora ovariae infecting the golden shiner Notemigoneous crysoleucas is an example of transovarian parasite transmission (Summerfelt and Warner, 1970).

McVicar (1975), Olson (1976) and Weissenberg (1968) transmitted Glugea species to fish held in the laboratory. Weidner (1973) and Stunkard and Lux (1965) suggested that invertebrate filter-feeders may serve as natural vectors or transport hosts for fish microsporidians. Olson (1976) determined a low level of Glugea stephani infection occurred by ingestion of spores directly. McVicar (1975) transmitted G. stephani through spore-carrying vectors and by injection of spores into the peritoneal cavity of adult fish. Weissenberg (1968) did not determine whether Glugea anomala was initiated by a spore-carrying vector or by direct ingestion of the spores.

G. hertwigi is an obligate intracellular parasite completing its life cycle within a single host cell (Debaisieux, 1920; Honiberg, 1964; Kudo, 1966; Moniez, 1920; Theolohan, 1891). After ingestion by a smelt, the spore stage is activated to extrude the polar filament
apparatus and discharges the infective sporoplasm stage intracellularly into the submucosal environs of the intestine (Dissanaike and Canning, 1957; Ishihara, 1968; Kramer, 1960; Lom and Vavra, 1963a; Vavra, 1976a; Weidner, 1972, 1976c). There are essential differences in the effect of microsporidan invasion on the host cell (Sprague, 1969; Sprague and Vernick, 1974; Weissenberg, 1976). One such effect is exemplified by the cytopathology of Glugea infections. Initially the parasite does not cause host cell degeneration but stimulates hypertrophy and abnormal development (Weissenberg, 1968, 1976; Weidner, 1976b). Hypertrophic growth is a common response to intracellular parasite invasion; however, the enlarged host-cell nucleus usually remains undivided (Jirovec, 1930; Moulder, 1979; Mrazek, 1910, Petri, 1976; Sprague, 1969; Weissenberg, 1949, 1965, 1976). On the other hand, development of Glugea is unique since it stimulates amitotic multiplication of the host-cell nucleus (Loubes et al 1976; Sprague and Vernick, 1968; Weissenberg, 1976). In his initial descriptions of G. anomala, Weissenberg (1921, 1949) followed by light microscopy transformation of the host cell. He referred to the association between an hypertrophied host cell and developing intracellular parasites as a "xenoma" (Weissenberg 1922, 1968, 1976).
The host cell component is induced to undergo extensive growth during vegetative development of the parasite. Eventually the parasite differentiates into mature spores which fill the central region of the xenoma. By this stage, a combination of host animal response and parasite growth transform the "xenoma" into a thick-walled "Glugea-cyst" filled with innumerable spores (Sprague and Vernick, 1968; Weidner, 1974, 1976b, Weissenberg, 1968, 1976). The "Glugea-cyst" or cell-hypertrophy tumor represents the most complex formation of an host cell and microsporidan parasite (Nigrelli, 1953; Sprague, 1969; Sprague and Vernick, 1974; Weissenberg, 1976).

The microsporidan life cycle has two distinct phases: (1) schizogony, during which the number of parasites rapidly increases by asexual multiplication and the infection is spread through the host cells and (2) sporogony, during which additional multiplication, involving autogametic recombination occurs and resistant spores are formed (Kudo, 1966, Vavra, 1976b; Sprague, 1977; Loubes, 1979). Among the microsporida life cycle stages of schizogony and sporogony differ in their structure and relationship to the host cell; however, many species are similar in the site of infection, number of parasite generations and the duration of the two
developmental phases (Cali, A. 1971; Gray, Cali & Briggs, 1969; Hazard and Anthony, 1974; Orimeres and Sprague, 1973; Sprague, 1977; Vavra, 1976b). Similarity of features does not imply similarity of biological characteristics (Loubes, 1979). Biological and biochemical characteristics are known for only a small number of microsporida and as yet are not used in their taxonomy (Fowler & Reeves, 1974a, 1974b; Sprague, 1977; Sprague and Vernick, 1971). Morphological features such as spore size, number of coils in the intrasporal polar filament, and length of extruded filament, and life cycle characteristics such as presence of a single nucleus or a diplokaryon in any life stage, formation of a pansporoblast or parasitophorous vacuole, and number of spores from a sporont are used as taxonomic determinants (Burges, Canning and Hulls, 1974; Canning, 1970; Fowler, 1974b; Hazard and Anthony, 1974; Loubes et al, 1976, 1979; Loubes, 1979; Milner and Beaton, 1977; Ormieres and Sprague, 1973; Sprague, 1977; Sprague and Vernick, 1971; Weiser, 1976).

The minutia of the microsporida urges morphological investigation at the ultrastructural level. Although the genus Glugea was established in 1891 by Thelohan, much of our present knowledge remains based on light microscopic examination of mature cyst infections (Debaisieux,
1920; Kudo, 1924, 1966; Moniez, 1920; Weissenberg, 1949, 1968, 1976). The few electron microscopic studies of Glugea have been concerned with stages of spore formation and spore structure (Loubes et al, 1976; McLaughlin, 1969; Sprague and Vernick, 1969; Vernick, Tousimis and Sprague, 1969). Lom and Vavra (1963a, 1963b) were first to publish electron microscopic studies on several genera of microsporidan spores, including Glugea, but gave details concerning only Pleistophora. Sprague and Vernick (1969) published a limited study of the fine structure of Glugea weissenbergi development. The authors admit the poor condition of their specimens and their descriptions have raised considerable controversy (Vavra, 1976a). Weidner (1976b) was first to publish a fine structure and cytochemical characterization on the periphery of the Glugea stephani-induced xenoma.

This study is the first ultrastructural investigation of Glugea-induced xenoma development. Observations made during this study produced data on: (a) the determination of the ecology of parasite transmission; (b) the establishment of a reliable laboratory method of inducing parasite infection; and (c) an understanding of the general life cycle, parasite-host cell relationship and ultrastructure of Glugea hertwigi development within host cells.
MATERIALS AND METHODS

Adult smelt

Spawning female smelt were collected with a cast-net from Wheatley, Ontario, on Lake Erie and from the Jones River, near Plymouth, Cape Cod, Massachusetts. A total of 150 fish from each location were examined internally for the presence of Glugea hertwigi cysts. The intestine and ovaries from infected and non-infected fish were excised, cut into small pieces and fixed in pH 7.4 phosphate buffered gultaraldehyde overnight at 4°C. After several buffer rinses, the material was post-fixed in phosphate buffered 2% osmium tetroxide for 2 hrs at 4°C, dehydrated in ethanol and embedded in Epon. One micron sections were cut on a Dupont-Sorvall MT-2B microtome and stained with 1% toluidine blue. Parasite cysts were removed from adult smelt, homogenized, pelleted in pH 7.4 phosphate buffer, stored at 4°C and used for the transmission experiments.

Transmission experiments

Eggs and milt were stripped from spawning smelt, mixed 1:5 respectively and kept in a well-aerated nylon
mesh cone. Naturally fertilized eggs were also collected from the riverbed. Anticipating large mortalities, a non-infected smelt population was located in Long Pond, Cape Cod, Massachusetts. Several years' examination of smelt from this pond had indicated they were completely free of *G. hertwigi* infection. Young smelt (20-25 mm) in Long Pond were attracted at night to a strong light at the surface and collected by hand-net. All fish were maintained at the National Marine Fisheries Service Aquarium, Woods Hole, in 20°C filtered fresh water taken from the Jones River well above the spawning sites. All fish were maintained on a diet of phyto- and zooplankton seined from Long Pond. The following methods of parasite transmission were attempted.

**Experiment 1.** Laboratory reared and collected smelt were exposed to a suspension of *G. hertwigi* spores placed in the tanks. The water was well aerated but not filtered for the following 48 hrs.

**Experiment 2.** Laboratory reared smelt (10 mm, 6 weeks post spawning) were fed spore-carrying zooplankton (cladocerans and copepods) on 2 consecutive days. The plankton was first exposed to a spore suspension for 30 min, washed once with water, examined to ensure the
presence of spores in their digestive tracts and then fed to the smelt.

**Experiment 3.** Collected smelt (15 mm, approximately 8 weeks post spawning) were fed spore-carrying plankton as above.

**Experiment 4.** Smelt from Experiment 1, 4 weeks after exposure to a spore suspension, were fed spore-carrying plankton as above. As a control, a number of laboratory reared and collected smelt were maintained unexposed to spores.

**Development of Xenomas**

Young smelt from all tanks were observed and photographed with a Wild M4 Makroskope 5 days, 1, 2 and 3 weeks after spore feeding. Intestinal tissue from experimental and control smelt were prepared for light and electron microscopy as outlined above. Serial, 2 micron thick sections were cut from 10 day old xenomas and stained for periodic-acid-Schiff's (PAS) positive material according to Huger, (1969). After a 1 hr incubation in IN HCl at 55° C, the sections were thoroughly rinsed and stained in Schiff's reagent for 3 hr, rinsed briefly in 1% sodium metabisulfite, and counter stained with 1% toluidine blue.
RESULTS

**Adult smelt**

In spawning females, *Glugea hertwigi* cysts were found in the ovaries of more than 50% of the infected fish in Lake Erie and in 25% of infected fish in the Jones River during 1977, 1978 and 1979. The ovaries of Lake Erie fish were heavily infected, whereas those of the Jones River were lightly infected. Light microscopy and ultrastructural examinations of infected ovaries showed that *Glugea* parasites were not within egg cells.

**Parasite transmission**

The results of the experiments are summarized in Table I. Infections were obtained from both methods of spore transmission; however, the intensity of infection differed (Fig. 1).

**Experiment 1.** Laboratory reared and field collected fish were exposed to suspensions of *G. hertwigi* spores. Four weeks after exposure, 40% of the smelt exhibited one or two *Glugea* cysts in the posterior region of the intestine. These cysts were uniformly dense and protruded from the submucosa distending the peritoneal cavity. The
**TABLE 1**

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Source of smell</th>
<th>Sample size</th>
<th>Method of exposure</th>
<th>Period of incubation</th>
<th>Number of smelt infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>mixture of lab reared and collected</td>
<td>20</td>
<td>spore suspension</td>
<td>28 days</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>lab reared</td>
<td>5</td>
<td>spore-carrying plankton</td>
<td>7 days</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>collected</td>
<td>14</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td>4*</td>
<td>mixture</td>
<td>8</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
</tr>
</tbody>
</table>

* Smelt from Experiment 1, carrying light infections 28 days after exposure to a spore suspension.
Figure 1. Young specimens of the smelt, Osmerus mordax, experimentally infected with *Glugea hertwigi*. The yearly incidence of natural infection nears 90% in juveniles with consequent vast mortalities. Seven days post infection, xenomas (white arrows) develop proximate to larger 2-week-old "*Glugea* cysts" (black arrows). Bar represents 2.5 mm.
fish fed continually throughout the experimental period. Several fish died during the period, were examined and one was found infected with a single Glugea cyst.

**Experiment 2.** Laboratory reared fish were exposed to spore-carrying plankton. One week after exposure all fish exhibited numerous small xenomas beginning just behind the stomach and continuing along the entire length of the intestine to the vent (Fig. 2). Gradually the fish stopped feeding, had difficulty swimming and all died by 16 days after exposure to the spores.

**Experiment 3.** Collected smelt were exposed to spore-carrying plankton. The results were similar to those in Experiment 2; however, these fish stopped feeding and died at about 25 days after exposure to the parasite.

**Experiment 4.** Smelt from Experiment 1, 4 weeks after being exposed to a spore suspension, were fed spore-carrying plankton. One week after feeding, all fish exhibited numerous small xenomas along the intestine as well as the large posterior xenomas (Fig. 3). All fish died within 2 weeks after exposure to the spore-carrying vectors. Controls were examined periodically and found free of infection.
Figure 2. Young smelt, 12 days after feeding on spore-carrying plankton. Numerous xenomas (arrows) begin just behind the stomach and continue along the gut to the vent. S, stomach; sb, swimbladder. Bar represents 180 μm.
Figure 3. Young smelt previously infected after exposure to a spore suspension developed numerous new xenomas (arrows) along the intestine when fed spore-carrying plankton. X, xenomas from exposure to spores directly. Sb, swimbladder. Bar represents 750 μm.
Parasite development

Light Microscopic Observations: Parasite growth was rapid at 20°C. Examination of the intestine from a heavy infection (Experiments 2, 3, and 4) showed the extensive tissue involvement. Infections protruded to the serosa and were easily dissected away intact (Fig. 4). Extensive host cell hypertrophy was the obvious feature of one micron sectioned material (Fig. 5). Xenomas ranged from 20 to 50 μm in diameter. Smaller xenomas contained one or two greatly enlarged host cell nuclei; whereas the larger xenomas were multinucleate with many nuclei lobed or branched. At 1 week the parasite formed vegetative colonies peripheral to the host cell nuclei. After 2 weeks of growth there was considerable increase in host cell hypertrophy and Glugea maturation to spores (Fig. 6). Xenomas ranged from 100 to 125 μm in diameter with host cell nuclei, cytoplasmic components and vegetate G. hertwigi particularly obvious in the peripheral region of the xenomas; spore stages of the parasite were common in the central region.

Electron Microscopic Observations: Glugea parasite invasion into host cells occurs without apparent effect on the integrity of the host cell. Host cell responded
Figure 4. Whole intestine 1 week after exposure to spore-carrying plankton. Xenomas (arrows) protrude to the serose and are easily dissected away intact. Bar represents 80 µm.
Figure 5. Cross section of intestine similar to that shown in Figure 4. At least 40 xenomas (arrows) 20 to 50 μm in diameter develop within the submucosa and protrude from the mucosa. Infected host cells hypertrophy and contain early schizont stages of the parasite. E, epithelium. One micron Epon section; 1% toluidine blue stain. Bar represents 50 μm.
Figure 6. Cross section of intestine from lightly infected smelt 2 weeks after feeding on a spore suspension. Progressive hypertrophy increased the xenomas to 100 to 125 μm in diameter. Xenomas develop in the submucosal layer causing mechanical distension of the epithelium (E). Sporogony stages and free dense spores fill the central region of the xenoma. One micron Epon section; 1% toluidine blue stain. Bar represents 50 μm.
to invasion with general hypertrophy of the cytoplasm. Host cells maintained a normal complement of cytoplasmic organelles such as mitochondria, ribosomes, endoplasmic reticulum and scattered vesicles (Fig. 7). Host-cell nuclei characteristically transformed from spherical to branching shapes with one or more diffuse nucleoli (Figs. 7 and 8). Condensed chromatin, karyokinesis or spindle microtubules were not observed in the host-cell nuclei during xenoma development (Figs. 8 and 9). Host-cell nuclei and mitochondria had close proximity to the vegetative population of parasites throughout xenoma development (Figs. 8 and 9). An extensive increase of host endoplasmic reticulum (ER) occurred and Figure 10 shows that layered arrangements of the ER paralleled the surface of schizonts.

The formation of the capsule was a distinguishing feature in the development of 10 to 14 day old xenomas. Hypertrophied host cells appear to elicit a cellular, inflammatory response from the host. Seven days after parasite invasion, lymphocytes and fibrocytes border the xenoma surface (Fig. 7). Light microscopy showed that 7 day old xenomas had shape plasticity (Fig. 5). As the xenomas matured from day 10 to day 14, numerous host fibroblasts were observed to interdigitate with one
Figure 7. Xenoma, 5 days after parasite infection. The hypertrophied host cell-parasite complex consists of host nuclei (N), early schizont stages of the parasite (Sc), numerous mitochondria (M) and an extensive network formed by the endoplasmic reticulum (arrows). Host nuclei are lobed and undergo amitotic blebbing (bold arrow). Host lymphocytes (L) and fibrocytes (F) infiltrate the site of infection. Nu, host nucleoli. Bar represents .74 μm.
Figure 8. Xenoma from intestine shown in Figure 4. Host nuclei (N) bleb while parasite schizonts develop into multinucleate plasmodia (PSc). Note numerous mitochondria (M) surrounding schizonts (Sc). Bar represents .67 µm.
Figure 9. Schizont (Sc) surrounded by lamellar profiles of host endoplasmic reticulum (arrow) and mitochondria (M). Note narrow isthmus between lobes of host nucleus (double arrows). Bar represents .38 μm.
another. Layers of collagen fibers covered the xenoma surface with both circular and longitudinal orientation (Fig. 11).

The youngest parasite stages observed by electron microscopy were amoeboid, uninucleate schizonts measuring 3 x 5 μm (Figs. 7, 8 and 9). This stage had centrally located nuclei with dispersed chromatin and a homogeneous nucleoplasm surrounded by a typical double envelope. The cytoplasm was limited by a unit membrane and contained areas of unequal electron density. Several schizonts were seen in each xenoma; these stages were situated within the host cell cytoplasm (Figs. 7 and 8). The transformation of uninucleate schizonts into schizont plasmodia was gradual. Schizonts became elongate and irregular in dimensions. The nucleus enlarged and diversification of the cytoplasm and cell limiting membrane occurred (Fig. 10). Figure 12, a cross-section of schizont plasmodia, shows these proliferative forms roughly ovoid and bounded by 3 distinct layers. The outer most was a thin single unit membrane which covered an intermediate lucent layer over a thick inner envelope. This trilaminar boundary was unique to this stage. The cytoplasm attained homogeneity, contained few inclusions, scattered vestiges of endoplasmic reticulum and numerous ribosomes. Golgi,
Figure 10. Early schizont plasmodium (PSc) surrounded by overcoats of host endoplasmic reticulum (arrows). Bar represents .28 μm.
Figure 11. Host response to xenoma 14 days after parasite infection. Longitudinal and circular layers of collagen fibers (arrows) secreted by host fibroblasts (F) encapsulate the xenoma. Bar represents 0.18 μm.
smooth-surfaced vesicles and mitochondria were not observed at this stage. As in all other microsporidians, nucleoli were not observed and condensed chromatin was uniformly distributed throughout a double membrane bound nucleus (Fig. 12).

The outer most membrane of schizont plasmodia formed occasional tubular elaborations which gave the appearance of a trabecular network (TBN) extending throughout the host cytoplasm, but particularly along the confines of the cisternae of the endoplasmic reticulum (Figs. 13, 14 and inserts). In cross section, the TBN exhibited a single, double-layered annulus that had a denser outer layer (Fig. 13, insert). Condensed material within the TBN was in perpetuity with the middle layer of the parasite boundary (Figs. 13 and 14, inserts).

Cytokinesis was evident in elogate schizont plasmodia (Fig. 14). The TBN junctions were found at various sites along plasmodia including the point of cytoplasmic constriction (Figs. 13 and 14). Karyokinesis proceeded as acentriolar pleuromitosis maintaining a nuclear envelope throughout the division cycle (Fig. 15). Microtubules approximately 0.025 μ in diameter attached to chromosomes and formed an intranuclear spindle that terminated at the centriolar plaque on the nuclear membrane.
Figure 12. Schizonts (Sc) surrounded by host endoplasmic reticulum. These stages are not observed within parasitophorous vacuoles. Parasite boundary delimited by 3 layers; a standard outer membrane, intermediate lucent layer and a thick inner envelope (arrows) N, nucleus. Bar represents .27 μm.
Figure 13. Cross section of schizonts (Sc). These stages characteristically display a trabecular network extending into the host cytoplasm from the surface of the developing parasites (arrow). Condensed material within the cisternae of these trabeculae appear to have continuity with the middle layer of the parasite boundary (insert). Bar represents .24 μm (a), .14 μm (b).
Figure 14. Schizont plasmodia (PSc). Trabecular junction is at the point of schizont cytokinesis (asterisk) (insert). Bar represents .34 μm (a), .24 μm (b).
A cross-sectional view of this structure displayed a hollow, barrel-like form (Fig. 15, insert). During division, the spindle plaque, and adjacent nuclear membrane appeared to invaginate into the nucleus (Fig. 15).

Elongate schizont plasmodia, with a single row of nuclei, became the dominant feature of the xenoma shortly before sporogony (Fig. 16). These plasmodia had an outer envelope similar to mature schizont; however, the glycocalyx appeared thinner. Cytoplasmic organelles were not discernible but condensed chromatin and the presence of centriolar plaques indicated recent karyokinesis. Multinucleate schizonts appeared to be the last stage in schizogony prior to sporogony.

Parasitophorous vacuoles (PV) formed in the cytoplasm of the host cell component when xenomas reached a diameter of about 90 μ (Fig. 6). The end of schizogony and the onset of sporogony was marked by several morphological features (Figs. 17 and 18). Chromatin dispersed within the nucleoplasm and appeared as in interphase. The cytoplasm lost density and contained small vesicles, tubular structures, Golgi, flattened profiles of endoplasmic reticulum and numerous free ribosomes (Figs. 17 and 18). An irregular PV vacuole formed around schizont plasmodia as cytokinesis divided each nuclei and a portion of
Figure 15. Asexual multiplication of schizont nucleus within schizont plasmodium. Microtubules (MT) form spindle to a centriolar plaque (CP) on the surface of the nuclear membrane. Hollow barrel-like appearance suggests true centriole (insert). M, mitochondria; ER, endoplasmic reticulum of host cell. Bar represents .20 µm (a), .15 µm (b).
Figure 16. Plurinucleate plasmodium (PSc). N, nucleus; S, spore. Bar represents .53 μm.
Figure 17. Onset of sporogony. Cytoplasmic constrictions (arrows) of plurinucleate plasmodium (PSc) forms primordial sporonts (Sp). Note pores along host nuclear membrane (bold arrows). Bar represents .44 μm.
Figure 18. Division of plasmodium (PSc) into primordial sporonts (Sp). Note scalloped surface of sporont (double arrows). Cytoplasm contains ribosomes and loose membrane lamellae (arrows). Chromatin (Ch) condensed within nucleus (N). Bar represents .38 μm.
surrounding cytoplasm into primordial sporonts (Figs. 17 and 18). The boundary of the PV was apparently absent or poorly delineated. There was an increase in the number of pores between the host-cell nuclei and host-cell endoplasmic reticulum adjoining PV's.

Structural changes of the sporont boundary and cytoplasmic components were rapid. Irregular patches of glycocalyx organized onto the plasma membrane of sporonts (Fig. 19). The initial manifestation of these patches took place during formation of primordial sporonts (Figs. 17 and 18). After division of the schizont plasmodia, the sporonts remained proximate to the periphery of the PV boundary; these stages did not affix to the boundary or develop tight junctions to the vacuolar envelope (Fig. 19). Glycocalyx deposition was preceded by the differentiation of the sporont Golgi apparatus and endoplasmic reticulum. The Golgi was composed of small vesicles loaded with electron dense material. Nuclei had dispersed chromatin and were centrally located within sporonts (Fig. 19). As sporogony continued, chromosomes condensed and a series of nuclear divisions took place (Fig. 19). Karyokinesis was again observed within membrane bound nuclei (Fig. 20). Chromosomes attached to microtubules terminating on the nuclear membrane at the centriolar
Figure 19. Early sporonts (Sp) adjacent to parasitophorous vacuole membrane. Outer surface forms patches of unequal density. Bar represents .32 μm.
plaque (Fig. 20). Small perinuclear vesicles were often observed proximate to the centriolar plaque; however, no continuity was observed between the vesicles and the spindle apparatus. Extensive layers of rough endoplasmic reticulum developed within the differentiating sporont cytoplasm. The glycocalyx became organized as a continuous coat measuring 20-30 μm on the sporont surface (Fig. 20).

Cytokinesis did not directly follow karyokinesis in the sporont; rather, the sporont became a multinucleated plasmodium (Fig. 21). Early sporogonial plasmodia had eccentric nuclei, rough endoplasmic reticulum (RER) with long flattened lamellae and a Golgi complex with extensive membrane cisternae adjacent to the RER (Fig. 21). A conspicuous feature within parasitophorous vacuoles was the presence of amorphous material (Figs. 21, 22 and 23). This material was observed in the vacuolar cavity throughout sporogony. Shape of the vacuolar material varied from tubules to granules of different size and density with a distinct tendency to assemble itself into tubules (Figs. 17, 21 and 22). Sporogonial plasmodia became very irregular in shape. Nuclei of these forms displayed chromatin condensed into a unique ring configuration (Fig. 22). The chromatin ring was observed within a single membrane bound nucleus and consisted of granules and a
Figure 20. Sporont during karyokenesis. Condensed chromatin (Ch) attach to intranuclear spindle microtubules (MT) that terminate at centriolar plaque (CP) on nuclear membrane. Note extensive endoplasmic reticulum (ER) and sporont surface (double arrows). Bar represents .23 μm.
Figure 21. Early sporogonial plasmodia (PSP). Tubules and amorphous material accumulate within parasitophorous vacuole (arrows). Outer surface of plasmodia thicken (double arrows) and Golgi (G) form membrane lamellae adjacent to endoplasmic reticulum (ER). Bar represents .42 μm.
Figure 22. Sporogonial plasmodium (PSp) with condensed chromatin in unique configuration within nuclei (N). Note tubules in pansporoblastic cavity (arrows). G, Golgi. Bar represents .40 μm.
few dense strands (Figs. 22 and 23). The nuclear membrane remained intact during this period. Membrane cisternae of the Golgi and endoplasmic reticulum expanded into lamellar profiles (Fig. 23).

Nuclear activity decreased concurrent with cytokinesis of the sporogonial plasmodia. Cytokinesis was incomplete dividing the plasmodia into sporoblast buds which remained connected by a short cytoplasmic bridge at the center. Sporoblast buds were seen in groups of two, three and most often four (Fig. 24). The sporoblast cytoplasm was similar to the sporont stage but the membrane lamellae were tightly coiled and interpreted as primordia of the polar filament (Fig. 24). The loss of ground substance during fixation seen in Figure 25 afforded an opportunity to observe the Golgi and endoplasmic reticulum during sporoblast differentiation. Dense aggregates of vacuolar and vesicular Golgi lay interspersed within an expanded system of endoplasmic reticulum. Several small uniform tubes formed inside this complex and appeared hollow in cross-section (Fig. 25). Sporoblast maturation occurred within an ever-increasing parasitophorous vacuole. Typically the cell wall of the sporoblast had the same structure as the sporogonial plasmodium, but measured 50 to 60 μm.
Figure 23. Cross section of nuclei within sporogonial plasmodium. Chromatin condenses to form unique circular array. Golgi (G) membranes are continuous with endoplasmic reticulum (ER) (arrow). Bar represents .34 μm.
Figure 24. Sporogonial plasmodium cleaving to form at least 4 sporoblasts (Spb). Chromatin disperses within nucleus (N). Arrows indicate cleavage furrows. Bar represents .30 μm.
Figure 25. Cytokinesis of sporogonial plasmodium. Narrow isthmus (asterisk) connects primordial sporoblasts. Outer surface becomes uniformly dense (bold arrow). Vesicular Golgi (G), distended endoplasmic reticulum (ER) and vesicles (arrows) form in a cytoplasm devoid of other organelles. Bar represents .29 μm.
Sporogony and consequent PV expansion was initiated in the central zone of the xenoma; after sporogenesis, fusion of vacuoles constructed a central storage space of large dimension (Fig. 6). The mass of stored spores restricted the growth area to the xenoma periphery. In such a stage, the central area of sporogenesis was surrounded by schizonts, plasmodia and a margin of host cell components (Fig. 26). Individual parasites moved from the PV boundary as cytokinesis separated the sporoblast buds. Host-cell nuclei exhibited numerous pores to the host endoplasmic reticulum which constituted a thin stratum between the PV and host cell components. A large number of host cell mitochondria and nuclei surrounded the PV (Fig. 26).

A loss of cytoplasmic density was characteristic of sporoblasts in early sporogenesis (Fig. 27). Golgi vesicles, vacuoles and cisternae were observed in close association with extensive rough endoplasmic reticulum (RER). The primordial polar tube appeared to form from Golgi and RER (Fig. 27, insert). Late sporoblast stages exhibited increased cytoplasmic density, general cellular compaction and elongation with the nucleus toward one end (Fig. 28). A unique feature of elongated sporoblasts was a crenulated surface topography (Fig. 28).
Figure 26. Several stages during Glugea hertwigi development. Sc, schizont; PSc, schizont plasmodium; Sp, sporont; Spb, sporoblast; PV, parasitophorous vacuole; N, host nucleus; Nu, nucleolus; arrows indicate nuclear pores. Bar represents .44 μm.
Figure 27. Young sporoblasts. Extensive rough endoplasmic reticulum fills the cytoplasm (arrows). Golgi (G) and associated particulate material have close association with endoplasmic reticulum. N, nucleus; CP, centriolar plaque. Bar represents .56 µm (a), .36 (b).
The polar cap structure originated prior to polar filament formation and appeared as an irregular vacuole limited by a unit membrane (Fig. 28). The vacuole was partially filled by vesicles of the Golgi apparatus and was situated in close proximity to the sporoblast nucleus (Fig. 28). The filament proper originated within the polar sac whose perinuclear position moved toward the anterior pole of the spore as the coils of the polar tube were formed (Fig. 29). Membrane sheaths from the endoplasmic reticulum surrounded the filament proper as coils spiraled within the spore posterior (Fig. 29).

The spore wall was a trilamellar structure consisting of an outer dense exospore, an electron transparent middle endospore and an inner plasma membrane (Fig. 29). No surface ornamentation, appendages or substructure were observed; however, the exospore remained crenulated until final spore maturation. The endospore varied from 50-200 μm in thickness, being thinner at the apical pole of the spore (Fig. 30).

Figure 30 demonstrates the structure of mature G. hertwigi spores. Several planes of section were required to visualize all mature spore structures. Spores were oval and had average dimensions of 3.9 x 2.3 μm. The extrusion apparatus was composed of the polaroplast,
Figure 28. Sporogenesis. Vacuole (V) is closely associated with nucleus (N). Polar filament (PF) (arrow) is anchored to the polar sac by Golgi. PV, parasitophorous vacuole. Crenulation (assumed artifact) is common appearance during sporogenesis. Bar represents .40 μm.
Figure 29. Spores. Surface wall has outer dense envelope and an inner lucent layer (double arrow). Membrane sheaths surround the polar filament (arrows). G, Golgi; PS, polar sac. Bar represents .22 μm.
Figure 30. *Glugea hertwigi* spores. W, wall; PF, polar filament; Pol, polarplast; Pov, vesicular polarplast; N, nucleus; PC, polar cap. Bar represents 0.34 μm.
polar tube, and posterior vacuole. The polaroplast appeared as tightly-packed arrangements of membrane pleats. These pleats extended from the anterior end of the spore as folds around the descending polar tube. The polar tube anchored to the inner spore wall, descended through the polaroplast and turned to form 10-12 coils arranged in rows around the posterior end of the spore. In cross section, the polar tube consisted of several concentric layers. The infective sporoplasm with a single nucleus filled the middle third of the spore interior.
DISCUSSION

Transovarian parasite transmission is known from a number of microsporidan species (Kudo, 1966). Recently, Summerfelt and Warner (1970) demonstrated a Pleistophora ovariae infection in viable eggs of the golden shiner, Notemigoneous crysoleucas. Although the ovaries of spawning female smelt were often loaded with Glugea hertwigi parasites, thorough examinations indicate G. hertwigi are not present in germinative or egg cells. Previously, there has been limited success in experimental peroral transmission of fish microsporidans (Delisle, 1969; McVicar, 1975; Summerfelt and Warner, 1970, Stunkard and Lux, 1965). However, Olson (1976) successfully transmitted Glugea stephani to the english sole, Paraphyrys vetulus in water temperatures above 15° C. Several authors are convinced that transport vectors are necessary to concentrate Glugea spores for natural transmission of certain fish microsporidosis (Haley, 1957; Putz and McLaughlin, 1970; Stunkard and Lux, 1965). Small filter-feeding animals may serve as transport hosts and in addition, may stimulate the spores to hatch and infect the fish. Weidner (unpublished observations) has observed
such a phenomenon with *G. stephani* in the winter flounder, *Pseudopleurenectes americanus*. In this study, transmission of *G. hertwigi* to both laboratory-reared and collected smelt was successful at 20° C either by direct spore consumption or ingestion of spore-carrying vectors; however, a major magnitude of difference exists in the intensity between direct spore and vector transmitted infections. Vector transmission produced massive infections along the entire intestine in all test subjects.

Smelt are selective predators, taking cyclopoid and calanoid copepods and several species of cladocerans as their first food (Reif and Tappa, 1966; Siefert, 1972). Presumably, the natural *G. hertwigi* infection occurs through the ingestion of spore-carrying filter feeders by very young smelt. Release of spores from infected adult smelt occurs via two routes. Scarborough (unpublished observations) has observed the expulsion of parasite xenomas from ovaries during spawning. In this manner, female adult smelt may unwittingly concentrate *G. hertwigi* spores in the immediate vicinity of developing young. Further, infected smelt carrion were seen being preyed upon by small crustaceans in the nursery areas after spawning. Nepsky and Dechtiar (1972) found that
heavily infected adult smelt were unable to recover from spawning stress; mass mortalities in the spawning grounds consisted of infected adults.

Massive infections of *G. hertwigi* consequent to ingestion of spore vectors are fatal to both collected and laboratory reared smelt. Mechanical distention of the intestinal tissue and starvation are thought to be the cause of death. *Osmerus mordax* is a difficult species to raise in the laboratory and the minimal condition of the reared fish probably precluded a greater susceptibility to the effects of multiple infections. Young smelt tolerate light infections for at least several weeks and likely carry them into adulthood. Nepszy and Dechtiar (1972) found that *G. hertwigi* colonies can remain in smelt for much of the host's life, and stress will significantly increase the mortality rate in these fish over uninfected smelt.

It is well documented that the life cycle of microsporidans begins with injection of the sporoplasm through a spore tube into a host cell (Lom and Vavra, 1963a; Vavra, 1976, Weidner, 1972). It is assumed that the discharging tube of *G. hertwigi* spores penetrates through the gut basal lamina delivering the parasite into submucosa cells. Although Weissenberg (1968, 1976)
believed the host cell to be a presumptive macrophage, the cell types which can support *G. hertwigi* development remain undetermined. The present observations indicate that massive infections will produce xenomas easily separated from the submucosal layer of the intestinal wall. Xenomas were observed associated with various visceral organs, including cells below the peritoneal lining. It is not known how *G. hertwigi* enters ovarian tissue; presumably, initially infected host cells enter the blood stream and are delivered to favorable environs for growth, such as the highly vascularized ovaries. *Nosema michaelis*, a microsporidan infecting the blue crab, *Callenectes sapidus*, undergoes vegetative growth in the gut wall and these cells subsequently circulate to muscle tissue for continued development (Weidner, 1972).

Moulder (1979) believes that the host-parasite interface is the juxtaposition in space and time of the regulatory mechanisms of the host and parasite. Although they have separate origins, the host-cell and *Glugea* spore become structurally and physiologically integrated. Once inside the host cell, parasite adoptions are concerned with avoidance of destruction and the maintenance and control of host-cell function (Moulder, 1974, 1979). Some intracellular parasites, e.g. *Leishmania donovani*,
are resistant to lysosomal enzymes; while others, e.g. Toxoplasma gondii and Encephelatozoon cuniculi appear to block lysosomal fusion with the phagocytized parasite vacuole (Jones and Hirsh, 1972; Jones et al, 1972; Moulder, 1979; Shadduck and Pakes, 1971; Trager, 1970; Weidner, 1975, 1976a). G. hertwigi multiplies while in direct contact with the host's cytoplasm. This is a common survival mechanism among microsporidians and a distinct feature of Glugea (Canning and Nicholas, 1974; Loubes, 1979, Loubes and Akbarieh, 1977; Loubes et al, 1976; McLaughlin, 1969; Sprague and Vernick, 1969; Weissenberg, 1976; Youssef and Hammond, 1971).

The interfacing association observed between G. hertwigi and host cell mitochondria and endoplasmic reticulum is seen in other obligate intracellular parasite infections. The regular overcoating of vacuoles containing T. gondii and E. cuniculi and the apposition of vegetative G. stephani with host-cell mitochondria and endoplasmic reticulum is thought to play a significant role in providing nutrients to the parasites (Gelderman et al, 1968; Hirsch et al, 1974; Jones and Hirsch, 1972; Jones et al, 1972; Pakes et al, 1975; Weidner, person. comm.). The trabecular network of vegetative G. hertwigi may be involved in molecular transport at the host-parasite
interface. The observation of continuous karyokinesis of the schizont, formation of multinucleate schizont plasmodia, absence of parasite mitochondria and parasite cytoplasmic simplicity supports the conclusion that the parasite relies exclusively on host-cell function for metabolites during schizogony.

Initially the host cell is not destroyed but stimulated to an abnormal hyper-growth. Cell hypertrophy is a frequent response to intracellular parasitism; however, amitotic proliferation of the host-cell nuclei is a feature unique to Glugea. In contrast, the host cell remains uninucleate in the hypertrophy of glomerular cells in Esox lucius invaded by Pleistophora oolytica, in the hypertrophy of ganglion cells of Lophius lophius invaded by Spraguea lophii, and in the hypertrophy of fibroblasts of Pleuronectes sp. invaded by lymphocytis virus (Canning, 1976; Loubes et al., 1976; Nigrelli, 1953; Sprague, 1969; Weissenberg, 1949, 1968). Giant nuclei are present in neoplastic tissues infected with Stempellia simulli, Pleistophora debaisieux, Thelohania chaetogastiis and Thelohania solenopsae (Knell et al., 1977; Loubes and Maurand, 1976; Weiser, 1976).

The mechanism of G. hertwigi influence and stimulation of the host cell to amitosis and hypertrophy is unknown.
Pavan et al (1969), (1971) and Roberts et al (1967) have described microsporidan invasions of Sciaridae and the effect on polytene chromosomes. Characteristically the size of the nucleus and chromosomes increased under parasite influence. The total amount of DNA and the amount of RNA synthesis was greatly enhanced. There was an increase in the number of mitochondria and in the activity of the host endoplasmic reticulum. Although infected cells behaved abnormally, they did not degenerate and successfully survived insect metamorphosis to form giant juvenile cells. Fisher and Sandborn (1964) isolated a type of juvenile hormone that caused giant cell stages in infected insects.

The enlargement of the nucleus in cells infected with microsporidans is thought to be a consequence of polyteny and generalized hyperfunction of the entire genome leading to an overall increase in DNA-dependent RNA synthesis (Diaz and Pavan, 1966; Roberts et al, 1967). Petri and Schiødt (1966) and Petri (1966, 1976) report the nuclei of Yosida rat sarcoma cells infected with E. cuniculi are characterized by an extraordinary number of large nuclear pores. According to current views, large nuclear pores provide a direct channel for RNA transit to the endoplasmic reticulum (DuPraw, 1970). Preliminary studies
by de la Torre and Gemenez-Martin (1975) on induced amitosis in onion root-tip provides evidence that transcription of ribosomal cistrons is necessary for the normal progression of mitosis and that any changes in the rate of RNA transcription at a particular point of the cycle can be a mechanism leading to amitosis. Band and Mohrlok (1974) found that Acanthamoeba sp. grown in deficient media exhibited an increase in nuclear number and size and that cytokinesis following karyokinesis was delayed. The induced amitosis was due to the metabolism of nucleotides as an energy source and consequent "unbalanced RNA synthesis:" Gross (person. comm.) has determined that the time and plane of cell division during sea urchin embryogenesis is controlled by levels of mRNA coding for histones. The present morphological evidence of nuclear edema, diffuse nucleoli, organelle proliferation, and the formation of nuclear pores in host-cell nuclei infers that during schizogony, G. hertwigi may in some way influence host RNA synthesis and by this mechanism induce cellular hypertrophy and nuclear amitosis.

In the present study, the interpretation of host-parasite interface is broadened to include the interaction of the xenoma and/or its products and the host's defense
mechanisms. The portal of cell entry for the microsporidan excludes the elaboration of specific antibodies to the sporoplasm; however, hypertrophic growth of the xenoma and consequent mechanical distention of host tissue provokes an inflammatory response from the host. This cyst-forming response benefits the host in localized containment of the parasite. The capsule formation seen in Glugea infections parallel that observed in many helminth diseases (Lumsden, 1979). There is local accumulation of lymphocytes, eosinophils and fibrocytes. The combined influence of metabolic by-products of the parasite and eosinophils induces fibrocytes to fibroblast differentiation and collagen deposition (Jackson, 1968; Warren, 1974). The chronic phase persists with proliferative fibrosis (Thomas, 1964). As seen in the mechanical distention of the intestine by G. hertwigi xenomas, fibrosis itself is potentially pathogenic where tissues of a given organ are displaced by collagenous connective tissue. Abundant blood vessels within the capsule is a common feature of Glugea-cysts (Sprague and Vernick, 1968; Weidner, 1976b; Weissenberg, 1968, 1976). In an examination of cysts produced by G. stephani, Weidner (1976b) found the xenoma glycocalyx acted as a sieve allowing passage of metabolites between the xenoma and host.
The xenoparasitic complex is a special category of host-parasite interactions distinctly different from simple infections (Canning, 1976; Weiser, 1976a). The term "tissue parasite" has been suggested as a substitute for the term xenoma (Loubes, 1979). The new terminology would encompass several types of cell-hypertrophy tumors (Sprague, 1969). Xenoparasitic complexes with microsporidia are represented by 3 types: (1) syncytial xenomas with host cells confluent in one common syncytium; (2) Glugea-cysts with a unicellular origin, and (3) neoplastic xenomas in which the parasite provokes an increase in the number of host cells inside the lobe of infected tissue.

The syncytial xenoma produced by Icthyosporidium sp. in the spot, Leiostomus xanthurus, has been studied by Schwartz (1963), Sprague (1969) and Sprague and Vernick (1974). The tumors result from fusion of neighboring cysts to produce a compound lesion of spectacular dimensions. After fusion of the cysts a massive invasion of host connective tissue divides the parasitic mass.

The transition between a syncytial and neoplastic xenoma is the cell-hypertrophy tumor (Weiser, 1976). This type of microsporidan infection is often seen in fish and occurs in several microsporidan genera as well as in Glugea. Kabata (1959) recognized the swelling of gill
filaments in *Gadus aeglefinus* to be a result of *Nosema branchiale*. Canning (1976) and Putz et al (1965) described the xenoma as a product of a single cell invasion. *S. lophii* parasitizes the ganglion cells of *Lophius* sp. and the formation of a cyst has been described by a number of authors (Canning, 1976; Loubes et al, 1979; Weidner, 1974). *Pleistophora cepedianae* (Putz et al, 1965) in *Dorsoma cepedianum* and *Nosema pimephales* (Fantham et al, 1941) in *Pimephalus promelas* produce large cysts in the body cavity. *Pleistophora hyphessobryconis* causes enlargement of skeletal muscle bundles to many times the normal diameter (Lom and Corliss, 1967). *Nosema invadens* elicits an inflammatory response and cyst formation in *Lepidoptera* sp. (Kellen and Lindgren, 1973).

Neoplastic xenomas usually occur without an enveloping cyst wall and are common to members of the genus *Pleistophora* (Canning, 1976; Sprague, 1969). *Pleistophora salmonae* in *Salmo gairdneri* occur as diffuse infiltrations in the connective tissue of the gill lamellae (Putz et al, 1965). The infections exhibit rather extensive epithelial proliferation (Canning, 1976). *P. ovariae* causes the same type of host neoplasm in ovaries of *N. crysoleucas* (Summerfelt and Warner, 1970). *Pleistophora typicalis* causes extensive damage in skeletal muscle of *Cottus* sp.
Pleistophora macrozoarcididis produces large neoplastic masses in deep musculature near the vertebral column of Macrozoarces americanus (Nigrelli, 1953).

Some aspects of development are common to all microsporidan infections (Vavra, 1976a, 1976b). Microsporidan schizonts are initially simple cells limited by a unit type membrane with one rather compact nuclear component (Canning and Nicholas, 1974; Lom and Corliss, 1967; Vavra, 1976b; Weidner, 1970). The presence of two schizont generations in G. hertwigi is similar to that reported for a number of microsporida (Gray et al, 1969; Vavra, 1976b; Youssef and Hammond, 1971). The first generation is represented by small cells and small, rounded plasmodia. In the second generation the parasite grows into long plasmodial cylinders with several large nuclei. Occasionally an infected host-cell is filled with sprouting multinucleated schizont-cylinders (Weissenberg, 1976). Unlike other microsporidan development, the present observations indicate a considerable simplicity of the cytoplasm during schizogony of G. hertwigi. The development of an endoplasmic reticulum and/or primitive Golgi apparatus is reported in schizonts
of *Nosema* sp., and *Pleistophora* sp. (Lom and Corliss, 1967; Milner, 1972; Weidner, 1972; Youssef and Hammond, 1971).

The limiting membrane of many microsporidan schizonts exhibits structural evidence of an active interaction with host cell cytoplasm. The surface area of the schizont membrane can be substantially increased by vesicular or tubular projections interdigitated with the host cell cytoplasm (Codreanu and Vavra, 1970; Vavra, 1976b). Sprague and Vernick (1968) reported the presence of an "annulate lamellae" in *Glugea weissenbergi* xenomas. They observed the lamellae continuous with all host cell and parasite components via the host endoplasmic reticulum. The trabecular network (TBN) observed in xenomas of *G. hertwigi* is analogous to the "annulate lamellae" reported by Sprague and Vernick (1969). Weidner (person. comm.) has observed such a trabeculae in *G. stephani* xenomas. Sprague and Vernick (1969) observed a very small granule or "gemmule" continuous with the annulate lamellae and suggested that this represented the earliest stage of parasite development. Electron microscopic examination in the present study failed to reveal any such stage.

It is likely that the condensed material within the TBN is the granular material observed by Sprague and Vernick (1969); however, present evidence cannot support their
interpretation. When *G. hertwigi* was made to discharge into unfertilized oocytes of *Stronglyocentrotus purpuratus* an extensive array of lamellae containing dense material formed within the egg cell in the region of the parasite (Scarborough and Byrd, unpublished). Elaboration of the TBN between host-cell components and the parasite may be for the purpose of nutrient exchange.

The trilaminar boundary observed in *G. hertwigi* schizont plasmodia has not been observed in the development of other microsporidans. Usually the schizont boundary remains as a simple unit membrane (Vavra, 1976a, 1976b). The continuity of the trabecular network with the outer envelope of *G. hertwigi* schizonts suggests that the outer layer may be a closely applied envelope during growth but not a true limiting membrane. Furthermore, the TBN appears to be continuous with the endoplasmic reticulum of the host cell.

Typically, centrioles are not observed in microsporidan parasites (Vavra, 1976a). A specialized area on the nuclear membrane serves as an attachment point for the intranuclear spindle apparatus. In the present study, microtubular spindles occurring in the dividing nuclei of the schizont and sporoblast are similar to those reported for *G. weissenbergi* by Sprague and Vernick (1968),
for *Nosema nelsoni* by Sprague and Vernick (1969) for *Nosema algerae* by Canning and Sinden (1973) and Vavra and Undeen (1970), and for *N. apis* by Youssef and Hammond (1971). The structure has morphological similarity with the kinetic center of yeasts (Sprague and Vernick, 1971; Moens and Rapport, 1971). The persistence of the centriolar plaque without attached microtubules in *G. hertwigi* sporoblasts was also observed in *Nosema* sp. by Walker and Hinsch (1972). Perinuclear vesicles are commonly observed adjacent to the spindle plaque (Vavra, 1976a, Vavra and Undeen, 1970; Youssef and Hammond, 1971). Their significance is unknown.

There is some evidence from microsporidians grown in tissue culture and from insect hosts subject to various conditions that the passage from schizogony into sporogony is due to the influence of environmental conditions (Fowler and Reeves, 1974b; Hazard and Anthony, 1974; Ishihara, 1969; Sprague and Vernick, 1971; Vavra, 1976b). It is not known if the environmental condition of the host or host-cell influences *G. hertwigi* sporogony. It is observed that sporogony is initiated after the majority of schizonts are in the multinucleate plasmodial stage and fill the central region of the xenoma.
The occurrence of irregularly spaced patches on the outside of the parasite limiting membrane and the appearance of a clear space between the parasite and the adjacent host-cell cytoplasm are used to mark the onset of sporogony and to identify the sporont (Cali, 1971; Vavra, 1976b). These outer patches have been described as "ramified cisternae" by Codreanu and Vavra (1970) as a "ragged outer coat" by Krinsky and Hayes (1978), as "incomplete amorphous material" by Canning and Nicholas (1974), as "tubular projections" by Colley et al. (1975), as "secretion products" by Loubes and Maurand (1976), and as "wall material" by Sanders and Poinar (1975). According to Canning and Nicholas (1974) and Sprague and Vernick (1969) the coat material is produced by the Golgi vesicles and endoplasmic reticulum of the sporont. The appearance of a Golgi apparatus and endoplasmic reticulum in *G. hertwigi* sporonts is concurrent with the disposition of the spore coat. Glycoprotein is known to be synthesized in endoplasmic reticulum and conjugated with carbohydrate in the Golgi complex (Peterson and Leblond, 1964). Therefore, it is likely that substances needed for the elaboration of the spore coat are manufactured by the parasite. In some species the thickening of the sporont membrane is due to the appearance of an additional unit
membrane (Overstreet and Weidner, 1974). During sporoblast differentiation the extramembranous material or membrane sheaths are disseminated more uniformly until a continuous thick coat is formed (Vavra, 1976a). In some microsporidan species this exospore becomes decorated with appendages or tubular structures. In *Pleistophora* sp. numerous flexible tubules radiate in all directions (Szollosi, 1971). Smaller tubules described as bristles (Sprague et al, 1968) occur at the surface of sporoblasts and spores of *N. michaelis* (Weidner, 1970) and of *Ormiersia carcini* (Vivares et al, 1977). Dwyer and Weidner (1973) demonstrated that these bristles have some of the characteristics of microtubules. These microtubules may participate in the flow of material to and from the parasite (Lom and Corliss, 1967; Overstreet and Weidner, 1974). The sporoblasts and spores of *G. hertwigi* are not decorated and have a smooth surface. The Golgi complex and endoplasmic reticulum continue to develop and become the dominant cytoplasmic feature of sporonts and sporoblasts of *G. hertwigi*. The increased activity of these organelles provides the substance for exospore formation during schizogony. The mode of transport of the exospore material to the parasite surface is unknown. There is no evidence in the present study of surface pores described by Canning and Nicholas (1964).
G. hertwigi sporonts transform into multinucleate plasmodia from which individual sporoblasts are cleaved by multiple fission. This development has been reported for a number of microsporidan species (Codreanu and Vavra, 1970; Cossins and Bowler, 1973; Gassouma and Ellis, 1972; Sprague et al, 1968). The clear space that separates G. hertwigi sporonts from the host cytoplasm increases to form a vacuolar space during the formation of sporogonial plasmodia. The vacuole is likely not bounded by a membrane of parasite origin; rather, it appears to be delimited by membranes of the host endoplasmic reticulum and is described as a parasitophorous vacuole (PV). A pansporoblast, being the complex of a sporogonial plasmodium, vacuolar cavity, and limiting membrane of parasite origin is reported for numerous microsporidans (Sprague, 1977; Vavra, 1976a). Overstreet and Weidner (1974) hypothesize that a vacuolar cavity acts as a "growth chamber" and regulates the flow of material from host to parasite.

A common feature shared by both pansporoblast and parasitophorous vacuoles is the presence of some electron dense material within the vacuole cavity (Canning and Sinden, 1974; Knell et al, 1976; Liu, 1973; Loubes and Arbariek, 1977; Pakes et al, 1975; Weidner, 1970, 1975, 1976a). This material apparently is of parasite origin
and is termed "secretion" by Vavra (1976). The appearance of this material varies and is observed as granules and tubules in the present study. Tubular formation within the PV is also seen in *N. michaelis* (Sprague and Vernick, 1979; Weidner, 1972) in *Pleistophora debaisieux* (Vavra, 1976) in *Encephalatozoon cuniculi* (Weidner, 1976a) and in *Thelohania* sp. and *Pleistophora* sp. (Gassouma and Ellis, 1972). What function the secretion material has remains unknown. Since sporoblast appendages function to transport material to and from the parasite (Dwyer and Weidner, 1974), these tubules may transport material within the PV cavity.

In some microsporidians, cells with 2 closely adjacent nuclei (the diplokaryon) appear at the onset of sporogony (Canning and Sinden, 1973; Canning and Madhavi, 1977; Cossins and Bowler, 1974; Vavra, 1976a, 1976b; Weidner, 1970). The presence or absence of the diplokaryon during certain stages of microsporidan development has recently been suggested as a taxonomic character (Loubes, 1979; Weiser, 1976b). The diplokaryon was not observed during any stage of *G. hertwigi* development.

Whether unikaryon or diplokaryon, the behavior of the sporont nucleus is the most controversial part of the microsporidan life cycle (Vavra, 1976b). There is no
useful evidence that karyogamy occurs in microsporidans. Sprague and Vernick (1968) report what appears to be nuclear fusion in *G. weissenbergi*; however, this interpretation is admittedly tentative. Loubes (1979) observed lengthy nuclear condensation in sporogonial plasmodia of several species of microsporidans. The nuclear structures observed by Vavra (1976a) in *Gurleya chironomi* are thought to represent synaptonemal complexes. These structures resemble chromatin strands in the ring-nuclei seen in *G. hertwigi* during early sporogony. Similar ring-nuclei were observed by Kellen and Lindgren (1973) in *Nosema invadens* and by Sprague (1940) in *Collosporidium periplanetae*; however, these authors made no comment as to their implication. The occurrence of synaptonemal complexes which in turn indicate the presence of meiosis, would establish sexuality in the microsporidans. Since there is no information about the DNA content of the nucleus of *G. hertwigi* or of any other microsporidan during any life stage, this question remains unanswered.

The single cells resulting from the division of the sporogonial plasmodia are sporoblasts. Multiple fusion and the formation of sporoblast buds that remain connected by a cytoplasmic bridge until sporogenesis is common in microsporidians that form a plasmodia during sporogony
(Codreanu and Vavra, 1970; Cossins and Bowler, 1973; Gassouma and Ellis, 1972; Vavra, 1976b). It is in the sporoblast that spore organelles begin to form. As seen in the present study the sporoblast cytoplasm contains extensive endoplasmic reticulum and a Golgi apparatus. All 3 elements of a "classical" Golgi occur in G. hertwigi (Dalton, 1961; Sprague and Vernick, 1974; Vernick et al, 1977).

The final event of the microsporidan life cycle is gradual transformation of the sporoblast to the spore. The present electron microscope study demonstrates that the polar filament and polar sac are the spore organelles differentiated first. The last event is formation of the thick endospore layer of the spore wall. Sporogenesis is recognized to be a complicated process (Vavra, 1976b). It is a rather rapid process; generally not many transforming sporoblasts are observed in ultrathin sections. Although the density of the spore and problems encountered during EPON embedding render electron microscopic studies difficult, there have been a number of exhaustive ultrastructural studies on sporogenesis and the structure of the mature spore (Burges et al, 1974; Colley et al, 1975; Erickson et al, 1968; Gassouma and Ellis, 1973; Jensen and Welling, 1972; Liu 1973; Liu and Davies,
Although there is no unanimous agreement concerning the origin of the polar filament, recent data and the present observations support the idea that it originates from the Golgi zone. The filament is built from the basal end. The bulbous part representing the future polar sac appears as a vacuole in the vicinity of the nucleus. The coils are synthesized and their formation progresses from the basal part to the terminal end. Membrane sheaths from the endoplasmic reticulum are added after coil formation. This mode of filament development is in agreement with the observations of Jenson and Wellings (1972), Loubes and Maurand (1976), Walker and Hinsch (1972) and Weidner, (1970). During G. hertwigi sporogenesis the polar sac vacuole is surrounded by vesicular Golgi and continuous with elements of the endoplasmic reticulum; however, it could not be determined if the spore polaroplast was a derivative of the Golgi, the endoplasmic reticulum or both.

The taxonomic character of disporous development has been applied to the genus *Glugea* (Canning and Nicholas, 1974; Kudo, 1924, 1966; Sprague and Vernick, 1972). This condition was not commonly observed in this study of *G.*
hertwigi. Loubes (1979) provided evidence from his ultrastructural survey of several microsporidan genera that the number of spores from a sporont division can be highly variable. Loubes et al. (1976) found that G. habrodesmi was at least tetrasporous and occasionally octosporous. Loubes (1979) points out that nuclear configuration, presence or absence of sporogonial plasmodia, the presence of a pansporoblast or parasitophorous vacuole, type of cyst formation and host specificity are constant characters. The high temperature used in this study likely influenced the rate of G. hertwigi maturation and may well have influenced the spore number.


Loubes, C. and M. Akbarich. 1977. Étude ultrastructurale de Nosemoides simocephali n. sp. (Microsporidie), parasite intestinal de la daphnie Simocephalus vetulus Muller, 1776. Z. Parasitenkunde. 125-137.


Olson, R. 1976. Laboratory and field studies on Glugea stephani (Hagenmuller), a microsporidian parasite of pleuronectid flat-fishes. J. Protozool. 23(1): 158-164.


__________. 1968. Intracellular development of the microsporidia Glugea anomala (Moniez) in hypertrophiying migratory cell of the fish Gasteroteus aculeatus L., an example of the formation of "xenoma" tumors. J. Protozool. 14: 44-56.


VITA

Ann L. Scarborough was born August 2, 1949 in San Diego, California and graduated from the Academy of Our Lady of Peace High School in 1967. She moved to San Francisco and attended the University of San Francisco in 1967 and 1968. She returned to San Diego, attended the University of California (UCSD), and graduated with a Bachelor of Arts degree in Biology. After 3 1/2 years as a Research Associate at UCSD Medical School Department of Pathology, she moved to Baton Rouge, Louisiana. In 1975 she entered Louisiana State University Department of Zoology and Physiology as a graduate student and received a Master of Science degree in 1977. She was awarded a Doctoral Dissertation Grant from the National Science Foundation in 1977 and continued in the Department of Zoology. She is currently a candidate for a Doctor of Philosophy degree.