Studies of a Fungus Parasite that Infects Blue Crab Eggs

by Curtis L. Newcombe*
CRANBROOK INSTITUTE OF SCIENCE BLOOMFIELD HILLS, MICHIGAN

and M. Rosalie Rogers**
UNIVERSITY OF WISCONSIN MADISON, WISCONSIN

• A microscopic fungus growth was observed on the surface of the blue crab *Callinectes sapidus* Rathbun in 1941 (Sandoz, Rogers, and Newcombe, 1944). The identity of this organism was established in 1942 by Professor John N. Couch of the University of North Carolina, who very kindly examined material sent to him from our laboratory (Couch, 1942). The unusual character of this growth early attracted attention. But it was not until evidence of its injury to the crab eggs was obtained by Sandoz and Rogers (1944) that a concerted effort was made to learn about its life history, its occurrence on and in the individual eggs as well as on and in the egg mass of the crab, and also about its occurrence in crabs at different parts of their natural range in Chesapeake Bay.

The question of the possible relation between the existence of the fungus parasite and the unusually low level of the crab catches in Chesapeake Bay during the period 1940-43 became a problem of more than academic interest alone. Hence, an integrated program of research embodying the aforementioned problem was started in 1942 at the Virginia Fisheries Laboratory in Yorktown.

This paper embodies a general review of the investigations on the occurrence and commercial importance of the parasite conducted under the direction of the senior author. A detailed treatment of the results will be presented elsewhere.

To appreciate the possible relation between crab catches and the egg parasite, one must recall that practically all larval crabs originating in Chesapeake Bay are produced near its mouth. While immature, they migrate

* Formerly Biologist and Director of the Virginia Fisheries Laboratory, College of William and Mary.
** Formerly Research Assistant, Virginia Fisheries Laboratory.

up the rivers and the bay. Then mating takes place and the females return to the lower bay to spawn (Churchill, 1917; Newcombe, 1945). Thus, the egg-bearing population exists in a relatively restricted aggregate which condition might be expected to favor the spread of an egg infection (fig. 1).

The identity and nature of the infection was at first in doubt. Hatching experiments by Sandoz and Rogers (1944) showed that eggs with considerable infection either failed to hatch or hatched abnormally (fig. 5). This led to the hypothesis that the organism, assumed to be a parasitic fungus, occured inside as well as on the outside surface of the egg, and subsequent treatment with lactophenol revealed the fungal branches or hyphae within the egg and established the parasite as an internal fungus (fig. 2). Careful study of infected eggs by Professor J. N. Couch resulted in his establishing the identity and most of the life history stages of this parasite (Couch, 1942). Being an undescribed species Prof. Couch gave this fungus the name *Lagenidium callinectes* and stated that it appears to be, "the only member of the Lagenidiales so far described from a marine habitat." In 1929, Atkins, working in England, reported finding a related fungus within the eggs of the pea-crab, *Pinnotheres*, taken from sea mussels and pointed out that, "this fungus . . . attacks a marine invertebrate and, as far as I have been able to ascertain, it is the first member of the Saprolegniaceae which has been so described." The order Saprolegniales includes the water moulds that are commonly found on dead insects and attacking life fish, these producing a diseased condition. In 1939, Professor F. K. Sparrow described a new species of *Lagenidium* parasitic on eggs of fresh water rotifers (Sparrow, 1939). That *Lagenidium callinectes* Couch occurs in a marine habitat and within the crustacean egg is of particular interest. To facilitate further study, Couch attempted to cultivate the fungus on agar. His results were negative but he suggests that the fungus might grow on a nutrient agar made with salt water. This remains a fertile field for study.

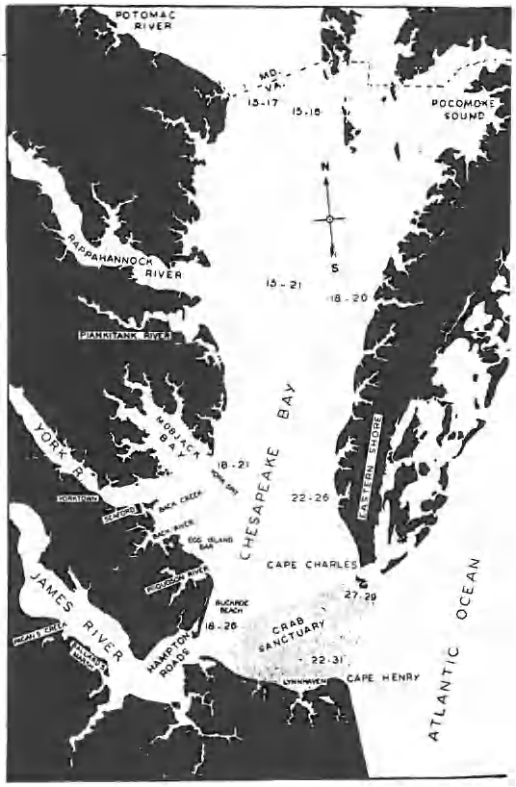

Fig. 1. Showing the Virginia waters of Chesapeake Bay and its tributaries and also the crab sanctuary established to protect egg-bearing crabs *(modified from Sandoz and Rogers, 1944)*. Latitude 36° 45'—38° 02'; longitude 75° 42'—76° 42'.

Certain characteristics of the morphology and development of this fungus as described by Couch (1942) are readily seen and possess general interest. The mycelium which may fill the egg consists of branched irregular hyphae which are sparingly septate and thin walled. In the process of spore formation and discharge, the end of a hyphal thread comes in contact with the inner egg wall and swells. According to Couch (1944) the part in contact with the wall forms a narrow tube that grows through the wall, immediately thickens, and elongates to form a large tube. After a period of growth of thallus the tip of the tube gelatinizes and protoplasm from a segment flows out from the

Fig. 2. Photomicrograph of blue crab egg showing internal hyphae of the fungus parasite (photography by Winfield and Goldstein).

mycelium to collect in a spherical undifferentiated mass surrounded by a delicate gelatinous envelope or "vesicle". Here, by progressive cleavage the spores are formed (fig. 4). Professor Couch states that when they are mature and the vesicle breaks they swim away sluggishly. As we shall point out later, the type of movement and behavior of the spores may partly explain the character of the distribution of the fungus in the egg mass or "sponge" of the crab. Infected eggs can readily be recognized by their smaller size as shown in the externally infected egg occupying a central position in figure 3. Some work remains to be done to provide a complete knowledge of the life history of this parasite.

Regarding the appearance of the fungus *on and in the individual egg,* it may be confined almost entirely to the interior or show up noticeably on the outside, depending on the stage of development. The mycelium within the yolk may be abundant or sparse. One or several exit tubes may be seen in a single egg and likewise more than one sporangium is not uncommon since each segment of mycelium may become a sporangium (fig. 4). In addition to *Lagenidium callinectes,* filamentous bacteria, suctorian protoza and other fungi are frequently observed attached to the egg surface. Fine, unbranched filaments which according to Professor Couch are classified in the Chlamydobacteriaceae, occur commonly.

We have studied the occurrence of the fungus *on and in the egg mass* with a view to estimating the degree of mortality of eggs at different places and at different seasons. Previously, numerous experiments were conducted to discover a method of rearing crab larvae from the egg and to provide an understanding of the effect of various environmental factors on the hatching of crab eggs (Lochhead, Lochhead and Newcombe, 1942; Lochhead and Newcombe, 1942; Sandoz and Rogers, 1944). Abnormal factors sometimes produced an abnormal larva, often called a prezoea, instead of the normal zoea as the first larval stage is called (fig. 5). Therefore, it was not surprising to find that the majority of eggs infected with the parasitic fungus either failed to hatch or gave rise to prezoeae, none of which was ever observed to develop into a normal first stage zoea.

In the beginning recognition of an infected crab "sponge" (egg mass) was dependent upon microscopic examination. Later, however, conspicuous surface patches of characteristic color were observed, thus providing a ready index of heavy infection. Rogers (1945) showed that the infection was almost always superficial occupying a layer about three millimeters in thickness over the periphery of the sponge and rarely penetrating much deeper into the egg mass (fig. 6). This surface condition obtains both in heavy and light infections. In a few sponges infected eggs were found within the sponge mass but not in significant numbers. Seemingly, spread of infection over the surface is rapid, whereas, the rate of penetration toward the interior of the sponge is extremely slow.

What may constitute the barrier against penetration of spores deeper so as to infect

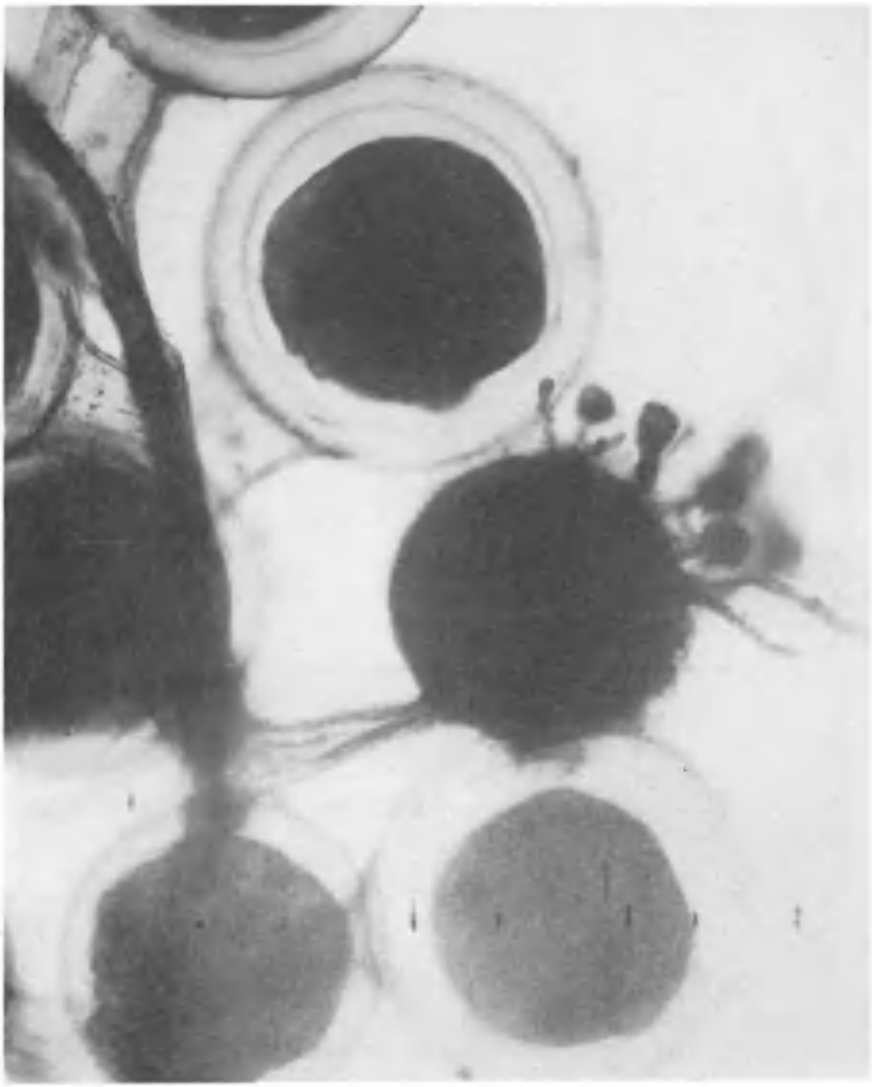

Fig. 3. Photomicrograph showing external hyphae and sporangia of a heavily infected crab egg with a normal sized egg (dia. 0.25 mm) on each side (*photography by Winfield and Goldstein*).

the inner eggs has puzzled us. The stages of development of eggs throughout the infected sponge are quite similar, indicating uniformly favorable conditions for the survival and growth of the eggs. The light factor, of course, differs between the peripheral layer and the inside. Professor Couch refers to the sluggish movement of the zoospores. Perhaps a better knowledge of their behavior might suggest an explanation for the parasite being restricted to the surface layer. Since the eggs are attached during only about two weeks, the period is limited for infection by and spread of the parasite.

Sponge crabs, the females bearing an egg mass, may be collected in summer at considerable distances from the mouth of the Bay, such as the Rappahannock River and some distance above Pagan Creek in the James River (fig. 1). The sponges are usually orange or bright yellow in color indi-

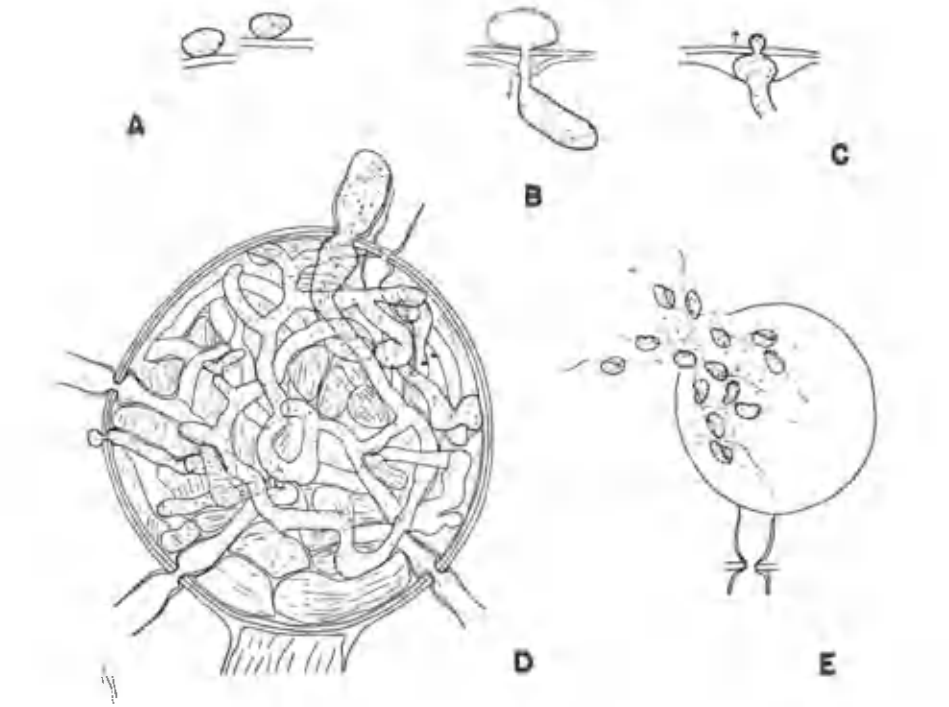

Fig. 4. Diagram of stages in development of the parasite showing, *a*, spores encysted on egg membrane; *b*, spore cyst empty, germ tube elongating; *c*, early stage in formation of exit tube; *d*, a crab egg heavily infected with the branching hyphae showing one sporangium at the top about ready to discharge its contents and four empty exit tubes; and *e*, discharge of spores from ripe sporangium (after Couch, 1942).

cating that they are freshly laid and that the crab is migrating down the Bay or river to waters of higher salinity for the purpose of hatching. Our records indicate that practically no infection occurs near the up-bay and up-river limits of sponge crab distribution, namely, the Rappahannock River and Mobjack Bay areas. We have observed it to occur occasionally near the mouth of the York River and more often at Buckroe Beach. However, in the Cape Charles—Cape Henry—Willoughby Spit—Old Point area, roughly designated in figure 1 by the crab sanctuary, a large proportion of the egg bearing crabs, ranging as high as eighty percent per sample and averaging an estimated sixty percent, was infected during a large part of the period of observation (Rogers, 1945). One August collection of seventy-seven sponge crabs from the area of the sanctuary off Lynnhaven contained fourteen sponges sufficiently infected with patches of fungus growth to make it visible to the naked eye. Expectedly those showing least infection were the orange and bright yellow or youngest eggs.

By establishing the following designations for degree of infection—slight, moderate, heavy, very heavy and uninfected—it was hoped that definite seasonal and locality characteristics of infection with respect to eggs in different developmental stages might be revealed. In general, however, the data are inconclusive and do not permit of detailed analytical treatment. Certain points are of interest. There is no evidence to indicate that the spores of the parasite display a preference for eggs in any particular stage of development. If such a preference does exist, its effect as indicated by percentage

infection of the eggs of different ages is concealed seemingly by the pronounced effect on degree of infection produced by variation in the time that the eggs are exposed to the spores. At present there is no way of determining how long crabs bearing eggs of a certain age have been in the infected area. Interpretation of the data becomes difficult since information is lacking on the following points: 1, time that the crab must be in the infected area to become parasitized; 2, time that the observed crabs have been in the exposed area; and 3, stage of development that the crabs were in when they reached the infected waters. With Mrs. Sandoz numerous field and laboratory observations were made with respect to transmittance and infection of the different developmental stages. While the infection was found to spread rapidly in laboratory cultures, little is known of the degree of spread of the infection to uninfected eggs under natural conditions. Since the percentage of infected yellow sponges which vary from about 1 to 5 days in age may be as high as 40, it is clear that a period of one or two days is quite significant as far as time required for infection in nature is concerned. Throughout the open season, sponge bearing crabs migrate into the sanctuary from less saline waters. Some have an orange or light yellow colored sponge on arrival, whereas some are far advanced in development and about ready to hatch, hence, they may not be in the infected area long enough to get parasitized. It appears that the heaviest infection may be expected on those sponge crabs that have been exposed to the fungus the longest time regardless of the developmental stages of the eggs. Probably, therefore, infection is more often in the brown and black sponges because of the longer time that they are likely to be exposed rather than because they are less resistant to the parasite.

No definite annual variation in degree of infection has been demonstrated due, perhaps, to an insufficient number of observations. An effort was made to recognize possible seasonal variations in the character and degree of infection. However, such

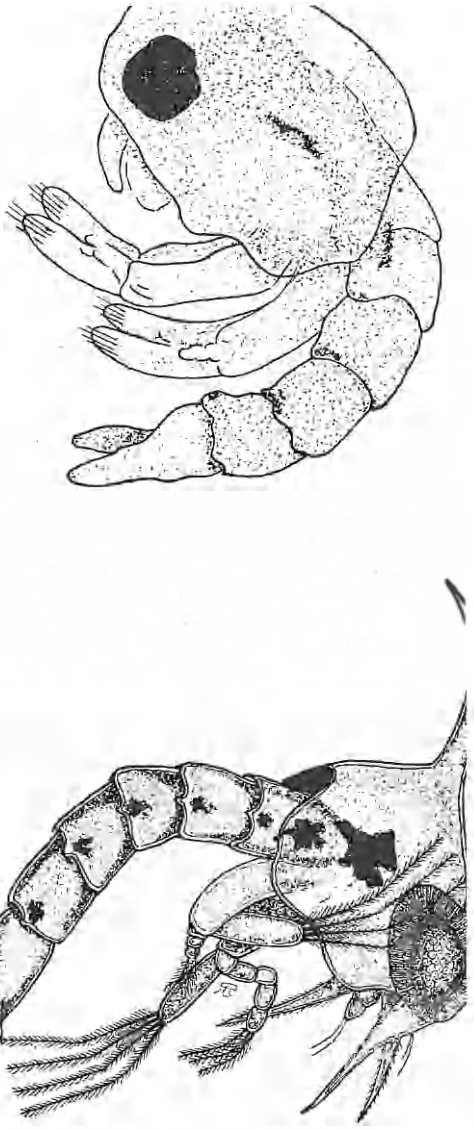

Fig. 5. Abnormal or prezoeal blue crab larva (above) and zoeal larva (about 1 mm in length) (drawn by Robertson).

attempts were not conclusive due in large part to the difficulty of employing sound statistical methods of sampling the crab populations. The results of certain preliminary quantitative estimates are given in the masters degree thesis by Rogers (1945).

We have observed heavily infected sponge crabs from just outside of the mouth of the

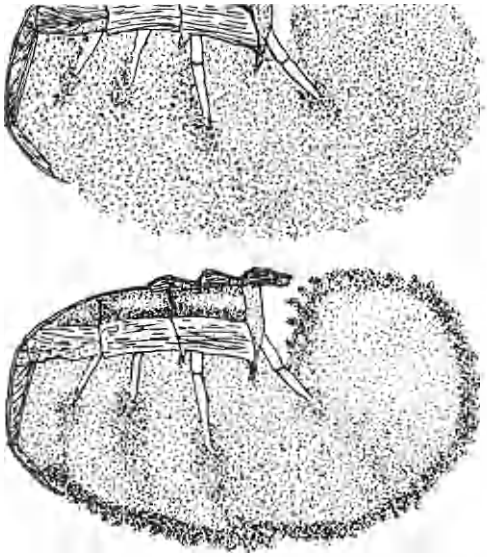

Fig. 6. Uninfected and infected (below) egg mass or "sponge" of the blue crab. The dark peripheral zone indicates area of infection *(drawn by Rogers).*

Bay, south of Cape Charles. But, apart from these data, there appear to be no records showing the absence or extent of presence of this parasite outside of Chesapeake Bay. The works of Smith (1938) and Galtsoff (1933) who investigated the effect of a fungus-like parasite on the commercial sponge industry of the Bahamas are of particular interest.

Localization of the infection to the peripheral zone of the sponge minimizes the likelihood of *Lagenidium callinectes* Couch being responsible for significant declines in the abundance of crabs. Random samples, however, should be taken each season so as to recognize any possible annual fluctuation in the degree of fungus infection that, thus far, may not have occurred.

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