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Distribution of the Fungus Lagenidium callenectes Couch and its Effect on Eggs of the Blue Crab

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DISTRIBUTION OF THE FUNGUS LAGENIDIUM CALLINECTES COUCH AND ITS EFFECT ON EGGS OF THE BLUE CRAB

by

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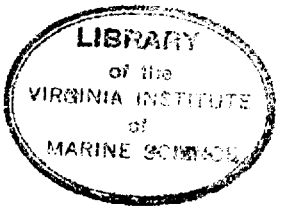

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DISTRIBUTION OF THE FUNGUS LAGENIDIUM CALLINECTES COUCH, AND ITS EFFECT ON EGGS OF THE BLUE CRAB

INTRODUCTION

During recent years the catch of blue crabs, Callinectes sapidus Rathbun, in Chesapeake Bay has shown unusual fluctuations. Since the meat of the blue crab is the source of a major industry in the states of Maryland and Virginia, this unpredictable condition presented a problem of commercial importance. Attempts are being made to analyze factors responsible for these catch fluctuations.

In 1941 a parasitic fungus was first observed on the eggs of many blue crabs obtained from natural spawning areas in Chesapeake Bay (Sandoz, Rogers, and Newcombe 1944). Dr. John N. Couch of the University of North Carolina kindly examined infected samples and recognized the parasite to be a new species, which he called Lagenidium callinectes Couch (Couch 1942).

The present study was undertaken to determine the relationship, if any, between a low production of crabs and presence of the parasite Lagenidium callinectes. The program of study was organized in two phases as follows: (1) in the laboratory, work was directed toward finding factors which influenced transmission of the fungus from one egg to another and from one crab to another; (2) statistical methods were adopted to locate areas in the Bay where infection exists. In examining individual egg masses (sponges), the amount of infection, depth of infected portions, and general condition of the sponge were considered.

METHODS

Growth and Transmittance of Lagenidium callinectes. This series of observations was conducted in the laboratory to determine factors responsible for the spread and development of the parasite. Water from the York River was used in a variety of containers, which included large glass finger bowls, white porcelain pans (20 x 4 cm.) and large aquaria (75 x 45 x 45 cm.). All aquaria were provided with a continuous flow of river water. Light was supplied from a north window; temperature ranged from 20 to 30 degrees C; salinity ranged from 18 to 22 c/cc. Examinations were made every one or two days. Experiments were set up in the following ways:

1. Several infected and uninfected egg-bearing crabs, selected from commercial catches at Seaford and Hampton, Virginia, were placed together in aquaria.

2. Healthy and infected eggs from two different sponges were placed at either end of porcelain pans and on opposite sides of large finger bowls. Running water from aquaria containing infected crabs was collected in pans into which normal eggs were then introduced. For controls, healthy eggs were placed in pans of water and females with sponges were placed in aquaria.

3. Infected and uninfected sponges in various stages of development were suspended in the York River near shore (Sandoz, Rogers, and Newcombe 1944). A small cage (30 x 13 x 25 cm.) constructed of window-screening was used to protect the sponges and keep them afloat. One side of the cage was fastened with hooks, and inside at the top was a row of staples along the middle rib. Individual ploopods, detached from the sponge, were threaded with string near the base of the protopodite and were attached to the hooks. The cage was buoyant enough to remain afloat thus subjecting the eggs to changes of light and wave currents.

Observations were made on the relative amounts of fungus growth in different salinities and the rate of transmittance of infection from egg to egg along the filaments of the pleopods. A series of salinities ranging from pond water up to the approximate concentration of sea water was prepared, using pond water and salt extracted from York River water.

In filaments selected for these salinity tests, the fungus had attacked all eggs extending 2-3 mm. along the distal end; below this point eggs were developing normally. One filament was placed in each Petri plate in 50 cc. of water. To prevent the exhaustion of oxygen, the dishes remained uncovered, thus furnishing an adequate supply from the air.

In nature other species of egg-bearing crabs have not been observed with this infection, so studies were conducted in an effort to determine whether or not this parasite has a specific affinity for eggs of Callinectes sapidus. Strands of infected blue crab eggs were placed with healthy eggs of several other forms in Syracuse watch glasses containing York River water. The other species of crabs included Pinnotheres ostreum Say, Sesarma cinereum Bosc., Neopanope texiana Smith, and Libinia emarginata Leach, all of which are found in the waters or along the shores where infected blue crabs occur.

Distribution and Degrees of Fungus Infection. In order to establish the areas in Chesapeake Bay where L. callinectes is present, samples of sponges were regularly collected from the commercial crabbing boats at Hampton and Seaford, Virginia. Preliminary sampling was done during the summer of 1943, the results of which indicated the waters where infection occurs. Early in 1944 weekly sampling of 20 to 25 sponges was begun. The sponges were preserved in 10% formalin as soon as the boats docked, only a few hours at most after the crabs had been removed from the water. Samples thus obtained were caught in the Lynnhaven-Hampton Roads waters and the Egg Island-York Spit waters.

Relatively few sponge crabs are found north of York Spit. These samples were taken to the laboratory for microscopic examinations. The data thus obtained demonstrated the occurrence of fungus in various areas of the Bay, the number of sponges infected per age group, and the degrees of infection per sponge. In this work, the age of the eggs was designated by the sponge color: yellow, brown, or black, representing 1-5 days, 6-11 days, 12-15 days, respectively.

To determine the degrees of infection on a sponge several methods were attempted before an effective system was devised. First, eggs were taken at random from the outside of a sponge and examined microscopically. A count totalling 500 eggs was made to estimate the percentage of exterior infection. Then about half the sponge was cut away and the proceedure repeated on eggs from the interior. It was found that infection did not penetrate to the interior; so to save time, it was decided to continue these observations only on the exterior. Where infection was observed, several filaments were detached at the base and examined for the progression of fungus along the strand. These methods of computing degrees of infection involved a high probability of error in view of the enormous number of eggs per sponge. It was necessary, therefore, to abandon this plan of estimating the percentage of diseased eggs, since it was impossible to count an appreciable number in every sponge to determine an accurate percentage. Satisfactory results were obtained by setting up a standard based on visible areas of infected eggs. When the fungus has spread through many eggs in a given area, the diseased portion will assume a brown color on yellow sponges and a grayish color on brown sponges. The following classification was adopted

for differentiating the infected sponges in routine collections:

Slight - fungus present in microscopic examinations but no areas of infection visible to the naked eye.

Moderate - presence of visible areas of infection (which may be one or more) but no more than half the sponge visibly infected.

Heavy - more than half of sponge periphery visibly infected, but with one or two small areas where infedtion has not become heavy enough to be seen.

Very heavy - a complete peripherial infection with no areas of healthy eggs visible.

These criteria apply only to the outside of a sponge. Previously, it had been substantiated that the fungus does not penetrate deeplyso these standards are indicative of superficial infection. From all the samples collected, four sponges were selected which demonstrated the different degrees of infection. From each pleopod of these sponges, 25 filaments were detached at the base and examined microscopically. Observations were made on the depth of fungus penetration, the general condition of interior eggs, and the possibilities of an appreciable hatch of larvae despite the exterior coat of infection. This examination also indicated certain characteristics of the disease in spreading over and into the sponge,

RESULTS AND DISCUSSION

Characteristics of the Disease. Knowledge of the life history of Lagenidium callinectes as described by Couch (1942) has been a valuable aid in studying the effect of this parasite on the eggs of the crab. The several developmental stages indicated by Couch were observed in this study and it has been possible to maintain the organism under laboratory

conditions thus providing a better understanding of how the egg is destroyed.

When germination of the zoospore begins, a delicate germ tube is sent through the egg membrane. This tube has been observed to grow rapidly into a network of branched mycelium that soon fills the entire egg. From the mycelium, hyphae pass through the egg membrane to the outside and form fruiting bodies, which contain numerous spores. Mature sporangia soon rupture and discharge new spores to continue the cycle of infection. When the nutrient material of the disintegrating egg has been exhausted by the fungus, the mycelium appears to break up into heavy-walled resting cells which seem to be resistant to adverse conditions. However, neither germination of these cells nor a sexual phase of reproduction has yet been observed. It has been found (Couch 1942) that an infected egg soon gives definite indication of being abnormal. Compared with normal eggs, diseased ones are opague and dwarfed. An infected egg is reduced from about 290 microns in diameter to approximately 231 microns.

Frequently, stumpy thumb-like projections of external hyphae appear on the infected eggs. The number of hyphae varies greatly. Usually there are one or two on an egg, but frequently nine or more projections were observed from one perspective. Laboratory and field studies have shown that eggs in any stage of development are susceptible to infection by L. callinectes.

Microscopic examinations are required to determine whether or not a single egg is infected. However, a large group of such eggs in the same region of a sponge assumes a discoloration that is readily seen

without the aid of a microscope. This discoloration, due to the opacity of the eggs is sharply contrasted to normal eggs. In a yellow sponge, the infected areas are light brown; whereas, in more mature brown or black sponges, the infected portions are gray.

Laboratory cultures showed that transmission of infection from one egg to another is extremely rapid. Often an entire pan of eggs would be destroyed by disease in 3 or 4 days, even when the first day showed very few infected eggs. Similarly, experiments have determined the conditions under which the fungus may be transmitted. In aquaria, healthy egg-bearing crabs quickly became infected when diseased crabs were introduced. In one case, water from an aquarium inhabited by a single infected female was used in a hatching pan containing only normal eggs. Within 2 or 3 days L. callinectes was seen and a majority of the eggs soon became infected. In other experiments in which diseased and normal eggs were placed at opposite ends of a pan, the fungus was observed to infect the normal eggs after about 2 days.

Infected sponges which were suspended in the York River failed to hatch. During the experiment the number of infected eggs increased while the uninfected ones under the same conditions hatched normally, the zoeal larvae escaping and leaving behind their empty, transparent egg cases.

For experimental purposes normal eggs were usually selected from the Seaford catches where diseased crabs were seldom observed. There is no record of infection in the York River, consequently, the chances of fungus having been introduced to the Seaford or the York River waters are slight. Examination of controls never showed fungus growth.

Damage to the Egg Mass. Egg masses vary a great deal in size, averaging about 75 mm. wide, 50 mm. long, 40 mm. deep. The eggs are fertilized as they leave the oviduct, then they slip down the filaments of the pleopods and remain attached there throughout the incubation period. Upon hatching, the larvae swim away leaving their discarded egg cases behind still fastened to the filaments.

An infected sponge takes on marked characteristics, because the opacity of diseased eggs gives a brown or gray appearance to infected portions. Any spong, regardless of its age, may become infected, for the spores of L. callinectes are irrespective of the embryonic stage which they attack. Several spores may simultaneously or in rapid succession come in contact with the same sponge, germinate immediately, and in a short time create visible areas of infection. In most instances sponges possess several diseased patches, each of them begun by different spores. This conclusion is based on the examination of a large number of moderately infected egg masses, which had diseased patches on opposite sides, or on separate pleopods. In most of the sponges where a slight infection was present, diseased eggs were found widely distributed over the periphery. At any time previous to hatching an egg may become infected by spores; hence, the greater the number of spores present in the water, the more quickly will a sponge reach the very heavy degree of infection.

Microscopic examinations have shown that the infection of L. callinectes is dangerous only to the periphery of a sponge. In fungus-infected crabs all eggs from the tip of the strand down to the 3 mm. point are usually infected, but below this peripheral layer eggs are normal. Occasionally, a few scattered interior eggs may be diseased.

In only one sponge were infected eggs observed at the base of a filament but this filament was only 13 mm. in length and located at the outer end of the pleopod.

Eggs within a sponge are packed closely together. The filaments, which are found only on the distal side of the pleopods, vary in length from approximately 3 to 22 mm. The longer ones extend from the base of the pleopod while the short ones are at the tip. After eggs have been extruded, this arrangement of length variations permits none of the filaments to be buried within the mass. The volume of eggs is so great that the apron is pushed away from the cephalothorax until it extends posterior to the carapace. There is a free flow of water around the sponge. Apparently, the outer eggs are forced to serve as buffers for the others. Commensals and parasites come in contact with these eggs first. Interior eggs lie closer together and do not seem to permit a rapid flow of water within the sponge. However, the interspaces are large enough for water to seep around the eggs. This movement of water is further aided by activities of the mother crab, such as vigorous jerking of her abdomen and frequent stirring of the eggs with her walking legs. These exertions increase water currents around and within the sponge. L. callinectes gains a foothold rather quickly, but never seems able to penetrate to great depths within the mass. The compactness of the sponge is responsible for slowing down the flow of water and appears to serve as a filter for straining out foreigh matter. The stirring activity of the parent crab may provide slight oppoftunity for fungus spores to infect eggs in the interior, because in a few cases infection was found at a distance of 5 or 6 mm. down the filament. Incidences of inner infection, although

uncommon, nevertheless provide positive evidence that conditions below the surface of a sponge are suitable for fungus growth. Observations have shown conclusively that embryonic development throughout a sponge continues simultaneously. This proves further that there is an adequate supply of aerated water in the interior. Hence, it seems likely that the external eggs may serve as buffers and that the arrangement of the sponge facilitates its use as a filter for the flow of water among egg-bearing filaments.

L. callinectes is found in nature thriving on outside eggs. An infected area increases rapidly in diameter while its penetration is much slower. A filament in such an area usually has 100 per cent diseased eggs at the distal end and extending several millimeters inward. This diseased portion is recognized by the gray, wax-like appearance of the eggs. Under the microscope the infection can be seen in its various stages. In very heavy infections the most distal eggs have had their nutrient material exhausted by the mycelium and resting cells have formed; the egg membranes may have started to disintegrate. Adjacent eggs to these have become very opaque and dwarfed and possess external hyphae and sporangia. The dieased eggs which are lowest on the strand are in the earlier stages of infection with only one or two empty spore cells on the outside; the internal mycelium is still developing, and very few or no external hyphae are visible.

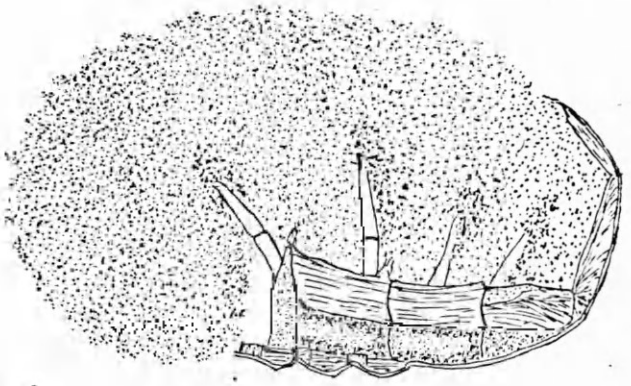
Under laboratory conditions Couch (1942) found that an egg could become infected and completely filled with mycelium in 48 hours. Should conditions in nature be optimum, it seems likely that a sponge could actually be destroyed in a short time, because there are unlimited numbers of spores constantly being liberated which may infect the eggs. How-

ever, from these investigations it is evident that in Chesapeake Bay the virulence of the parasite is retarded. The infection, despite its constantly increasing number of spores, does not progress from egg to egg along the strand farther than 3 mm. during the two-week incubation period of the crab eggs.

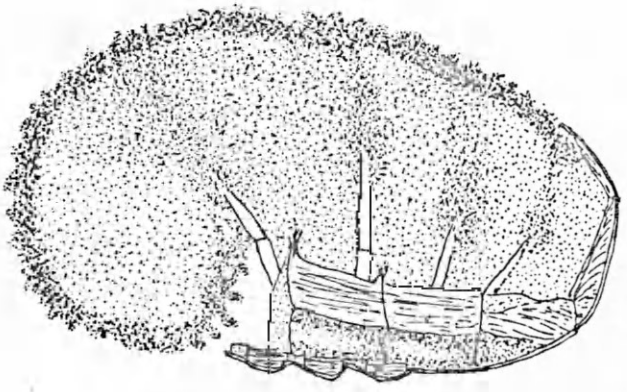
While the fungus moves down a strand, the outermost eggs are being destroyed and the inner ones are progressibely becoming infected. As the distal egg membranes disintegrate, the tip of the filament would be expected to become bared. However, during this investigation no observations were made where the filament had been bared due to the falling away of diseased eggs. Yellow sponges with very heavy infection were examined as well as many black sponges in the same condition. Yet, in no case was as many as 50 per cent of the eggs destroyed. If it were possible for a newly-formed sponge to become infected and perhaps half the eggs killed, it is likely that at least some of the heavily infected black sponges examined would have been partially destroyed. This was not the case, however, and even sponges with heavy external infection were observed to have the discarded egg cases of a normal hatch within. On a basis of these observations, it seems doubtful that L. callinectes is an important detrimental factor. True, many eggs are killed by it, but even heavy peripheral infection does not retard development of embryos in the interior.

An average-sized sponge is estimated to have 2,000,000 eggs with about 10 eggs per millimeter of filament. The average length of all the filaments of a sponge is approximately 12 mm. In very heavy degrees of infection, if the eggs in the distal 3 mm. of all the filaments were infected, estimates based on these figures would allow about a 25 per cent infection of the total number of eggs in the sponge. Then converted to

FIGURE I Egg Masses of Callinectes sapidus Rathbun Showing a Normal and a Diseased Sponge. (Longitudinal section).



Normal Sponge Without Infected Eggs



Diseased Sponge With Peripheral Infection

whole numbers there would be at least 1,500,000 eggs which do not become infected and which as has been shown will develop and hatch normally. It is concluded therefore that this fungus disease has not been responsible for recent fluctuations in crab populations (Figure I).

On the other hand, if various seasonal phenomena should affect the waters of Chesapeake Bay and create more optimum conditions for the fungus than have prevailed in the summers of 1941 to 1944, L. callinectes may possibly become a parasite of major importance.

In sizable samples it would be expected that the per cent of fungus occurrence would progressively increase from the yellow to the black sponges. Since the brown and black egg masses are older, fungus spores have had more opportunity to gain a foothold. Table I gives the results from observations of the Lynnhaven-Hampton Road samples. The fact that brown and black sponges are decidedly higher than yellow ones in the number of diseased sponges is accredited to their longer exposure. The factors which influenced the decline of infection in July cannot be determined. There are many conditions which could have been responsible, but it seems more probable that a brief period of adverse weather might have been the chief cause.

The length of time that a sponge remains in the water increases proportionately the probabilities of infection. Therefore yellow sponges are expected to contain the least amount of infection and black sponges the heaviest. Table II gives the results of these observations. Yellow sponges as shown here may become diseased very soon, since they display a small percentage of heavy and very heavy degrees of infection. The increase of infection in brown sponges is proportional to the greater amount of time these eggs have been exposed to spores of the parasite. The group of black sponges, however, shows a decrease in heavy infection.

TABLE I Percentage of Yellow, Brown, and Black Sponges Infected by Lagenidium callinectes Couch during June, July, and August 1944. Hampton Roads-Lynnhaven Area.

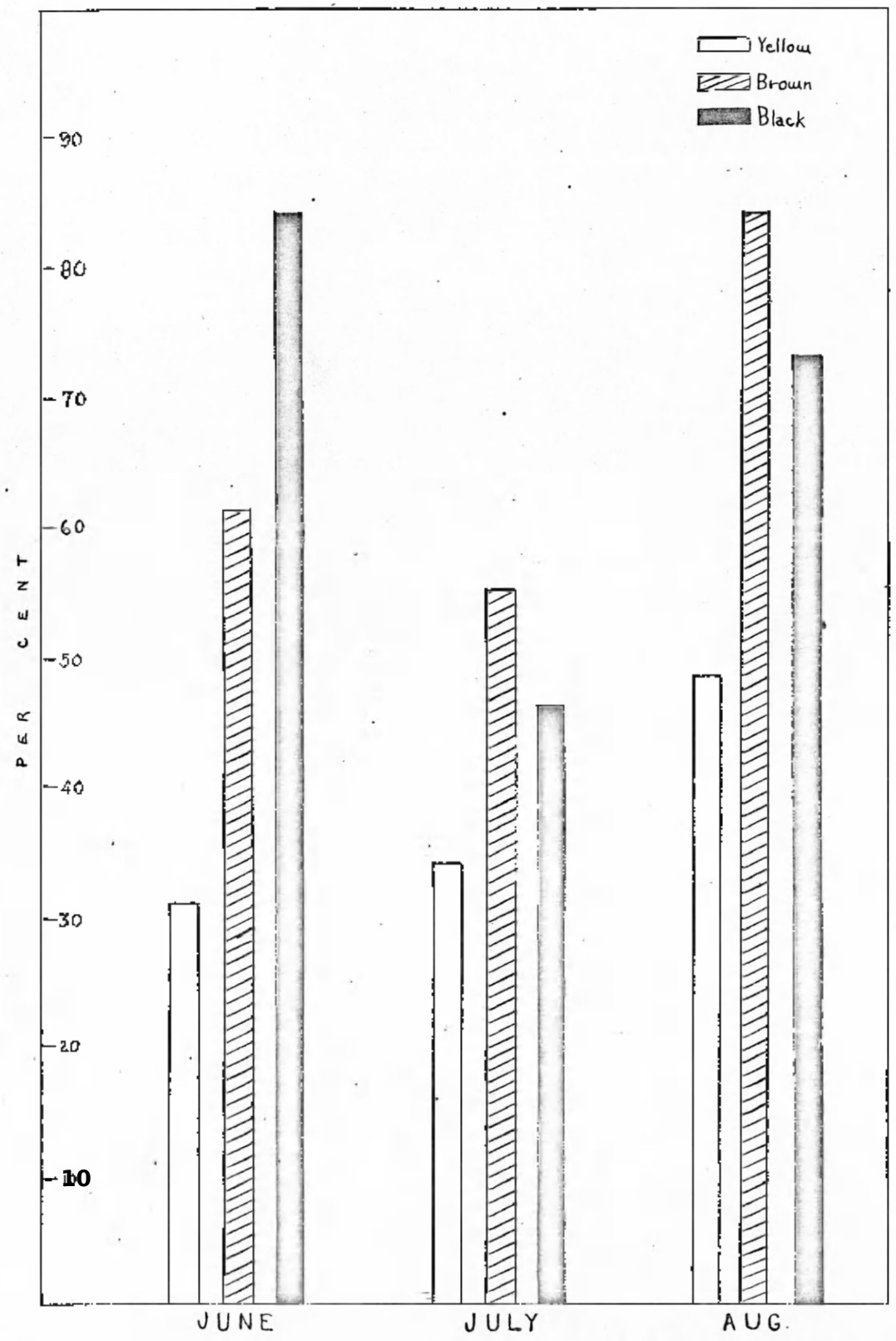
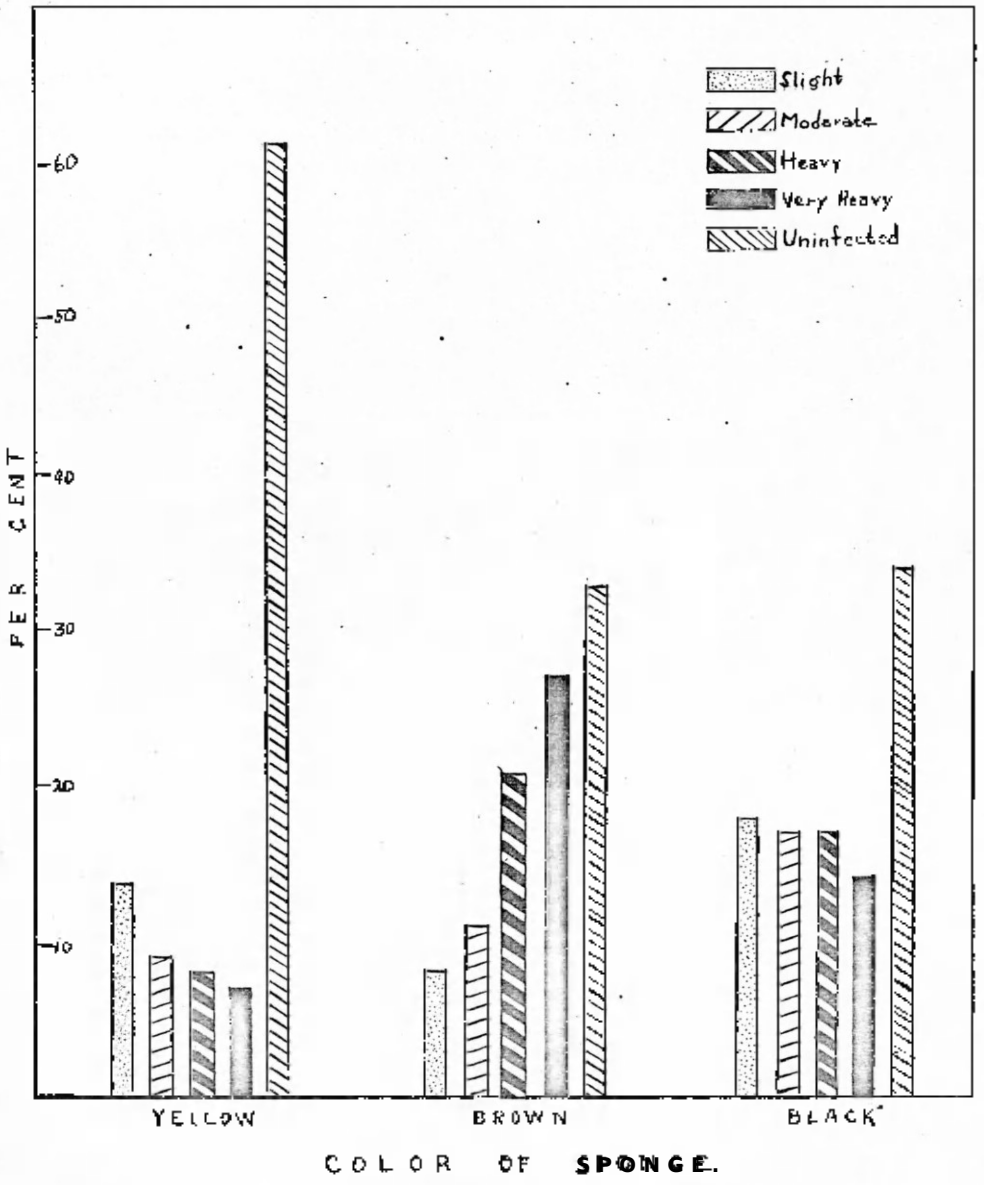


TABLE II Percentage of Yellow, Brown, and Black Sponges Infected by Lagenidium callinectes Couch during June, July, and August 1944, Hampton Roads-Lynnhaven Area.

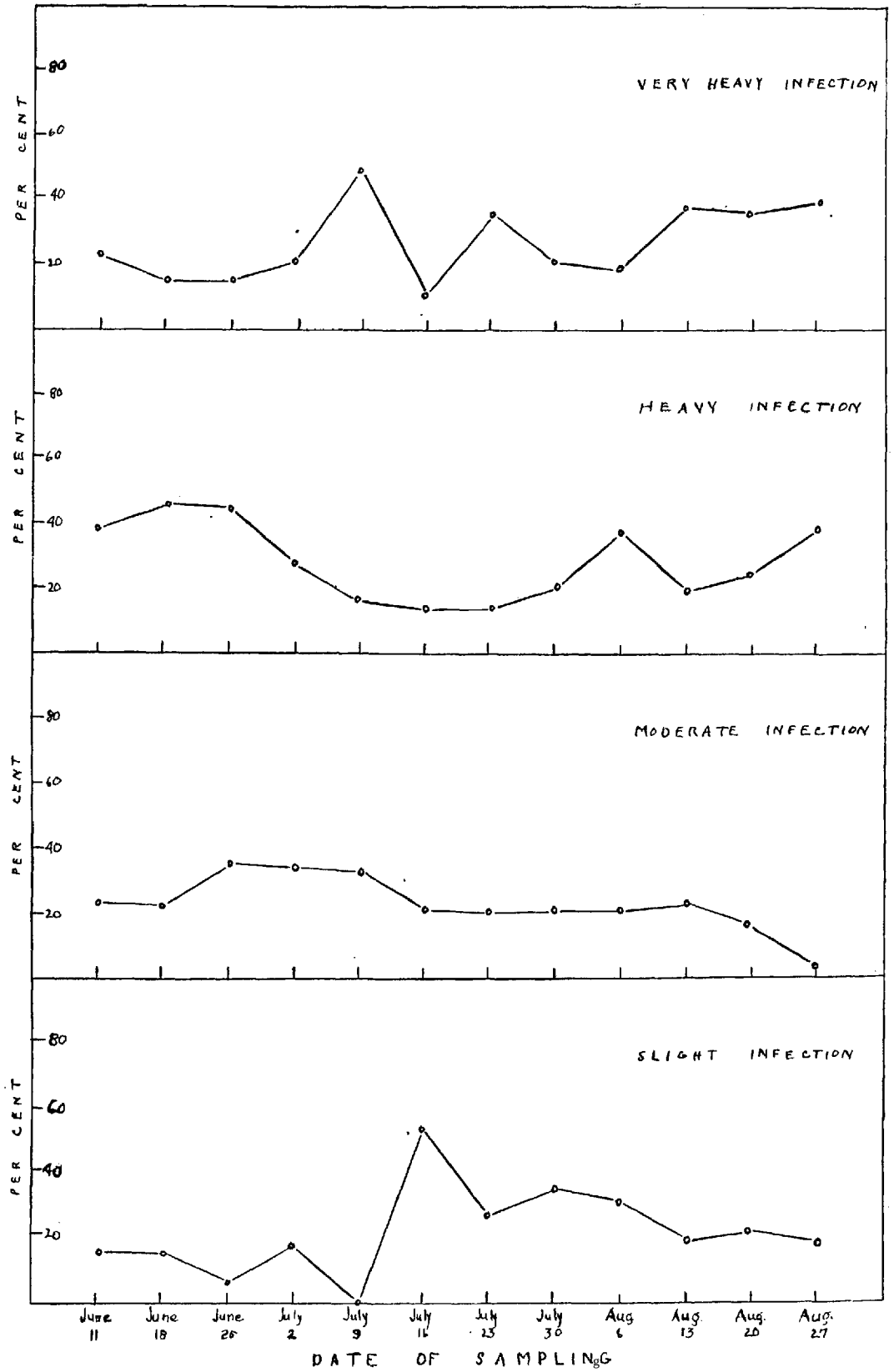


From the data, it has been impossible to arrive at a definite explanation regarding this phenomenon. L. callinectes occurs in the same number of sponges in both groups, but the browns are heavily infected whereas, approximately half of the specimens in the oldest group are only slightly or moderately infected. It is evident that the spores of the parasite do not display a preference for eggs in any particular stage of embryonic development. Consequently, black and brown sponges are equally vulnerable. There has been no indication in the older embryos of physiological factors which combated the spores successfully. Since the percentage of fungus occurrence is equal in the two groups and since there is an appreciable amount of heavily infected sponges, the present assumption is that the heavier infection still occurs as frequently among the black eggs as among the brown. Due to random sampling, it may have happened that fewer heavily infected sponges were studies, hence the results found in Table II.

Estimates of the four degrees of infection have shown that in every 100 infected sponges each degree occurred on the average of 25 times. This indicates a continuous cycle for the parasite. From what is known of L. callinectes, its progress in spreading from one sponge to another would be expected to keep the spawning crab population infected to various degrees. If the spores were greatly affected by changes in natural conditions as could be caused by weather this balance would presumably be altered. Table III gives results of these comparative studies. In all the groups there is fluctuation but the mean for each is approximately 25 per cent (Table III).

These studies have shown that L. callinectes is a superficial parasite; its damage in the heaviest cases being much less than 50 per cent of the eggs of a single sponge. The instances of very heavy infection among the infected black sponges is only 14 per cent, which gives evidence that a

TABLE III Percentage of Four Different Degrees of Infection by Lagenidium callinectes Couch Occurring in Blue Crab Sponges during the Summer 1944. Hampton Roads-Lynnhaven Area.



time factor must be important in holding this disease in check. If more than two weeks in natural summer spawning conditions is required for incubation of crab eggs, it is quite likely that L. callinectes could become a menace. However, in Chesapeake Bay where the incubation period is brief, the fungus does not spread rapidly enough to destroy large numbers of blue crab eggs. Observations have shown that this infection of external eggs does not affect the normal development of other eggs which have escaped infection. Their growth continues and hatching occurs at the normal time. In laboratory hatching experiments, uninfected egg-bearing filaments yield about 90 per cent hatch (Sandoz and Rogers, 1944). Numerous empty egg cases observed on sponges removed from spawning grounds also indicate a high hatching percentage of uninfected egg masses in their natural environment. When a sponge becomes infected, however, diseased eggs do not hatch, but among the uninfected eggs on the same sponge the hatching percentage seems to remain high.

In the laboratory, when infected eggs were present on the filaments, zoea which hatched normally often became infected with fungus. It is believed that this infection could not have occurred before hatching because the mycelium (48 hours under laboratory conditions)* may fill an entire egg in a short time. In all probability the embryonic development would have been disturbed within a few hours at most after penetration of the spore. The zoeae possess a very thin exoskeleton quite similar in appearance and thickness to the egg membrane which spores attack easily enough. It seems more probable that zoeal infection occurs following a normal hatch. None of the larvae taken from plankton have ever been observed with fungus infection. Infected zoeae have been seen in laboratory hatching pans, where the larvae must swim about in spore infested water. Under natural conditions larvae hatch from the sponge of the mother crab as she rests on the bottom in warm shallow water; immediately the young begin to move toward the sur-

face due to positive phototropism. In this way they swim away from the old sponge where infection may have contaminated the surrounding water with many motile spores. Moreover, when the fungus invades the zoeae, the larvae soon weaken and become unable to swim which results in their death. Providing that such infection does occur in nature, this would explain the absence of diseased individuals from plankton.

Factors Affecting the Fungus. From laboratory experiments this fungus gives evidence of a wide range of salinity tolerance. In all salinities, from 5 to 30 o/oo, the hyphal growth and spore formation proceeded rapidly. In pond water (salinity 0 o/oo) in a two-day period there was some development of external hyphae and a few small abnormal sporangia. During a two-day period in salinities 15, 20, 25, and 30 o/oo there was such heavy growth that the eggs appeared to be enveloped in a fine white down. New eggs also became infected. In salinity 20 o/oo where the parasitic growth was extermely heavy, a typically infected crab egg was observed with seven sporangia, four exit tubes, and four hyphae, all visible from one perspective.

L. callinectes can withstand sudden changes in salinities. The sponge crab used in this experiment was taken from Lynnhaven where the salinity is about 27 o/oo. She was carried in a moist basket to the laboratory; eggs were cut from the sponge and placed in York River water (salinity 20 o/oo) for about an hour. When the salinity series was set up, the sponge filaments were transferred directly to pond water and salinities of 5, 10, 15, 20, 25, and 30 o/oo. In no case except pond water was there apparent detriment to the fungus growth. Development in a salinity as low as 5 indicates that it may be possible for L. callinectes to become gradually conditioned to thrive in slightly brackish water.

Low temperatures were observed to retard fungus development somewhat. This was first noticed in hatching experiments in 1942. When diseased eggs were placed in the refrigerator (15 to 16 degrees C.) the fungus development and spore formation were delayed. This temperature, however, did not prevent sperulation and the spores continued to swim about, but their movement was sluggish.

It is not known just how L. callinectes overwinters. Lower Chesapeake Bay is the only place where it has been found as yet. There is frequent freezing in this region during the winter season. In as much as the reappearance of this fungus has been observed every season since 1941, it must maintain a resistance to freezing temperatures. Whether it seeks a secondary host for the winter is not known.

There is also a possibility that the fungus may be brought up by the Gulf Stream each season since L. callinectes appears on crabs which come into Chesapeake Bay from the ocean and on crabs which are taken off Virginia Beach, just outside the entrance to the Bay. These ocean-going crabs are identified by the presence of heavy commensal growth; bryozoan, sponge, barnacle, and algal growth on the carapace; and gill infestation: stalked barnacle (Octolasmus mulleri Coker) and a Nemertine (Carcinonemertes sp.). Should the fungus come into the Bay with these crabs, an annual occurrence of the infection, as widespread as these investigations prove, would necessitate a tremendous influx of infected females into Chesapeake Bay. In such a case L. callinectes must originate in the warmer waters along the southern coasts of this country. But, because it is so widespread over the spawning grounds of the Bay, the theory of oceanic origin seems less likely than perhaps a secondary host or a specialized overwintering stage in Chesapeake Bay.

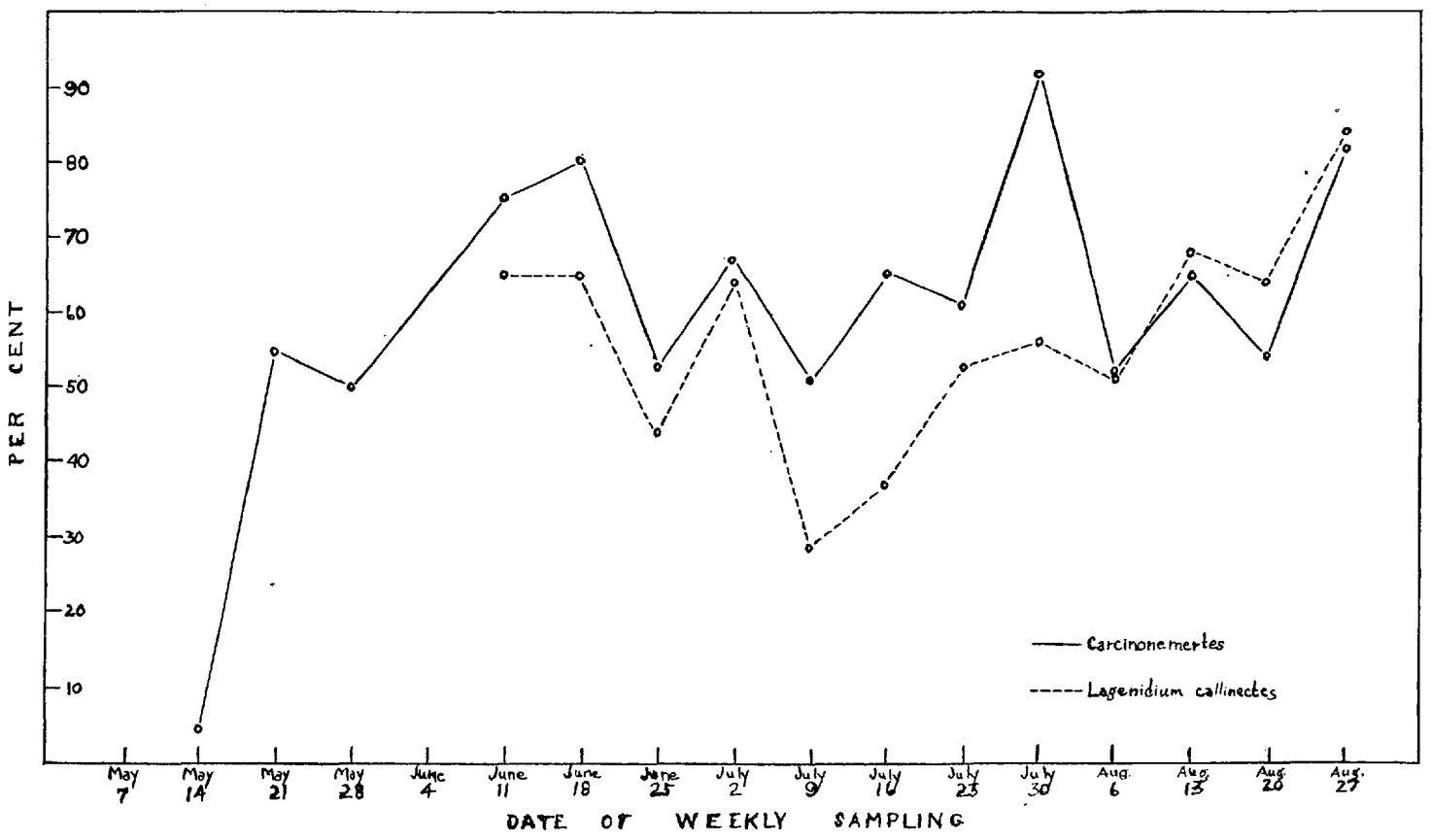
Other Organisms on the Crab Sponge. In addition to fungus other organisms, either parasitic or commensal, are frequently found living in the sponge. These organisms, though quite common, seem to do very little damage to the eggs. A vorticelloid form and an Ephalota sp. are often attached to the eggs of the peripheral portion of the sponge.

When fungus was first observed on the eggs of Callinectes sapidus, a hair-like growth longer than the diameter of a crab egg was noticed. Some eggs showed a profuse growth of such filaments, and at first were confused with the parasitic fungus. However, this filamentous growth later was recognized as a chlamydobacterium, but it has not been identified as yet.

In 1944, while conducting crab studies at this laboratory, Dr. Sewell H. Hopkins found a parasitic nemertine, Carcinonemertis sp., to be very abundant on the gills of the blue crab. This worm was likewise observed embedded in the sponge where it deposited its own eggs in a case entwined around the filaments. In these observations it was noticed that the nemertine and the fungus frequently occurred together. (Table IV). The growth factors for Carcinonemertis and L. callinectes appear to be similar, since the results show a corresponding periodic fluctuation of the two. The worm feeds on blue crab eggs and may be responsible for more damage than the fungus. It embeds itself in the interior of the sponge to feed and deposit its eggs.

Occurrence of Lagenidium callinectes in Other Species. Other species of crabs taken from the littoral zone and waters where the blue crab is common have not been observed to bear infected eggs. This is probably due to the fact that the specimens examined came from the Seaford area where fungus does not occur frequently and from the York River where it has never never

TABLE IV Percentage of Blue Crab Sponges Infested by Lagenidium callinectes Couch and a Carcinonemertes sp. during the Summer of 1944. Hampton Roads-Lynnhaven Area.



been observed.

Laboratory experiments were carried out in an effort to infect eggs of other crabs. Within 2 to 5 days, the fungus was transmitted to eggs of Pinnotheres ostreum Say and Neopanope texiana Smith. Though not contracting the disease during these investigations, it is still conceivable that eggs of Libinia emarginata Leach and Sesarma cinereum Bosc. may also become infected. Previous experiments, particularly with Libinia, have never been successful because the embryos died before hatching thus severely contaminating the water. Even though S. cinereum eggs remained alive in the laboratory for more than a week, the attempt to transmit L. callinectes was unsuccessful. Normal larvae of S. cinereum have been hatched out in the laboratory. Since it has been demonstrated that this fungus species is not an obligate parasite on eggs of Callinectes sapidus, examinations of sponges should be made on other crab forms of the Lynnhaven region.

Distribution of Lagenidium callinectes in Chesapeake Bay. The discovery of this disease among spawning blue crabs of Chesapeake Bay aroused a wide interest among fishermen and conservationists. The Commission of Fisheries of Virginia maintains a sanctuary in lower Chesapeake Bay where optimum conditions exist for development of blue crab eggs. Examinations of several samples of crabs from the sanctuary in 1942 indicated that a high percentage of infection occurred there. These results raised a question as to the value of protecting sponge crabs in this area. Furthermore, they pointed to a need for locating the waters where infection exists in order to determine whether the fungus is a general or localized parasite.

Extensive samples collected during 1941-44 have indicated that L. callinectes is quite common in waters extending from Hampton Roads to Cape Henry. However, the disease is not confined to these open areas. Samples from neighboring places also revealed the existence of the fungus in inlets of the region. Samples from several miles within the Lynnhaven River showed a high percentage of fungus occurrence. In August 1943, a sample from this river showed a 58 per cent infection; in July 1944, another sample showed a 57 per cent infection. In 1942, infected sponges were found from Pagan's Creek, a tributary of the James River. However in August 1944, a sample from Ballard's Marsh at the James River Bridge was not infected. In July 1941, the fungus was observed in a sample from Buckroe Beach, which represents the northerly limit of heavily infested waters.

In 1944, sponge crabs first appeared in commercial catches the second week in May. The fungus was not present until a month later; the first record being taken from a sample collected on June 11 in which 13 out of 20 sponges were infected. There seems to have been a simultaneous appearance of the fungus in both the Hampton Roads and Cape Henry areas. This would indicate that the organism is well distributed throughout the region, spends a quiescent winter and becomes active as soon as favorable conditions return. Egg-bearing crabs disappeared soon after August 31, 1944, until which time the fungus was present in more than 50 per cent of the specimens with a small increase during the last of August.

Samples from other regions of Chesapeake Bay have been examined. Throughout the Seaford area, infection is uncommon. Several samples taken during June and August showed a 2 to 3 per cent infection. In one sample taken off Egg Island Bar at the mouth of Back River, infection

occurred in 45 per cent of the sponges. For this region the figure is high, however, Egg Island Bar is located in waters adjacent to Hampton Roads and the sanctuary where there is infection. In the York River at Yorktown, no infection has been observed. One or two infected sponges have been taken from Mobjack Bay, Poquoson River, and from the mouths of the York River and Back Creek. The degree of infection in most cases was slight. This region, however, is not populated by great numbers of sponge crabs whose eggs have reached the hatching stage. The majority of spawning crabs are migrating toward the lower bay where hatching takes place, therefore more brown and black sponge crabs are found in the Lynnhaven waters. It is concluded that the general migration to the Capes of spawning females is responsible for retaining the infection in this one locality. When a female has completed spawning, the fungus probably ceases to live on that individual because hatching has depleted the food supply of the parasite. When the young crabs begin their northward migration, it is believed that the parasite remains behind since there is no evidence of an immature crab harboring the organism. The adult females are believed to die soon after spawning so it is extremely doubtful that spreading up the Bay from the Lynnhaven area could occur by migrations of infected females. The evidence at hand seems to show that the fungus is localized in waters where female blue crabs hatch their eggs.

Economic Significance of Lagenidium callinectes. From this discussion, Lagenidium callinectes has been shown to be a peripheral parasite of the egg mass of the blue crab. The data obtained show that this parasite is present in a large percentage of sponges (Table V). However,

TABLE V Percentage of Sponges from Lower Chesapeake Bay that were Infected by Lagenidium callinectes Couch during the Period 1942-1944.

Location	Year	Number of Sponges Examined	Distribution of Infection			Percentage of Infection
			Yellow	Brown	Black	
Lower Bay	1942	82	1	13	19	40
Lynnhaven Roads	1943	30	3	9	4	53
Lynnhaven Roads	1944	393	78	104	60	62
Lynnhaven River	1943	12	6	1	0	58
Lynnhaven River	1944	37	13	8	0	57
Hampton Roads	1943	15	1	8	4	87
Hampton Roads	1944	136	20	19	16	40
Ballards Marsh (James River Bridge)	1944	11	0	0	0	-
Seaford	1943	76	0	0	1	1
Seaford	1944	254	6	4	4	5.5
York River (at Yorktown)	1944	63	0	0	0	-
Rappahannock River	1943	6	1	13	19	40

while it is present and spreads rapidly among the peripheral eggs, penetration into the sponge is slow. Meanwhile, the healthy eggs of the interior, which in all cases represent at least 3/4 of the mass, continue their development and hatch normally.

This parasite, now evidently established within the spawning area, may possess the potential ability to destroy a large proportion of blue crab eggs. However, in the light of facts given here, prevailing natural conditions seem to hold the fungus in check at least during the years of this study. It is known that the parasite has a fairly wide temperature and salinity tolerance, but the incubation period for the crab lasts only about two weeks which appears to be too brief a time for the fungus to work deeply into the center of an egg mass.

This study has suggested the importance of obtaining more information concerning the environmental conditions that affect development of this unusual marine fungus.

SUMMARY AND CONCLUSIONS

1. Lagenidium callinectes Couch is a peripheral parasite of the egg masses of blue crabs of Chesapeake Bay.

2. The infection is heavier in brown and black sponges than in younger ones, due to the longer exposure of older sponges in spore infested water.

3. The fungus spreads rapidly over the periphery of an egg mass, but very slowly toward the interior.

4. The compactness of eggs in the interior of the egg mass seems to serve as a filter for the water moving through the sponge. This appears to prevent the infection of eggs in the interior.

5. Peripheral infection does not seem to retard the development of the eggs in the interior of the sponge.

6. Lower Chesapeake Bay is the localized area where L. cellinectes is known to exist.

7. Present evidence suggests that the fungus overwinters in lower Chesapeake Bay.

8. Eggs of other crabs have been artificially infected with L. callinectes Couch. However, infection of eggs of other crabs has not been observed in nature.

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