III. THE CAUSES OF DEATH IN ARTIFICIALLY-REARED LOBSTER FRY.

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Attempts to rear lobster fry through the first three stages of development under artificial conditions have been attended by many difficulties. Although protected from most of their enemies when kept in confinement, the fry are still subject to attacks of various kinds, and the elimination of these destructive agents must be accomplished before complete success in lobster culture can be attained. Cannibalism was one of the most conspicuous causes of loss in the early experiments, and an abundant growth of diatoms, other algæ, and protozoa, covering the bodies and appendages and interfering with movement and feeding, has destroyed large numbers throughout the progress of the work. A fatal fungus which attacks them has also been a factor difficult to contend with.

During the summer of 1902, at the request of Dr. Hugh M. Smith, then chief of the division of scientific inquiry of the U. S. Fish Commission, I undertook the study of these causes of death in lobster fry, with a view to suggesting remedial measures. Observations were begun as soon as the experimental hatching and rearing plant at Woods Hole was installed. This apparatus was operated under the direction of the special commission for the investigation of the lobster and clam, and is described in detail elsewhere in this report.

CANNIBALISM.

The first fry were placed in the bags May 19, 1902, but the fans were not set in motion until June 3. Cannibalism was especially marked when the fry were in crowded quarters or were allowed to collect in corners. It was evident that they must be kept in a receptacle large enough to allow each individual considerable space to himself; otherwise, if they did not devour each other, they suffocated and became foul. Moreover, they must be supplied with an attractive food with such frequency that they were not tempted to feed upon each other.

The first two of these conditions, plenty of room and continual motion, are well met by the rearing apparatus used by the special commission. The third—that of food supply—requires further attention.

On the securing of a proper food supply depends not only the prevention of cannibalism, but also, as we shall see later, what is of far more importance—the length of time required to pass through the early stages and the escape from the growth of diatoms.

In the experiments at Woods Hole the fry were fed for the most part finely chopped lobster liver, clams, and menhaden. None of these proved an ideal food; perhaps that of most value was the clams. Further experiments to discover a suitable food for the fry are greatly to be desired, as on this depends to a large extent the practicability of rearing the fry at all successfully. A further discussion of this important question will be found under a later section of this paper.

DIATOMS.

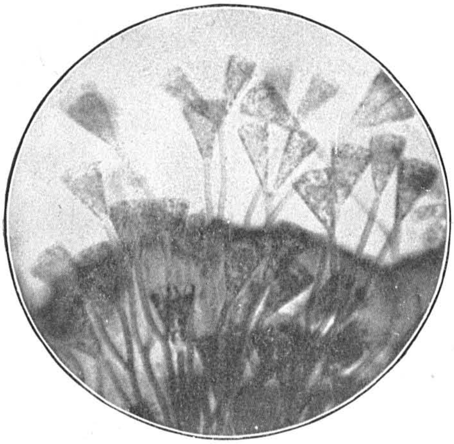
It was the abundant growth of diatoms on the surface of the body and on the appendages that first led to a consideration of the causes of death of the fry. It was supposed that the growth was a parasitic fungus, but microscopic examination soon showed that it was merely an external growth of a few protozoa and algæ, and very many diatoms of a few well-defined species. These did not at all endanger the life of the lobster, except in so far as they were a mechanical obstruction to his movements. They did not penetrate his chitinous shell and were all thrown off at each molt.

CORRELATION OF THE LIFE HISTORY OF THE FRY AND THE PRESENCE OF DIATOMS.

Absence of diatoms on adult lobsters and on eggs when attached to the female.—The lobsters and eggs examined came from the immediate vicinity of Woods Hole, from Gloucester, Mass., Block Island and Narragansett Pier, R. I., and from Noank, Conn., and no diatoms were found on the adults or on the eggs when attached to the swimmerets of the female. On the stalks by which the eggs are attached to the appendages of the female there are frequently colonies of a vorticella (*Zoothamnium elegans* D'Udekem), but these are seldom found on the eggs and never on the fry, and so have no bearing on the problem under consideration.

Diatoms on the eggs and fry in the hatching-jars.—When the eggs are placed in the McDonald hatching-jars the diatoms make their appearance within twenty-four hours. The species that first appears, in fact the only species that appears on the eggs while in the jars, is *Licmophora tincta*, the one destined to be the most abundant throughout the life of the fry (pl. IV, fig. 1).

In cases where the fry hatch within twenty-four hours after the eggs are placed in the jars, some of them, in a few hours after emerging from the eggshell, have a considerable number (14–25) of diatoms



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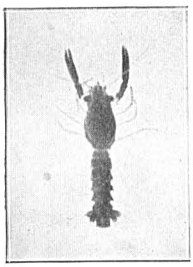

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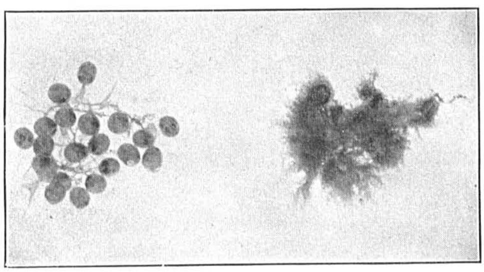

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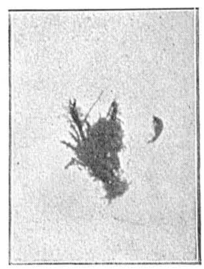

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on their appendages. Others remain perfectly clean. In the hatching-jars the growth of diatoms never becomes abundant either on the eggs, egg-stalks, or fry.

Diatoms on the fry in the rearing apparatus.—As soon as the eggs or fry are placed in the bags of the rearing apparatus, the growth of diatoms is greatly accelerated, and eggs, egg-stalks, and fry are soon covered with them (pl. IV, figs. 3, 6, and 7). The fry become brownish and shaggy to the naked eye, are impeded in their movements and in their feeding, and soon perish unless the act of molting intervenes to rid them of their unpleasant burden.

As already stated, in the hatching jars but one species of diatom is present (*Licmophora tincta*). Upon removal to the rearing bags the number of species increases, and although *Licmophora tincta* is always the most abundant at Woods Hole, other species also are present in large numbers. A list of the species and their relative abundance will be found in a later section.

Diatoms on the different stages of the fry.—The eggs and fry of the first, second, and third stages become badly covered and in many cases killed by the diatoms. When an individual survives and molts to the fourth stage he is less liable to infection. He is now a more active swimmer. He swims not merely to keep afloat, but to go swiftly from place to place, to retreat from danger, or to capture his food. His shell is harder, his limbs and appendages are larger, less feathery, less adapted for the attachment of the diatoms. His manner of life is changed. He now seeks the bottom and crawls about and hides under stones, shells, and seaweed, or even burrows in the sand—a manner of life that enables him to free himself to a large extent from any external growth. But even in this fourth stage certain individuals become covered with diatoms, particularly when they do not have access to a sandy bottom. Individuals of the fourth stage have been observed with a growth of algæ one-half inch long, and in one case an amphipod tube, with its living occupant, was closely attached to the carapace (pl. IV, fig. 5). In this stage, however, diatoms are not so troublesome and probably are never the cause of death, particularly if the fry are transferred to cars with a sandy or gravelly bottom.

DIATOMS AND THE PROCESS OF MOLTING.

As is well known, the process of molting usually takes but a few moments. Not infrequently, however, something goes wrong, the fry becomes entangled in the old shell, the struggle is quite prolonged, and often the lobster dies in the process. The method of molting is the same in all stages of the lobster; the old skin splits across the back between the thorax and the abdomen and the body is worked out from this opening, leaving the old shell with all the appendages intact. If the old shell is covered with diatoms, some of these are dislodged in the process of extracting the appendages, particularly if the struggle is prolonged, and become attached to the bristles or hairs of the clean appendages. Frequently the number thus attached is large.

RATE OF GROWTH OF THE DIATOMS ON THE FRY.

As soon as a lodgment is obtained upon the lobster the diatoms begin their active growth and soon spread over the whole body. Even a few hours after molting the fry may be badly infested and within two days are so covered with diatoms that they appear shaggy to the naked eye. Fry that molt become covered again within the same length of time. Figures 3 and 4 of plate v show the amount of growth that may take place in 48 hours.

SOURCE OF THE DIATOMS—THEIR NATURAL HABITAT.

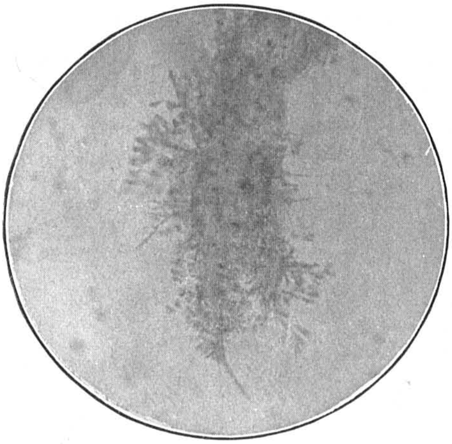
From the fact that the diatoms are not present on the eggs while still attached to the female and that they appear immediately in the hatching jars, we are led to suppose that they are present in the water when pumped through the pipes to the hatchery, and that perhaps they grow on the inside of the pipes or hatching jars.

Diatoms in the water pumped to the hatchery.—A quantity of water as it flowed from the supply pipe leading to the hatching jars was filtered and an examination made of the material collected by the filter. The diatom *Licmophora tincta* was present in considerable numbers and with it were the following species, though much less abundant: *Tabellaria* (*Striatella*) *unipunctata*, *Licmophora flabellata*, *Navicula*, (two species), *Pleurosigma* sp. There were present, also, some algal filaments, Vorticella, and fragments of copepods, isopods, and amphipods (*Caprella geometrica*).

The spores of these diatoms are so small that they can not be detected by direct microscopic examination, and the sessile varieties of diatoms, those present on the lobsters, do not grow in ordinary water cultures. It was therefore difficult to demonstrate the presence of spores in the water, but the fact that they are given off in large numbers during certain stages of the growth of diatoms makes their presence highly probable.

On the inside of the hatching jars and on the glass tubes that carry water to the bottoms of the jars, there is almost always a growth of algæ, and on the filaments of the algæ there is usually a growth of diatoms. As far as observed these diatoms were all of one species, *Diatoma hyalinum*. *Licmophora tincta* was never found growing there.

Diatoms in water outside of hatchery.—During September, 1902, a series of observations was made to determine the presence of diatoms in the water outside of the hatchery. In the tow collected on several



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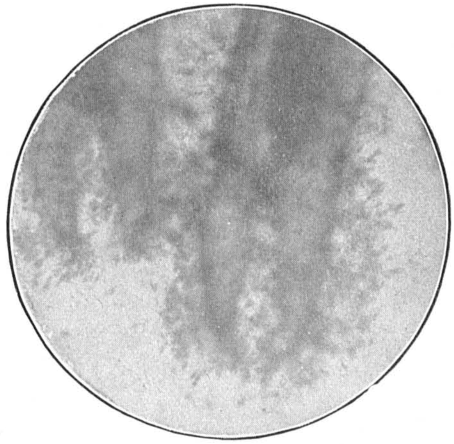

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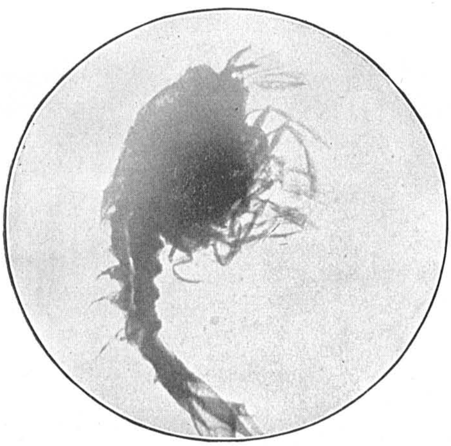

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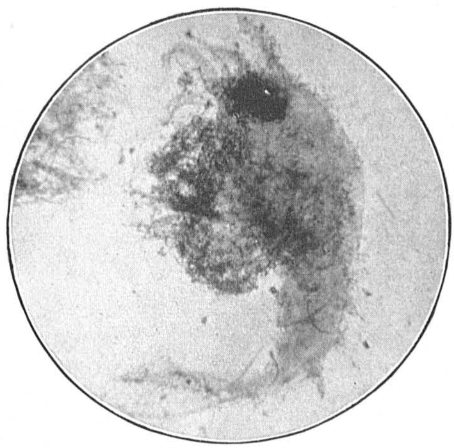

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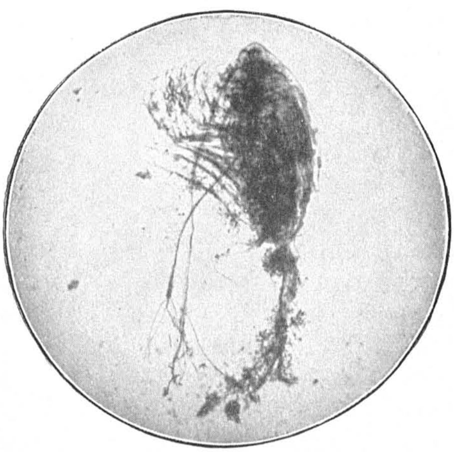

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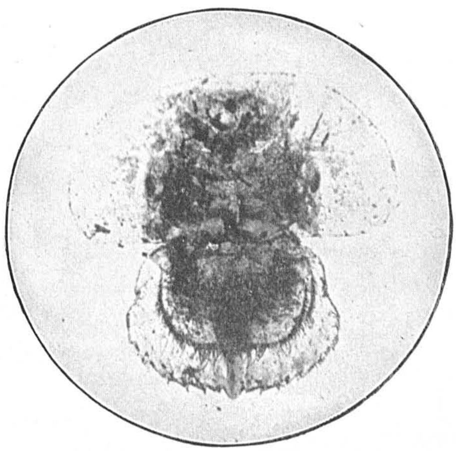

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days, at different times during the month, the following species were found, being named in the order of their abundance:

Chætoceros sp.
Rhabdonema adriaticum Kützing.
Tabellaria (Striatella) unipunctata Agardh.
Navicula sp.
Rhabdonema arcuatum (Lyngby) Kützing.
Licmophora tincta Grunow.
Pleurosigma sp.
Rhizosolenia gracilis H. L. Smith.
Rhizosolenia setigera Brightwell.
Grammatophora subtillissima Bailey.
Diatoma hyalinum Kützing.
Achnanthes longipes Agardh.

It thus appears that the water is filled with living diatoms of both the free-swimming and the sessile varieties, the latter ready to become attached whenever opportunity offers.

DIATOMS ON LOBSTER FRY IN THEIR NATURAL HABITAT AND ON OTHER CRUSTACEA.

The next question was, Do the diatoms become attached to lobster fry when the latter are unconfined—that is, under natural conditions? Only a few lobster fry could be obtained for examination. One taken in the tow near the Fish Commission wharf early in June did not show any diatoms. One of the third stage and two of the fourth or fifth stage, taken at the surface at 11 p. m., 40 miles south of No Mans Land, on July 30, 1902, showed no diatoms. Other crustacea have occasionally been observed to be covered with them. A copepod, probably *Corynura bumpusii*, covered with *Licomophora tincta*, was taken off the Fish Commission wharf by Mr. Vinal N. Edwards on June 24, 1902 (plate v, figure 5). On September 10, 1902, a young *Limulus polyphemus* was taken covered with another diatom, *Rhabdonema adriaticum* (pl. v, fig. 1). A large number of copepods, zoeæ, young squilla, etc., taken in the tow during September, were examined, but with the above exceptions no diatoms were found on crustacea under natural conditions.

Other crustacea besides lobsters, however, when kept in confinement are subject to diatom infection. Dr. M. T. Thompson, during the summer of 1900, found that certain experiments with larval hermit crabs (*Gleucothoe*) had to be abandoned because of the abundant growth of diatoms on the young crabs.

If any conclusion can be drawn from these few observations it is that although the water is filled with diatoms ready to become attached when opportunity offers, they do not ordinarily attack living lobster fry or other crustacea, unless some artificial condition, such as confinement or weakened vitality, is introduced.

DIATOMS ON OTHER SUBMERGED OBJECTS.

Any submerged object is capable of and usually does support a growth of diatoms after it has been in the water a short time. The spiles of the Fish Commission wharf show numerous species, among them *Licmophora tincta*, the one so abundant on the lobsters. The eel grass in all localities is covered with them. Collections from the eel grass show the following species, named approximately in the order of abundance:

August 5, 1902.
- Licmophora flabellata (Carmichael) Agardh.
- Licmophora tincta Grunow.
- Rhabdonema arcuatum (Lyngby) Kützing.
- Rhabdonema adriaticum Kützing.

August 30, 1902.
- Pleurosigma sp.
- Cocconeis scutellum Ehrenberg.
- Melosira sp.
- Nitzschia longissima (Brébisson) Ralfs.
- Rhabdonema arcuatum (Lyngby) Kützing.
- Rhabdonema adriaticum Kützing.

September 10, 1902.
- Licmophora flabellata (Carmichael) Agardh.
- Rhabdonema adriaticum Kützing.
- Rhabdonema arcuatum (Lyngby) Kützing.
- Nitzschia longissima (Brébisson) Ralfs.
- Synedra affinis Kützing.
- Pleurosigma sp.
- Amphora sp.
- Podocystis sp.

The nets of the fish traps, soon after being set, bear a great abundance of individuals and species, and the bags of the lobster-rearing apparatus are particularly well adapted for the attachment and growth of many species. The water set in motion by the fans is continually passing through the scrim of which the bags are composed, and the diatoms are filtered out. They become entangled in the fibrous material, and begin to grow rapidly. It is this growth that is the principal cause of the rapid fouling of the bags. At least once in every two or three days it was necessary to replace the bags in order to keep up the circulation of the water. The following species were found growing on the bags between May 29, 1902, and July 1, 1902:

- Nitzschia longissima (Brébisson) Ralfs.
- Licmophora tincta Grunow.
- Chaetoceros sp.
- Grammatophora marina (Lyngby) Kützing.
- Licmophora flabellata (Carmichael) Agardh.
- Rhabdonema arcuatum Kützing.
- Pleurosigma sp.
- Pleurosigma fascicola W. Smith.

Synedra gallioni Ehrenberg.
Synedra affinis Kützing.
Tabellaria (Striatella) unipunctata Agardh.
Navicula sp.
Cocconeis scutellum Ehrenberg.
Melosira sp.
Melosira nummuloides Agardh.
Cocconeis sp.

It seems as if in these bags we had an ideal method of growing diatoms in large numbers under the most favorable conditions for distributing them to the fry. The circulation of water is continually throwing the fry against the sides of the bags, and if these bags are foul with a growth of diatoms, the fry will become foul almost as soon as they are placed in the bags.

PRESENCE OF DIATOMS AT OTHER LOCALITIES.

At Woods Hole, as we have already seen, the species of diatoms that trouble the lobster fry are distributed quite generally in the water and on all submerged objects. It was desirable to determine whether the same conditions exist at other places along the coast, and examinations were made with this in view.

Conditions at Gloucester, Mass.—In the hatching jars at Gloucester, on the inside of the glass and on the tubes, June 21, 1902, there were found a very few *Licmophora tincta*, along with several other species (*Coscinodiscus* sp., *Cocconeis* sp., *Navicula* sp., *Rhabdonema arcuatum*). In no case were diatoms as abundant as in the same places in the Woods Hole jars, however. In fact, considerable search was required to find any, and none of the fry taken from the hatching jars at Gloucester showed a single diatom. They were perfectly clean, in decided contrast to the condition of the fry in the jars at Woods Hole.

Conditions at Wickford, R. I.—Experiments in lobster culture have been carried on for the past three summers (1900, 1901, 1902) at Wickford, R. I., at the floating laboratory and hatchery of the Rhode Island Fish Commission. The first year the fry were confined in large square bags made of scrim, fastened to a float and weighted at the lower corners. A few unsuccessful experiments were made with cars. One experiment was tried in which the water in the bags was continually stirred with an oar for six days. Doctor Mead states in the Report of the Rhode Island Fish Commission for 1901 (page 71) that "a larger proportion of fry was obtained from this experiment than from any other ever tried at Wickford, Woods Hole, or elsewhere, and also that one of the most encouraging results of the experiment was the clean and healthy appearance of the fry at all stages. The continual stirring prevented the accumulation of the parasites found on the bodies of nearly all specimens in the other lots." This seems to indicate that at times during this year some of the fry were troubled with a growth of diatoms.

During the next year, 1901, an apparatus similar to that already described in this paper was installed at Wickford with the cooperation of the U. S. Fish Commission. As far as can be learned very little trouble was experienced from the growth of diatoms during the year. The only statement in regard to diatoms in the report of the Rhode Island fish commission for 1902 is that "at certain periods during the summer a great quantity of diatoms and other small organisms, both plant and animal, are caught in the meshes of the scrim bags and there accumulate to such an extent that the circulation of the water is often interfered with." Nothing is said about their presence on the fry, and Doctor Mead states that they were not sufficiently abundant to be noticeable.

During 1902 a new style of apparatus was installed at Wickford, consisting of large square canvas bags, 12 by 12 by 5 feet, with small windows of copper netting, as described in the report of the Rhode Island fish commission for 1903. In the bottom of these bags fans revolved as in the other experiments. Fry reared in these bags remained clean until the first of July, while during the same summer at Woods Hole diatoms were abundant on the fry throughout the season. A few fry obtained from Wickford on June 30, 1902, showed a very few *Licmophora tincta* and some *Tabellaria* (*Striatella*) *unipunctata*, *Navicula* sp., *Rhabdonema arcuatum* and *Rhabdonema adriaticum*, but the diatoms were not sufficiently abundant to be noticeable to the naked eye. The fry infected had been hatched at Woods Hole and immediately transferred to Wickford. The first week in July diatoms began to be abundant on some of the fry at Wickford, in all cases the first affected being those hatched at Woods Hole, which, as we have already seen, were quite badly infected before leaving the hatching jars at that place. Fry hatched and reared at Wickford did not begin to show a growth of diatoms until after July 8, 1902. The same species were present on the fry hatched and reared at Wickford as on those hatched and reared at Woods Hole, but the relative abundance of the different species varied, as is shown by the following table, which gives the names of the eight most abundant species found on the fry of the two localities.

On fry hatched and reared at Woods Hole:

- Licmophora tincta Grunow.
- Diatoma hyalinum Kützing.
- Rhabdonema arcuatum (Lyngby) Kützing.
- Tabellaria (Striatella) unipunctata Agardh.
- Licmophora flabellata (Carmichael) Agardh.
- Synedra gallionii Ehrenberg.
- Synedra affinis Kützing.
- Grammatophora marina (Lyngby) Kützing.

On fry hatched and reared at Wickford:

Grammatophora marina (Lyngby) Kützing.
Synedra gallionii Ehrenberg.
Synedra affinis Kützing.
Tabellaria (Striatella) unipunctata Agardh.
Rhabdonema arcuatum (Lyngby) Kützing.
Rhabdonema adriaticum Kützing.
Cocconeis scutellum Ehrenberg.
Licmophora tincta Grunow.

On fry hatched at Woods Hole and reared at Wickford:

Grammatophora subtillissima Bailey.
Synedra gallionii Ehrenberg.
Synedra affinis Kützing.
Rhabdonema adriaticum Kützing.
Rhabdonema arcuatum (Lyngby) Kützing.
Tabellaria (Striatella) unipunctata Agardh.
Nitzschia longissima (Brébisson) Ralfs.
Licmophora tincta Grunow.

From a consideration of the conditions at Wickford it seems as if fry reared there were less liable to infection by diatoms than those reared at Woods Hole, even though the same species of diatoms are present at both places. The fry hatched at Woods Hole were the first to show a growth of diatoms when reared at Wickford, and perhaps introduced the troublesome species in large numbers to the rearing bags there. It is noticeable that the most abundant and troublesome species at Woods Hole was the eighth most abundant species on fry hatched and reared at Wickford.

The character of the material of which the rearing bags are made may have something to do with the abundance of diatomaceous growth, not only on the bags, but also on the fry to which the bags so readily distribute it. It is certain that the canvas bags used at Wickford in 1902 did not become foul for a considerable period, while the scrim bags used at Woods Hole had to be changed every few days. This may explain the later appearance of the growth on the fry reared at Wickford as compared with those reared at Woods Hole.

Conditions elsewhere.—Elsewhere than at Woods Hole and Wickford experimental rearing of fry has not been tried except in a very imperfect way. In 1900 some preliminary experiments were tried at Orrs Island, Maine, and Annisquam, Mass. In the former locality diatoms were abundant on the fry; at the latter they were present in less numbers. The higher temperature of the water and the consequent more rapid growth of the fry probably explains the comparative freedom from diatoms at Annisquam. The temperature there was sometimes as high as 76° F., and the lobsterling (fourth) stage was reached in ten days.

SEASONAL DISTRIBUTION.

Hardly enough data have been collected to draw any conclusions in regard to the seasonal distribution of the diatoms affecting the lobster fry. A few facts have been noted, however. During the time that lobster fry were being reared at Woods Hole, from June 1, 1902, until the middle of July, about the same relative abundance of species growing on the fry obtained from first to last. *Licmophora tincta* was always the most plentiful. *Licmophora flabellata* was occasionally present early in the season, but later, about July 5, 1902, it became much more abundant. In some cases this species was practically the only species attached to the carapace, *Licmophora tincta* and the other species being confined to the limbs and abdomen.

That the later appearance of the diatoms on the fry at Wickford had anything to do with their seasonal distribution is doubtful; the temperature of the water may have had some influence, but it seems hardly probable because of their absence the previous season, and also because Wickford temperatures are, as a rule, higher than those at Woods Hole. The explanation already given—the infection of the bags by the fry brought from Woods Hole—seems more reasonable.

SPECIES OF DIATOMS FOUND ON LOBSTER FRY.

Below is a list of all diatoms found on lobster fry hatched and reared at Woods Hole, in the order of their abundance:

Licmophora tincta Grunow.
Diatoma hyalinum (Kützing) Grunow.
Rhabdonema arcuatum (Lyngby) Kützing.
Tabellaria (Striatella) unipunctata Agardh.
Licmophora flabellata (Carmichael) Agardh.
Synedra gallionii Ehrenberg.
Synedra affinis Kützing.
Grammatophora marina (Lyngby) Kützing.
Grammatophora subtillissima Bailey.
Melosira sculpta Kützing.
Cocconeis scutellum Ehrenberg.
Actinoptychus undulatus Ralfs.
Hyalodiscus subtiles Castracane.
Coscinodiscus concavus Ehrenberg.
Navicula lyra Ehrenberg.
Navicula didyma Ehrenberg.
Nitzschia vivax W. Smith.
Nitzschia longissima (Brébisson) Ralfs.
Schizonema americanum Grunow.
Navicula sp.
Rhabdonema adriaticum Kützing.
Campylodiscus sp.
Actinoptychus sp.
Amphora sp.

STRUCTURE AND LIFE HISTORY OF DIATOMS.

The diatoms are a well-defined group of aquatic plants not closely related to any other. Perhaps they should be placed nearer the brown algæ, Phæophyceæ, than any other group, and might be defined as unicellular algæ, characterized by a silicification of the cell wall and by the presence of chlorophyl and a brown pigment, diatomin. Though unicellular, they may be united in chains or filaments, or, by the secretion of a gelatinous material in the form of an inclosing sheath or a supporting stipe, they may form colonies of characteristic shape adhering to plants or other submerged objects.

Cell structure.—Though the diatoms appear in a great variety of forms and sizes, their structure is essentially the same in all. The cell is inclosed in a shell composed of silica, consisting of two symmetrical parts or valves, which are in contact at their margins with an intermediate hoop or girdle. In some forms one valve fits over the other like the cover of a pill box. The girdle may be single or double or complex in structure, with one or more plates inserted between the top of the valve and the girdle. The siliceous shell is usually elaborately and exquisitely sculptured, the extreme delicacy of the details with which the valves are ornamented making the diatoms most beautiful objects under the microscope, and testing its highest powers.

The form of the diatom varies with the habits of the species. Most of the free-swimming forms are oblong, oval, or spindle-shaped; the fixed species are usually of different shape at their free and attached ends; the floating forms have special contrivances for increasing their buoyancy.

The cytoplasm is disposed, peripherally, as a lining to the cell wall; centrally, it may form a bridge across the center of the cell or may take the form of a stellate mass with a series of radiating threads extending out to the peripheral cytoplasm. The nucleus is in the peripheral cytoplasm close to the cell wall, is suspended by the protoplasmic bridge, or is in the center of the stellate mass of cytoplasm.

Chromatophores are always present in the cells. They are yellowish-brown in color and contain besides chlorophyl the peculiar pigment diatomin. The chromatophores vary in number in the different species and take the form of bands, granules, or rounded masses arranged irregularly or in radiating lines. The arrangement of the chromatophores is not constant even in the same individual. An amorphous mass may become divided into numerous granules of equal size and definite outline. There seems to be some definite relation between the arrangement of the chromatophores and the growth and division of the whole frustule. In some species a few round oil globules are also present in the cytoplasm.

Motility.—Many of the free-living diatoms have the power of movement. The mechanism of this motion has been variously explained: (1) As produced by pseudopodia of protoplasm extending through openings in the cell wall; (2) by the presence of cilia extending through the cell wall; (3) by endosmatic currents of water passing in and out of the cell. As the diatoms on the lobster were all fixed species, the matter of motion was not specially investigated in connection with the present question, although frequently in stained specimens apparent cilia were observed extending from all sides of the frustule. This same appearance has been noted in some of the motile forms, and it has been suggested, on what grounds I can not say, that these cilia are probably fungoid growths.

Reproduction.—The ordinary method of multiplication of the diatoms is simple cell division. The nucleus divides first, the chromatophores divide either before or after the division of the protoplasm, and two new cells are formed within the old pair of valves. Each of the new cells forms a new valve on its inner side, so that the new valves lie back to back along the line of division. In cases where the valves are of unequal size, as each old valve becomes the larger valve of the new diatom, it follows that after division the daughter diatoms are smaller than the original. In those species in which the valves do not increase in size this results in a great diminution in the size of the new diatoms, and the original size is again restored by the formation of "auxospores."

The formation of auxospores was at one time supposed to take place merely to compensate for the reduction in the size of the diatoms by repeated cell divisions. They are more properly considered as forms of reproduction. Two kinds must be considered—the asexual and the sexual auxospores. In the former the cell contents separate from the cell wall, increase greatly in size, with or without division and subsequent coalition, surround themselves with a membrane, and finally form a new diatom within, of the maximum size of the species. In the sexual method the cell contents escape from two cells, fuse, a true fertilization takes place, and a new diatom is formed from the resulting cell either at once or after a preliminary division.

The formation of swarm spores in the diatoms has not been observed, although in many species there are indications that some such phenomenon occurs. From the relation of the diatoms to other algæ in which this is a common method of reproduction it seems most probable that it does occur. A few species form resting spores. The protoplasm of the cell becomes condensed into about one-third of its normal volume, and a thick cell wall of definite and peculiar shape is formed about it. In this state the diatoms are extremely resistant and are able to await the return of more favorable conditions, when they reassume the original form of the species.

Licmophora tincta Grunow.—Of all the species found on the lobster fry the most abundant is *Licmophora tincta* Grunow. This form was found on every fry examined, and in most cases constituted over 90 per cent of all the growth present. It is also abundant, as we have already seen, in the water and on submerged objects generally. It seems to be particularly adapted for lodgment and growth on young crustacea of various sorts, however, especially when they are kept in confinement. The species occurs only in salt water, and though the genus was named by C. Agardh as early as 1827 and the species by A. Grunow some time later, yet it has appeared under many synonyms. The species are in many cases doubtfully distinct, and this may account for the fact that the early synonymy is inextricable. Following are some of the names:

Gomphonema tinctum Agardh.
Riphidophora elongata Kützing.
Riphidophora oceanica Kützing.
Riphidophora superba Kützing.
Riphidophora meneghiniana Kützing.
Podosphenia hyalina Kützing.
Podosphenia β Kützing.
Podosphenia racemosa Kützing.

In shape the frustules are more or less cuneiform in front view, convex in side view, inflected at the larger end. They show transverse striæ ranging from 27–28 per 0.01 mm. at the base to 30–31 at the center and 33 or more at the top. A pseudoraphé is easily apparent. The endochrome is arranged in a radiating manner about the nucleus and cytoplasm in the center of the frustule, or may appear as regular oval granules scattered throughout the frustule. The frustules are mounted on a gelatinous stalk, at first represented by a simple knob at the end of the cell, but this later grows out into a stalk which divides dichotomously as the cells divide, and finally forms a much branched stipes of considerable length and complexity. (Pl. IV, fig. 1.)

What were taken to be auxospores were observed in one or two instances (pl. IV, fig. 2), but no evidence of other spore formation, either swarm or resting spores, was seen, nor any evidence of conjugation.

OTHER GROWTHS FOUND ON LOBSTER FRY.

Algæ.—Although diatoms are the first and most abundant organisms that appear on lobster fry, they are by no means the only ones. On both Woods Hole and Wickford fry filaments of a green alga are frequently seen. This occurs principally on the fry of the first, second, or third stage, but individuals of the fourth stage have been observed with very abundant algal growths.

Protozoa.—On many fry are found, more or less abundantly, specimens of the stalked protozoan, *Ephelota coronata* Strethill Wright. This protozoan was observed on fry at Woods Hole on June 17 and July 3, 1902, and was probably more or less abundant throughout the season. At Wickford it was especially abundant early in July (1–8), sometimes as many as 86 individuals being found on one fry.

Crustacea.—One specimen of a tube-dwelling amphipod was observed at Woods Hole on the back of the carapace of a fry in the fourth stage. (Pl. IV, fig. 5.) In no case were the algæ, protozoa, or crustacea so abundant that they caused any serious inconvenience to the fry.

SUGGESTIONS FOR THE PREVENTION OF THE GROWTH OF DIATOMS ON THE FRY.

As will be seen from a consideration of the foregoing facts, the successful rearing of lobster fry depends to a large extent on the discovery of some method of combating or getting rid of the growth of diatoms. The following suggestions are derived from these observations:

Filtering the water in which the fry are kept.—This method of removing diatoms could of course be applied only to the water in the hatching jars; it would be a practical impossibility to filter the water in which the fry are kept in the rearing apparatus. Inasmuch as we have learned that the troublesome species of diatoms are present in the water as it flows into the hatchery, however, and that in many cases the diatoms become well established on the fry before the latter are removed from the jars, it would certainly retard and to a large extent prevent the rapid growth of diatoms during the first molts of the fry if the water supplied to the hatching jars were filtered. No very elaborate filter would be required. It would not be necessary to remove the smallest organisms, such as bacteria, though this would certainly be an advantage for other reasons. Experiment would determine the sort of a filter required. It might be that a settling basin would be all that is necessary for the removal of both diatoms and their spores.

Selection of other localities for the rearing apparatus.—Experimental rearing of the fry has been practiced at but few localities—Orrs Island, Me., Annisquam, Mass., Gloucester, Mass., Woods Hole, Mass., and Wickford, R. I. Diatoms occurred in all these localities, but were somewhat less abundant at Annisquam and Wickford. Whether this was due to a difference in the temperature of the water—the temperature at Annisquam and Wickford was somewhat higher than at the other localities—or to other conditions, can be determined only by experiment. It may be that there are other places along the coast where greater differences of temperature or other local conditions might still further reduce the growth of diatoms; the possibility of finding such a place warrants a series of trials in several localities. It must be borne in mind, however, that Woods Hole fry when they come from the hatchery are infected with the diatoms and that they are liable to introduce these diatoms to any locality where such an experiment is tried. Fry from Gloucester, on the other hand, are apparently free from infection when they come from the hatchery.

Changes in the rearing apparatus.—There is no doubt but that the scrim bags are to a large extent responsible for the rapid growth of diatoms on the fry. As has already been shown, the bags rapidly become foul from a growth of diatoms and other organisms filtered out of the water as it passes through them. The fry are continually coming in contact with the bags, and the diatoms, being easily dislodged, readily become attached to the feathery appendages of the fry. The use of canvas bags with copper netting windows, as tried at Wickford in the experiments of 1902, seems to prevent to a large extent the rapid fouling of the bags and the consequent growth of diatoms on the fry. A more frequent changing of the bags would perhaps bring the same result, but this method is hardly practicable. Perhaps there is some other material of which the bags might be made on which the diatoms would not grow so easily, or there may be some preparation, such as tar or oil, with which the bags might be coated, that would prevent the attachment of the diatoms. It is certain that the cleaner the bags the longer the fry remain free from diatoms. If the fry were received clean from the hatchery, or were hatched directly in the bags and the bags were kept perfectly clean, there would be little trouble from diatoms.

Sunlight and shade.—Diatoms are chlorophyl bearing plants, and consequently require sunlight for their best and most rapid development. Might not their growth be restrained by confining the fry in bags shielded from the direct light of the sun? Several experiments were made to determine this point. On June 22, 1902, an awning was placed about 3 feet above the level of the water over certain bags containing fry in the first stage. The number of diatoms on the fry under the awning steadily decreased, or at most did not develop further than their original condition, and the difference between the control fry in the sunlight and those in the shade was easily apparent to the naked eye. Some of the fry that molted from the first to the second stage were without a trace of diatoms apparent to the naked eye, while those in the sunlight showed a considerable growth. On the other hand, however, the fry themselves seemed to be influenced unfavorably by the absence of sunlight. They had less pigment in their shells and seemed much less active than those in the sunlight.

Further experiments were not tried at Woods Hole, but at Wickford some observations were made bearing on this point. During the summer of 1901 an awning shaded all the bags in which the lobster fry were reared. This awning was about 9 feet above the water, so that while the direct sunlight was excluded there was a considerable amount of diffused light. During this summer there was very little trouble from diatoms. In 1902 the awning was not used and there was a very abundant growth of diatoms. It may be that some other condition had something to do with this result, but it seems very certain that, although the exclusion of the greater part of the light may be injurious to the fry as well as to the diatoms, a cutting off of the direct sunlight, without excessive shading, is an important factor to be considered in all attempts to get rid of the great abundance of diatomaceous growth that ordinarily occurs.

Hastening the development of the fry.—The last and perhaps the most promising remedy that suggests itself is to hasten the development of the fry. A rapid series of molts prevents the excessive growth of the diatoms. If by means of proper care and proper feeding the fry can be so hastened through their early stages that the diatoms have no chance to develop to an injurious extent, the problem is solved in the most advantageous way. This economy of time will be felt not only because of its influence on the growth of diatoms but also in the running of the apparatus and in the attention of those employed to care for the fry.

The principal factors on which depend the rapidity of growth and the freqency of molting are temperature and food. Dr. A. D. Mead, of the Rhode Island fish commission, seems to think that the temperature is the most important factor, as will be seen from the following statement by him in the Report of the Rhode Island Fish Commission for 1901:

The average period between hatching and reaching the fourth stage for the entire eleven experiments at Wickford was a little óver 12 days. In each experiment the average duration of the first three stages varied from 9 to 16 days. In experiments conducted at Woods Hole the time required for these molts was considerably greater; of the first lot, hatched May 23, the fourth stage was reached by a few only on June 12, after an interval of 20 days. Indeed, on the twelfth day (the average time of reaching the fourth stage at Wickford) none had reached even the third stage at Woods Hole. The explanation of the variations in the length of time required for the first three stages probably lies in the differences of temperature of the water—the colder the water the slower the development.

It is not possible to say at present that the variations in the length of the early stages are due entirely to the differences in temperature, and it may be that other factors have more or less influence: but it is extremely probable that temperature is the main factor.

This conclusion seems justified to some extent also by the experiments of the special commission at other points on the coast, as follows:

Locality.	Temperature of water.	Time required for first three stages.
	°F.	Days.
Orrs Island	57-63	25-26
Woods Hole	63-65	22-25
Wickford	65	16
Do	72	9
Annisquam	76	10

On the other hand, the question of a proper food supply seems quite as important as the temperature. It is true the temperature affects animals, particularly the invertebrates of the salt water, to a large extent. Their activity in getting about, in finding and capturing their food, and the metabolism of that food depend altogether on the temperature; but unless proper food is supplied them their increased activity is of no avail, growth does not occur, and the temperature consequently has no influence on the rapidity of their development.

The temperature can, of course, be regulated only by changing the location of the rearing apparatus. The food supply can be varied at will. The experiments thus far made with different foods are not very satisfactory, and it seems to me that this question deserves more attention on the part of those engaged in rearing the fry. About all that can be said at present is that they will not eat finely chopped mussels, starfish liver, beef liver, scup, or herring; that they will eat finely chopped clams, periwinkles, blue crabs, lobster liver, and menhaden. Of these, the clams and menhaden have proved the most practicable, though at Woods Hole the former were difficult to obtain and the latter was found so full of oil that the water and bags were quickly fouled by it. Of course the natural food, consisting principally of small copepods, crustacea, diatoms, and algæ, is out of the question, because of the impossibility of securing it in sufficient abundance.

In spite of the fact that the food thus far used has not been particularly favorable, it has been found possible to reduce the critical period, under proper conditions of temperature and feeding, from 25 days to about 9 days. There is no reason why, with better conditions in the rearing apparatus, and better feeding, perhaps in localities better adapted as far as temperature is concerned, it would not be possible to still further hasten these changes. If, however, this shortest period were made the average period for all experiments, the growth of diatoms would not seriously menace the fry.

PATHOGENIC FUNGUS.

On June 30, 1902, it was noticed for the first time that many of the fry in some of the rearing bags were turning white and dying. The entire number in some bags eventually died. Upon investigation it was found that the bodies of the dead fry were filled with the mycelial filaments of a fungus.

This growth was found to begin in most cases in the third or fourth segments of the abdomen, where the first indication of its presence was the opaque, whitish appearance of these segments in contrast to their almost transparent normal condition. It soon spread throughout the body of the fry, destroying all the internal organs, until the chitinous shell was full of closely packed mycelium (pl. VI, fig. 1).

The fungus was isolated in pure culture. It grew on the ordinary bacterial culture media, and was also cultivated on salt water agar and on sterile potato and bean pods. In all cases the aerial growth was pure white. A colony growing on salt water agar is shown in figure 3, plate VI. The growth is a branching septate mycelium (pl. VI, fig. 2), which soon breaks up into a number of short segments resembling large bacilli (pl. VI, fig. 5), and probably representing arthrospores or conidia. In certain filaments the formation of what are apparently endospores was observed (pl. VI, fig. 4).

Some inoculation experiments were made with the fungus, but owing to the lateness of the season when the fungus was isolated young lobster fry were not to be had. Inoculations were made in fish, shrimp, and old lobsters, but were not very successful.

The fungus is to be classified, presumably, among the Oosporeæ, as one of the Hyphomycetaceæ of the fungi imperfecti. Its final identification and complete life history are to be worked out at some future time. Its origin is not known, but is probably traceable to the oily menhaden flesh with which the fry were being fed, thus getting into the intestinal tract. Another season's observations will be necessary to determine the origin, habits, and complete life history of the fungus, and until then no suggestions can be made as to methods of prevention.

CONCLUSIONS.

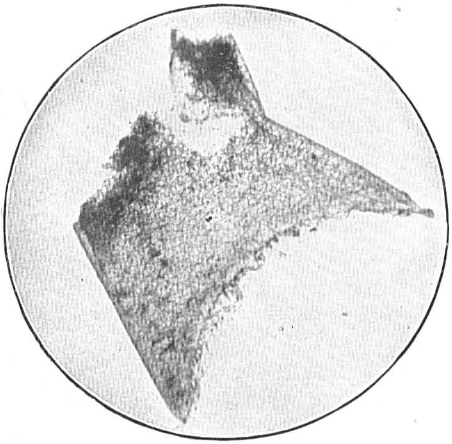
1. The principal causes of death in artificially reared lobster fry are cannibalism, an external diatomaceous growth, and a pathogenic fungus.

2. Cannibalism may be prevented by avoiding overcrowding and by providing some method of keeping the fry continually in motion.

3. The diatomaceous growth may be prevented or to a large extent reduced by—

(a) Filtration of the water supplied to the hatchery.

(b) Selection of a place for the location of the rearing apparatus where diatoms are least abundant.



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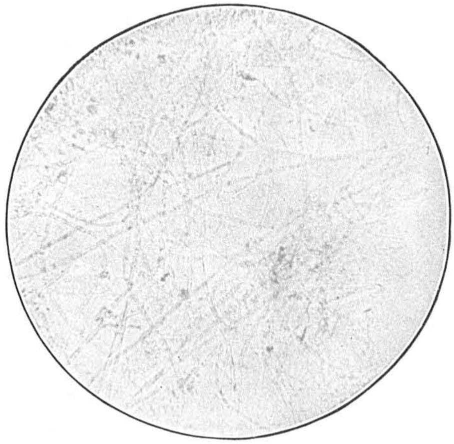

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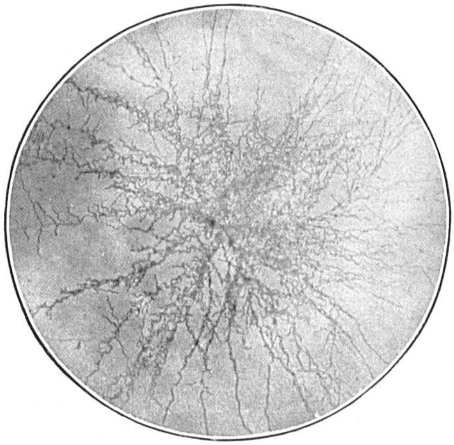

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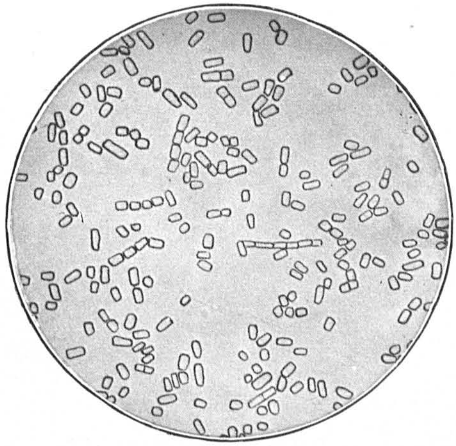

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(c) Keeping the rearing bags free from diatoms by changing the material of which they are composed, by coating them with some substance which will not permit the attachment of diatoms, or by more frequently substituting new ones.

(d) Regulation of the amount of light to which the fry are exposed.

(e) Hastening the development of the fry by locating the rearing apparatus where the most favorable temperature may be secured, and by supplying the most suitable food.

4. The pathogenic fungus, though known to be extremely fatal and disastrous to the successful rearing of fry if once introduced into the bags, has not been studied sufficiently to warrant any suggestions as to methods for its prevention.

EXPLANATION OF PLATES.

PLATE IV.

Figure 1. The diatom *Licmophora tincta* Grunow, growing on the back of a lobster fry. X 150.

Figure 2. The same, showing the cellular structure of the frustule and the formation of auxospores, X 200.

Figure 3. Clean lobster eggs and eggs covered with a growth of diatoms. Natural size.

Figure 4. Clean lobster fry of the fourth stage. Natural size.

Figure 5. Lobster fry of the fourth stage covered with diatoms and a tube-dwelling amphipod, Natural size.

Figure 6. Clean lobster fry of the third stage. Natural size.

Figure 7. Lobster fry of the third stage covered with diatoms. Natural size.

PLATE V.

Figure 1. Claw of lobster fry of the third stage covered with diatoms. X 50.

Figure 2. Claws of lobster fry of the third stage covered with diatoms. X 50.

Figure 3. Clean fry of the first stage. X 10.

Figure 4. Fry of the first stage two days later covered with diatoms. X 10.

Figure 5. Copepod, *Corynura bumpusii* Wheeler, covered with diatom *Licmophora tincta* Grunow. X 30.

Figure 6. Young *Limulus polyphemus* Linnæus covered with diatom *Rhabdonema adriaticum* Kützing. X 15.

PLATE VI.

Figure 1. Posterior segment of lobster fry of third stage filled with fungus mycelium. X 25.

Figure 2. Mycelium of fungus. X 200.

Figure 3. Colony of fungus on salt water agar. X 25.

Figure 4. Mycelium of fungus showing endospores. X 250.

Figure 5. Mycelium of fungus broken into arthrospores or conidia. X 250.