A NEW PARASITIC DINOFLAGELLATE FROM FRESH-WATER FISH
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This work was begun as an attempt to identify an ectoparasite that was causing substantial losses, especially of young fish, in fresh-water aquaria. The organism was soon determined to be a dinoflagellate. Since no fresh-water ectoparasites had been previously reported in this group, it became apparent that this was probably an undescribed species. Observations were therefore made in order to determine the details of its life-history and morphology. In most respects this organism is similar to previously described species of the genus Oodinium Chatton. It has therefore been placed in this genus with the name Oodinium limneticum sp. nov.

The natural habitat of this organism is not yet known. It may have come into the aquaria from local waters with live food (Chaoborus larvae and Daphnia) collections. It may, on the other hand, have come in with commercial importations of exotic aquarium fishes. If this is the case it may have come from almost any of the warmer parts of the globe, and it may since then have established itself in some localities of this country. There also remains the possibility that the natural habitat includes the waters in the vicinity of some commercial aquarium fish breeder in this country. The answer to this question will have to await future reports of the species.

Oodinium limneticum has been observed on nine species of fish representing three families. Two species of Cyprinidae, four from the Poeciliidae, and three of the Anabantidae are included. This is the second dinoflagellate parasite of vertebrates reported. The only other species is Oodinium ocelatum Brown (1931) which was described from the gills and skin of marine fish. The new species is the first record of a fresh-water parasitic dinoflagellate. Of the four previously described well recognized species of Oodinium,
the life-histories are known for only two, *Oodinium poucheti* (Lemmermann, 1899) and *Oodinium ocellatum*. Only that of *O. ocellatum* is known with reasonable completeness (Brown 1934 and Nigrelli 1936).

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**Materials and Methods**

Preliminary observation of the pathological effects of this parasite on nine different species of fish were made during the last two years. Fish of various ages were involved. The details are discussed in the section on pathology. All of these fish were kept in aquaria of five to 10 gallon capacities under temperature, food, and oxygen conditions which approached the optimum.

For the recent detailed observations on the morphology and life-history of the parasite only two species of fish were used as hosts. *Lebistes reticulatus* and *Xiphophorus helleri* were chosen chiefly because they were available in various ages and were convenient to work with in small containers. Partly grown young which could be kept in small culture dishes (10 cm. diameter) furnished most of the material.

Aquarium water that had been filtered and boiled was used for the cultures, and the parasite laden fish were introduced through several changes of distilled water to avoid contamination by other protozoa which might complicate the observations.

The chronological aspects of the life-history were determined by continuous observation of living active specimens mounted on microscope slides with large cover glasses ringed with petroleum jelly. Small paper wedges were used to prop the cover glasses so as to give a slightly larger volume of water. Nigrelli (1936) showed that development of *O. ocellatum* cysts may be arrested by too small a volume of water. In my observations on *O. limneticum* quite close correlation was obtained between the behavior of single mounted cysts and mass cultures in dishes. Normal division rate and formation of infectious gymnodinia occurred. Observations on the parasitic stage and on encystment were made by removing portions of the fins of parasitized fish and mounting on slides as described above. This proved very satisfactory since the transparent fin membranes made detailed study easy.

Starch tests were made with iodine-potassium iodide. This substance also proved useful in slowing activity of rapidly swimming gymnodinia when introduced in small quantity at the edge of the cover slip.

Most observations were made on living material but some of the morphological details were checked with acetocarmine and iron-haematoxylin preparations.

A special method for observing the attached parasite on the living host...
and for observing attachment of the gymnodinia to the host was devised. Newly born *Lebistes reticulatus* were used as hosts. The fish was mounted in a deep hollow ground slide with the caudal fin held between the shoulder of the depression and the cover slip. This prevented movement and yet allowed the fish to continue respiration. Observations could thus be made even under high power. This technique was especially useful in making periodic examinations of the growth of attached parasites.

Various details were recorded during the observations with the aid of photomicrographs and camera lucida drawings.

**Life-history of Oodinium limneticum**

The typical life-cycle of this species consists primarily of a parasitic stage during which rapid growth occurs, encystment, a series of mitotic divisions, and the formation of gymnodinian swarmers which are able to parasitize fresh-water fish. Although this general pattern is in agreement with the observations recorded for other members of the genus certain details observed in this work are unique. These will be noted later in the discussion. Variations from the normal cycle may occur as a result of interference. For ease of description, the most usual events will be reviewed before the variations are noted.

The parasitic stage begins as a pyriform body (Fig. 10) from $12 \times 7.5$ to $20 \times 13 \mu$ with a relatively smooth outline. It contains quite a number of chromoplasts and amyloid granules but little starch. The light green plastids are quite variable in size and shape ranging from 0.5 to 4.0 $\mu$ and from small spheres to larger lenticulate or irregular bodies. The spheroid or somewhat ellipsoid nucleus is about 6.0$\mu$ in diameter and contains numerous granules that are conspicuous even in living condition (Fig. 10). No ocellus and no flagella were observed. The smaller end of the parasite forms a stalk or peduncle (Figs. 1, 2, and 13) which is free of plastids. This structure is shaped like an inverted cone. It consists of a knobbed columella, which at first is cylindrical but later becomes conical, and a sheathing collar. The parasite attaches itself to the host by means of tentacle-like processes ejected from the knob of the columella. These so-called rhizoids seem to push their way between the cells of the host by pseudopodial action. The peduncle is about 3.0$\mu$ long at the outset and with the growth of the parasite enlarges to a maximum of about 15$\mu$.

One day after attachment the parasites average about 28$\mu$ in length. They reach about 38$\mu$ the second day, about 50 the third, and about 60 the fourth day at $25^\circ$C. The length constantly equals approximately 1.5 transdiameters (Figs. 1 and 10) up to the fourth day. From this time on the increase in diameter is more rapid than the increase in length so that a spheroid shape (Fig. 11) is approached. This shape is not completely attained, however, since the peduncle remains as a disconformity and the
rather flaccid nature of the parasite in its larger sizes causes slight irregularities in the outline (Fig. 14). The maximum size observed is about 80μ in diameter and 96μ long including the peduncle. The parasite may remain attached for a week or somewhat longer at 25°C. During its growth starch becomes very abundant in the parasite, and its periphery becomes crowded with chromoplasts that are quite olivaceous and more uniform in size (about 3.0μ in diameter) than at the outset. The nucleus increases to a diameter of 20μ.

A thin cellulose wall completely surrounds the parasite except for the pore at the end of the peduncle through which the rhizoids extend. This pore is sealed at the time of encystment by the secretion of a cellulose cap. A similar wall is present during all other stages of the cycle as well.

If the parasite has not been mechanically dislodged from the host beforehand it will withdraw its rhizoids pseudopodially into the knob in the peduncle when it reaches maturity. The peduncle is then retracted and dedifferentiated and the pore is sealed. Retraction of the peduncle seems to restore turgidity and produces a smooth spherical outline (Figs. 3 and 14), but no appreciable increase in volume was noted. Averages from ten individuals indicate that an individual 81 by 65μ rounds up to a diameter of 71μ.

The cyst thus formed promptly begins a series of equi-valent mitotic divisions. The first division plane is transverse and succeeding ones follow in a regular and synchronous manner (Fig. 15). During the first division each daughter cell secretes a complete new wall (Fig. 4). The original wall occasionally remains intact so that subsequent divisions result in palmelllas confined within approximately the same volume as the original cyst. In such cases the wall usually ruptures during some later division. Most com-

**Explanation of Plate I**

Figures 1–9 are camera lucida drawings of various stages in the typical life-cycle of Oodinium limneticum sp. nov. from living specimens. Finer details have been added free-hand. Figs. 3, 4, and 5 are ×500, all others are ×1000.

Fig. 1. Young parasite 60 hours after attachment.
Fig. 2. Almost mature parasite seven days after attachment.
Fig. 3. Encysted parasite after dedifferentiation of peduncle but before migration of plastids into the peduncular region.
Fig. 4. End of first division, original wall still intact.
Fig. 5. Third division completed but separation of the daughter cells has not yet occurred. The original cyst wall has disappeared.
Fig. 6. Last division completed but separation of gymnodinia has not yet occurred.
Fig. 7. A pair of completely formed gymnodinia after separation but still rolling over each other.
Fig. 8. A typical, active, infectious gymnodinium.
Fig. 9. Parasite at time of attachment showing transformation from gymnodinian-form.
monly, however, the original wall disintegrates at the end of the first division, so that as the daughter cells round up after each division they become completely free of one another. After the first division the old cell wall is used by the daughter cells so that a new wall is formed only across the division face (Fig. 5). Often the original cell wall dissociates as a result of bacterial action. This results in the formation of a rather poorly defined viscous matrix that tends to keep the division products loosely grouped for several divisions or until the formation of gymnodinia.

Divisions proceed in normal fashion through 2, 4, 8, 16, 32, 64, and 128-cell stages at intervals of 7 to 10 hours at 25°C. The first division may be completed in less time than this. The products of the last division average about 13μ in diameter. These go through another division during which gymnodinian swarvers are formed. The girdle is obscure but usually discernible just before separation occurs (Fig. 6). The process of separation of sister gymnodinia involves first a sudden shaping into the typical form. The two organisms are then free of each other but remain in contact for a short time during which the flagella lash slowly at first as the two bodies roll over each other (Fig. 7). In a few seconds activity increases and the gymnodinia separate completely and swim away in the typical rapid spiral manner. These swarvers (Fig. 8) are usually about 15μ long. They have a deep girdle and sulcus (which extends to the posterior pole), no ocellus, and an epicone that is larger than the hypocone. They represent the infectious stage of the cycle. Not infrequently (especially when they are larger than average) these gymnodinia will remain active for only a few seconds to a minute or two and then suddenly encyst. They then go through another division and give rise to a second generation of gymnodinia. These remain active for a longer period of time but none are known to have remained motile for more than 48 hours. At the cessation of activity these also encyst. What happens to these cysts was not observed since they remained dormant for several days.

On coming in contact with a fish the gymnodinium seems to adhere at first by the longitudinal flagellum, a protuberance then appears in the lower sulcal region and from this several short tentacles push their way out (Fig. 9) and move as if groping about. They soon push their way into the host tissue. Their growth is difficult to follow after they penetrate the host tissue, but they are probably pushed between the cells of the host by means

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**Explanation of Plate II**

Fig. 10. Photomicrograph of a group of young living parasites after one to three days of attachment to a fishes fin. Note form, plastids, and granular nuclei. ×700.

Fig. 11. Photomicrograph of several older living parasites. Some have been attached up to seven days and are almost mature; note the shape. ×450.
PLATE III
of pseudopodial action. During the attachment the lateral flagellum pulls loose from the membrane which holds it in the girdle and both flagella as well as the girdle and sulcus disappear. The cycle is thus completed.

This may be considered the normal life-cycle of *Oodinium limneticum* but various factors may interfere and cause certain modifications. The parasite may be mechanically dislodged or forced by the death of the host tissue to disengage itself at any time before it reaches maturity. When this occurs encystment usually follows promptly but in the smallest specimens it may be delayed for a short time. In some of these the tentacles were observed partly extended and groping. They could presumably reattach themselves if they were to come into contact with a satisfactory substratum. All those observed formed cysts and began to divide. These very small cysts (12–15 μ in diameter) on division may give rise directly to pairs of gymnodinia. Larger cysts go through more divisions, the exact number being roughly proportional to the volume of the parasite so that the resulting swarmers are nearly the same size. Formation of gymnodinia has been observed at all stages of division from the first to the 256-cell stage. There is some indication that the exact division which will give rise to the motile cells may be controlled to a limited extent by external factors such as oxygen supply and temperature. When oxygen supply was limited during the later divisions by crowding, by stagnant conditions, or by a small volume of water, division sometimes continued until the last cysts and the gymnodinia formed from them were smaller than those found under more favorable conditions. Sometimes gymnodinia formation failed altogether and divisions were halted at the early stages possibly by severe oxygen shortages. Gymnodinia show their preference for high oxygen supply by congregating near the surface of cultures. Temperature seems to have an inverse effect, with the larger gymnodinia being most abundant at the lower temperatures. The range in size of the gymnodinia observed is from 10 to 19μ in length.

**Explanation of Plate III**

*Fig. 12.* Photograph of a living, heavily parasitized, immature *Xiphophorus helleri* to show the reticulate pattern produced by the concentration of parasites at the margins of the scales. ×4.

*Fig. 13.* Photomicrograph of an almost mature living parasite attached to the fin of a fish. Note rhizoids, structure of the peduncle, plastids, and form. ×625.

*Fig. 14.* Photomicrograph of a group of encysted living parasites of various ages. ×200.

*Fig. 15.* Photomicrograph of the products of the second division of a cyst. The third division is almost completed but separation has not occurred. ×540.
**Taxonomy**

*Oodinium limneticum* sp. nov.

This species is ectoparasitic on the epidermal covering of any part of the body of fresh-water fish. The parasitic stage ranges from $12 \times 7.5$ to $96 \times 80 \mu$; pyriform when small becoming slightly irregular subspherical with growth; peduncle short, stout, consisting of a knobbed columella bearing fine rhizoids and surrounded by a collar of about equal length, contained five or six times in total length, and free of chromoplasts; no ocellus or flagella; abundant yellow-green chromoplasts, starch grains, and amyloid granules; cellulose wall complete except for the rhizoids and a pore through which they extend. A spherical cyst forms on separation from the host. Equal divisions result in two to 256 gymnodinia. Gymnodinia with cellulose wall, numerous yellowish-green chromoplasts and amyloid granules, no ocellus, and no striae; the length, 10 to 19$\mu$, is 1.25 transdiameters; girdle is deeply impressed with rounded sides, only slightly oblique; transverse flagellum almost completely encircling girdle; sulcus from girdle to antapex, broad, rounded sides, absent from epicone; epicone subspherical exceeds hypocone; hypocone as broad as, but shorter than epicone, broadly notched posteriorly and ventrally by sulcus; nucleus spheroid, about .35 transdiameters, with coarse granules.

The various genera of parasites included in the order Dinoflagellida are usually placed with the other unarmored dinoflagellates in the sub-order Gymnodinida (order Gymnodiniales of the class Dinophyceae according to botanical systems). Chatton (1920) in his comprehensive monograph has reviewed most of the literature concerning these organisms. Nigrelli (1936) has added the more recent literature to the review included with his valuable contributions to the life-history of *Oodinium ocellatum* Brown, 1931.

About twelve genera of parasitic dinoflagellates are now recognized. Many of them including *Oodinium*, were given family (Oodinidaceae etc.) ranking by Chatton. All of the known genera were later placed in the single family, Blastodinidaceae Chatton, 1906, by Kofoid and Swezy (1921). They included *Apodinium*, Blastodinium, Chrytriodinium, Oodinium, Parapodinium, Paulsenella, Schizodinium, Syndinium, and Trypanodinium all of Chatton, as well as two genera (*Ellobiopsis* and *Paradinium*) which according to Chatton (1920) are not dinoflagellates. *Haplosooon* was omitted by Kofoid and Swezy but added by Calkins (1926). Reichenow (1930) has since added *Endodinium* Hovasse and *Merodinium* Chatton (1923) to the list and transferred the entire group to the family Gymnodiniidae.

Of these twelve genera of parasitic dinoflagellates only five are ectoparasitic. *Apodinium* and *Parapodinium* are found only on pelagic tunicates. *Chrytriodinium* is parasitic on copepod eggs, and *Paulsenella* was found as a parasite on a diatom. Species of *Oodinium* have been described from
tunicates, pteropods, annelids, siphonophores, and fish. *Oodinium ocellatum* from marine fishes is the first dinoflagellate known to parasitize vertebrates. The species described in this paper is the second such parasite and the first fresh-water parasitic dinoflagellate. The present species has therefore been given the specific epithet limneticum and placed with its cohorts in the genus *Oodinium* Chatton (1912). This genus was defined by Chatton as follows: “Dinospores à hémisphère antérieur beaucoup plus développé que le postérieur. Pas de pigment xantho-chlorophyllien, mais un lipochrome. Formes végétatives fixée par un tronc absorbant fibrillaire. Parasitisme blastotrophe. Pas de scissiparité simple. Sporogénèse intervenant après libération du parasite à produits, homodynames épars.” Chatton cited *Oodinium poucheti* (Lemmerman, 1899) Chatton (1912) as the type. Comparison of the description of the new species with the above definition and with the description of the type species leaves little doubt that the present organism belongs in the genus *Oodinium*. As compared with the definition only the presence of xanthochlorophyll pigments and the absence of lipochrome granules in the new species are noticeably at variance. This is the case with some other members of the genus as well.

The parasitic stage of *O. limneticum* differs from that of *O. poucheti* in the presence of chromoplasts, absence of lipochrome granules, absence of cellulose covering over rhizoids, and smaller size. The gymnodinia stage differs in the presence of a conspicuous sulcus, presence of chromoplasts, and the presence of a cell wall. *O. limneticum* agrees with the type species in the general structure and shape of the parasite, total absence of an eye-spot, general shape of the gymnodinia with a larger epicone than hypocone, and general pattern of the cycle.

Chatton placed four definitely recognized species and three of doubtful validity in the genus *Oodinium*. *O. ocellatum* is the fifth valid species and *O. limneticum* the sixth.

*O. poucheti* has been partially described above. It was obtained from the tunicate *Oikopleura dioica*. Its life-history is only incompletely known. Observations on this species include: parasite 150–200, ovoid or spherical, complete cellulose covering including rhizoids, large vesicular nucleus, short robust peduncle containing fibrils and bearing rhizoids, no groove or flagella; the dinospores are naked with a girdle but no sulcus; lipochrome granules are present, but no eye-spot or chromoplasts.

*O. amylaceum* (Bargoni 1894) Chatton is known only in the parasitic stage from *Salpa mucronata* and *S. democritica*. It was first placed in the Foraminifera. It is characterized by an extensive arborization of the rhizoids. Amyloid granules are present.

*O. appendiculariae* (Brooks and Kellner) Chatton from the ascidian *Oikopleura tortugensis* was first mistaken for stages in the development of this host. Nothing but the attached parasites are known.
O. fritillaria Chatton from Fritillaria pellucida is known only in the parasitic stage. It is 80×130μ or 115μ in diameter. The nucleus is very large, measuring 75μ in diameter. A few lipochrome granules are present. The species is characterized by the broad disc (60μ in diameter) which terminates the peduncle and serves to attach the parasite to the host.

O. ocellatum Brown from marine fishes differs from all other known members of the genus in possessing a conspicuous eye-spot. This ocellus consists of a black bar lying parallel to a red bar with a highly refractile space between. It is present at all stages of the cycle. This species has been the only member of the genus to have its life-history determined with reasonable completeness. Only the details of transformation of the free swimming to the attached form are still obscure. The parasite is pyriform ranging from 12.4 to 103.7μ in length, but may reach 150μ on encystment. It possesses a cellulose wall, chromoplasts, erythromes, amyloid granules, a naked peduncle with fine rhizoids and a flagellum-like process, and an eye-spot. Palmella stages result in the formation of up to 256 dinospores that each possess two flagella but no wall, girdle or sulcus. These are transformed into typical gymnodinia with girdle, sulcus, transverse and longitudinal flagella, and cellulose wall. Encystment occurs after the parasite leaves the host. The first division of the cyst is longitudinal.

Two other forms have been included in this genus. From what is known of the parasitic stages they probably belong here, but like O. appendiculariae the information is so incomplete that they can not be subjected to a critical analysis. These are O. sp. Chatton (1920) and O. sp. Dogiel.

Nigrelli (1936) has proposed a tentative key to the known valid species of Oodinium based entirely on the characteristics of the parasitic stage since the rest of the life-history has not been determined for several species. This key may be easily modified to include O. limneticum.

Key to the species of Oodinium Chatton, 1912.

A1 Peduncle terminating in few rhizoids
B1 eye-spot present 1. O. ocellatum
B2 no eye-spot present
C1 chromoplasts present
C2 no chromoplasts present
A2 Peduncle ending in a broad disc 2. O. limneticum
A4 Peduncle ending in an extensive arborization of rhizoids 3. O. poucheti
A5 Peduncle ending in an extensive arborization of rhizoids

Pathology

O. limneticum has been found as an ectoparasite on many species of exotic fresh water fishes being kept in aquaria. The hosts on which it has been observed are as follows: Brachydanio rerio, Barbus phutunio, Lebistes reticulatus, Xiphophorus helleri, Platypterus maculatus, Mollienisia latipinna, Colisa lalia, Betta splendens, Trichogaster leeri. The parasite has been observed on all external portions of the body including fins, trunk, eyes,
mouth, and gills. Seen against a dark background with the unaided eye it is
definitely yellowish and is often present in such large numbers as to cause
the fish to appear dusted with sulfur. This is especially true of darker
colored fish. Against a light background, it appears white. The parasites
frequently attach themselves in greatest concentration along the outer
margins of the scales of the fish’s body. This produces a pronounced reticu-
late pattern (Pl. III, Fig. 12).

Although this organism attacks adult as well as very young fish its
virulence is greatest among the young. Young fry (up to two weeks) of the
labyrinth fishes (Colisa, Betta, Trichogaster) usually succumb in less than a
week after infection. Many young ovoviviparous fish (Lebistes, Xiphorus,
Platypoecilus, Mollienisia) die within two weeks of infection though some
live longer, and a few may eventually become free of the parasite and live
to maturity. Larger young and mature fish frequently maintain their vigor
and may show no apparent discomfort in spite of heavy infection, but a few
of these may also die. Those that succumb often develop noticeably spread-
ing opercula in the later stages, indicating heavy gill infection. These indi-
viduals may also exhibit some emaciation and folding of fins. Fish with
heavy infections of body and fins may have no gill infection.

It is probable that very small fish are killed directly by the loss of
organic matter to the parasite. Since larger fish are able to withstand very
heavy infection unless it involves the gills, it is likely that their death is
caused by interference with respiration. Organic loss from larger fish would
be relatively slight. No secondary infections have been observed. This is in
contrast to the conditions found in heavy infections of the ciliate Ich-
thyophthirius which are often followed by infections of the fungus Sapro-
egenia. Death of the host usually ensues soon after this parasite establishes
itself. Since Oodinium does not burrow into the epithelium as does Ich-
thyophthirius no rupture remains in the protective coat of the fish when the
parasite drops off. This probably accounts for the absence of Saprolegnia
infections.

Individual fish exhibit varying degrees of resistance and susceptibility.
A fish may become densely covered with the parasite, and remain so for a
week or longer until one or several parasitic cycles are completed. After
that no further infection occurs. This fish may then remain free of parasites
in spite of their presence in large numbers in the surrounding water and on
other fish. Some individuals appear to have a natural immunity since they
do not develop even an initial infection when the parasite is known to be
present.

It may be of interest to note the nature of some of the infections. All
45 mature Brachydano rio (zebra fish) in one aquarium remained para-
sitized for three weeks after which they were cured with methylene blue.
Only three died. Three mature Barbus phutunio (pygmy barb) remained
heavily infected for a month and showed no distress. They were cured with methylene blue. Rivulus cylindraceus in the same aquarium were not infected. Lebistes reticulatus (guppy), Platypoecilus maculatus (platy), and Xiphophorus helleri (swordtail) of various ages were observed with infections. The results were as reported above. Most of them were left untreated. A golden strain of guppy showed very low incidence of infection. Less than \( \frac{1}{4} \) of those exposed were parasitized. Over \( \frac{3}{4} \) of the normal gray strain became infected under the same conditions. Some of the swordtails were continuously parasitized for four months without succumbing or showing distress although the fin development seemed to be somewhat retarded. Most of the fish that were at least one month old when infected eventually became free of the parasite; most of the younger ones died. At one time 39 Mollienisia latipinna (mollies) one week old became infected and all died within 10 days. Over 100 fry of each of the labyrinth species, Colisa lalia (dwarf gourami), Betta splendens (Siamese fighting fish), and Trichogaster leeri (pearl gourami) became infected and all died except one dwarf gourami which lived to maturity.

As indicated by the list of species of fish which O. limneticum is known to have parasitized, its specificity must be very low. Three families of fish are included among its hosts. No others have been tested, but it is likely that many other fish and possibly other groups of animals are satisfactory hosts.

Various methods may be used to control the parasite. One method which has been used successfully and is practical in aquaria involves the use of the dye methylene blue. While the cysts are not affected by this substance in low concentrations the gymnodinia absorb the dye readily and are killed. In clean water .01 gram per gallon of water is usually effective. Both soil particles and plants absorb the dye, so that larger and repeated applications must be used when these are present. At temperatures of 25° to 30°C the time required for completion of the life cycle is much less than at lower temperatures, and treatment is most effective in this range. Cysts rarely remain longer than a week at these temperatures, but treatment should be continued at least that long after the last parasites have dropped off. Higher temperatures than those indicated may be used to further accelerate the cure but it is often difficult to maintain the vigor of the host under such conditions. Lower temperatures, on the other hand, reduce the activity of the fish making it an easier victim as well as causing the parasite to remain attached for a longer period of time.

**DISCUSSION**

Oodinium limneticum exhibits saprozoic nutrition during the parasitic stage. Whether the organism is entirely dependent on this mode of feeding
and is therefore an obligatory parasite is not definitely known. Many species of the Dinoflagellida are holophytic, some are holozoic, and other primarily saprozoic. In addition combined methods have been reported for some species. The mere presence of starch in the free living stages of parasitic species does not necessarily indicate holophytic nutrition. Food absorbed by the parasite from the host is stored as a huge starch reservoir which passes through the cyst stages to the gymnodinia and may be its sole food supply. The short period of motility (less than two days) observed here lends support to this possibility. The well developed chromoplasts, on the other hand, are always present and suggest the probability of photosynthesis. No ingestion of solid material was observed in this organism although starch grains were made available. A free living species of dinoflagellate which was also under observation at the time ingested large starch grains readily and egestion of waste products near the junction of the sulcus and girdle was observed as well. Nigrelli (1936) reported maintaining *O. ocellatum* in sterilized sea-water which strongly indicates holophytic nutrition. The length of time that the organisms were carried, the presence or absence of cysts, or the duration of motility were not indicated. It is most probable that the trend of evolution in this group of dinoflagellates has been in the direction of the parasitic habit and the morphological and physiological features associated with that mode of life. It would not be surprising therefore to find that some members of the group are able to combine holophytic and (or) holozoic nutrition with the saprozoic type, whereas other species may have quite completely substituted the latter for the former habits. The total absence of chromoplasts from several parasitic species lends support to this contention.

It was interesting to note that no appreciable increase in volume occurs during the encystment of *O. limneticum*. Observations have also been made on *O. ocellatum* regarding this feature. Brown (1934) stated that no increase in volume occurs during encystment, whereas Nigrelli (1936) observed a sudden swelling just before the first division of the cyst due to an imbibition of water. No other species of *Oodinium* have been observed with regard to this feature.

Kofoid and Swezy (1921) have pointed out that in general the behavior of the flagella during encystment of motile dinoflagellates is not well known. These authors suggested that the flagella are probably absorbed but that the possibility of their being discarded is not precluded. *Oodinium limneticum* affords satisfactory material for observing such details. Just before encystment in this species, the gymnodinium comes to rest. The transverse flagellum soon pulls free of the delicate membrane which holds it in the girdle and then waves and coils about aimlessly causing only slight jerking of the organism. Then as the body rounds up both flagella drop off and
drift away in tangled coils. The whole process usually takes only a few seconds, but occasionally it requires several minutes, and often it occurs so rapidly that the details can not be easily followed.

Interesting observations made on a mixed culture containing this parasite indicate some of its ecological relationships to other microorganisms. *Paramecium multimicronucleatum* was seen to ingest considerable numbers of the smaller cysts. In a few cases it was noted that cysts were egested intact after completing the normal cycle within the ciliate and on reaching the anal pore posterior to the cytopharynx. In other instances cytolyis was observed and digestion occurred. There is some variation in the thickness of the cellulose wall and it is possible that the cysts with thicker walls can not be digested during the time required for a normal cyclosis. It was rather surprising to note this behavior in *Paramecium* since its food is usually composed of very small particles consisting primarily of bacteria.

The cytopharynx is hardly as large as some of the cysts ingested which were about 14µ in diameter. Cysts often rolled along the groove and rebounded from the cytostome without being ingested. No active gymnodinia were ingested but one which struck the upper end of the oral groove began to encyst, was drawn down the groove, rebounded, was carried back to the groove, and was finally ingested after encystment was complete. Several other gymnodinia were seen to encyst soon after direct collisions with *Paramecium*. An unidentified rotifer was also noted to ingest the cysts of *Oodinium*.

Although observations which indicate sexual activity have been recorded for a few dinoflagellates, true sexual reproduction has not been completely described for any species. No sexual behavior was noted in the present species.

**Summary**

1. A new species of parasitic dinoflagellate, *Oodinium limneticum* sp. nov., is described together with its life-history, pathological, taxonomic, and certain ecological relationships.
2. It is recorded from nine species of exotic fresh-water fish being kept in aquaria. The natural habitat is not yet known.
3. The life-cycle consists of a parasitic stage attached to the epidermis of fish by rhizoids arising from a peduncle, encystment after leaving the host, and a series of equal divisions resulting in two to 256 gymnodinian swarmers able to infect fish.
4. A cellulose wall, chromoplasts, starch and amyloid granules are present. No ocellus, or lipochrome granules are present.
5. The taxonomic relationships and a key to the species of *Oodinium* are included.
6. The loss of the gymnodinian flagella during encystment is described.
7. Ingestion of *Oodinium* cysts by *Paramecium* is described.
8. Nutrition of the organism is discussed.
NEW PARASITIC DINOFLAGELLATE FROM FISH

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