A PROTOZOAL PARASITE (*CHLOROMYXUM THYRSITES*, SP. N.) OF THE CAPE SEA-FISH, THE "SNOEK" (*THYRSITES ATUN*, Euphr.).

By J. D. F. Gilchrist.

The Cape "Snoek" *Thyrsites atun*, a large pike-like fish, of great economic importance in South Africa, is frequently found to be affected in a peculiar way. The flesh becomes soft, and in some cases even assumes a liquid form. The fish in this condition are known to Cape fishermen as "Pap Snoek," and, as they constitute about 5 per cent. of the total catch of Snoek in the chief season for this fish (May–June), their occurrence is a serious loss to the fishing industry. The fishermen believe the condition is brought about by the fish not being properly killed when caught by the inexperienced and unskilled, the softening of the flesh being the result of the fish being bruised in struggling about in the bottom of the boat. Microscopical examination of the affected tissue, however, showed that the condition is more probably due to protozoal parasites resembling *Chloromyxum*. These had clearly four polar capsules, as in this genus, though certain facts in its life-history seem to indicate that it should be separated from this genus.

It is not improbable that the parasite is identical with a protozoal parasite found by Dr. J. Burton Cleland (Journal and Proc. Roy. Soc., N.S. Wales, vol. xliv, 1910) in the Australian "Barracoota," which is believed to be the same species of fish as the Cape "Snoek." The parasite brings about the same destruction of the tissue of the host, the fish so affected being known as the "Milky Barracoota." The spores fixed by drying were examined by Dr. Cleland, and from their general appearance were believed to represent a species of *Chloromyxum*.

In the year 1917 I drew up a short note on the subject, and exhibited some specimens of the parasite and affected tissue at a meeting of this Society. The complete life-history has not yet been ascertained, but, as suitable material and opportunity are not readily obtained for pursuing the investigation, the following results are recorded :

Various methods were employed, chiefly sublimate-acetic and others such as sublimate-alcohol-acetic, sublimate-formalin-acetic, 10 per cent. formalin, and osmic acid. Simple drying on the slide also afforded some useful information, and was a very convenient diagnostic method, along with methylene blue staining. In addition to smears of the tissue, sections of varying thickness down to 4 μ were made, and mostly stained with hæmatoxylin and eosin; staining intra-vitam with methylene blue was also found instructive with regard to some details.

The Spore.—The usual appearance of the spore in fixed material is very characteristic (fig. 1, *a*). The four unequal polar capsules were readily seen with methylene blue staining, and, in hæmatoxylin preparations, four nuclei associated with them were conspicuous; near the apices of the polar capsules was a greater or less amount of protoplasm (presumably the sporoplasm) (*sp*, in fig. 1, *c*). Surrounding the whole there usually appeared a

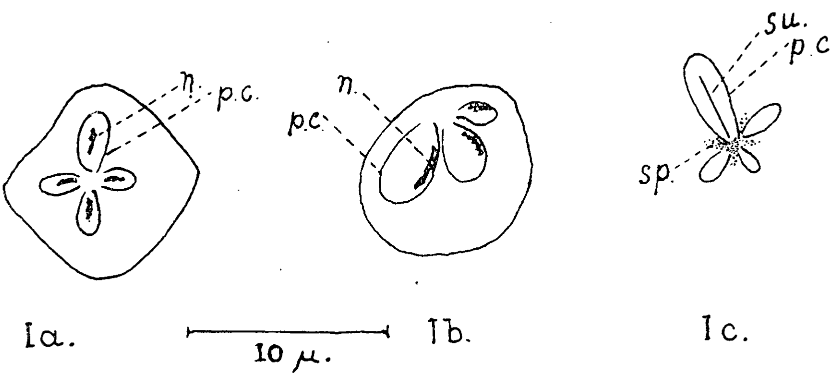

FIG. 1*a*–1*c*.—Spores of *Chloromyxum* preserved and stained.

quadrangular space, which might represent the outline of the spore. Though usually quadrangular it was often rounded or lenticular, and as frequently absent altogether (fig. 1). It was not till these bodies were examined in a living condition that the existence of a definite wall was determined, and its shape made out. They can best be seen in a living spore, which happens to be rolling about in the fluid in which it is examined. It was then seen that the spore was dome-shaped, with a broad, flattened base which is drawn out into four angular projections (fig. 2). These projections have clearly defined edges; their angles are sharp and acute, and do not stain with any of the reagents employed. They appear to be thin but solid in their distal half, an appearance which may, however, be due to the walls coming into contact with each other at these points.

The shape of the spore is not constant though usually of the stellate outline described. One of the rays opposite the larger polar capsule is often slightly longer than the other, and that opposite the smaller capsule is sometimes a mere blunt projection (fig. 2, *b*). The spore has therefore a bilateral symmetry, less marked, however, than the radial symmetry.

Exceptionally all the rays are blunt. Cases have been observed in which the whole spore wall assumes a vesicular form, and this may be an earlier or later stage of development.

That the spore wall is thin and somewhat membraneous seems to be indicated by cases in which the spore has been attacked by bacteria. After

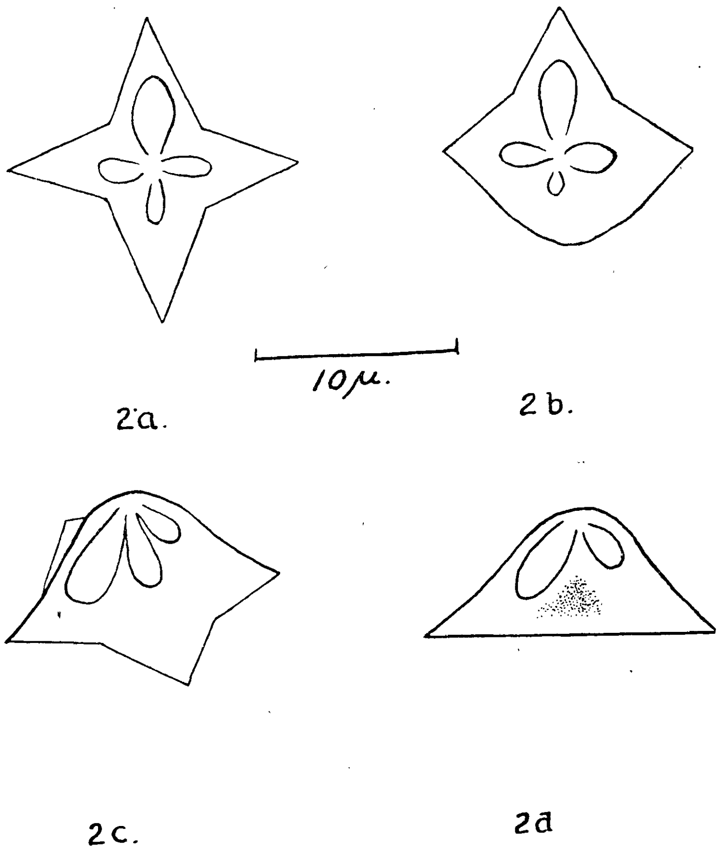

FIG. 2*a*–2*d*.—Spores of *Chloromyxum thyrsites* in the living state.

three or four days these usually appear, and, after attacking the muscular debris, attack the spores, removing the whole of the inside and leaving a very thin outer covering. In some respects this spore wall or sporocyst differs from what is found in Myxosporidia. In the first place, there is no indication of a suture dividing it into two shell-like halves, as in many other forms; and, secondly, it is not resistant to strong acids, as are the shells of other Myxoporidian spores. Further, it is readily ruptured when well shaken in water.

The size of the spore varies, but is usually about 12 μ from the tips of opposite projections. The height is about 8 μ.

Polar Capsules.—These are the most conspicuous features of the spore, both in the fresh and preserved condition. They readily stain with methylene blue in both cases. They are separate bodies, appearing, when alive, of a bluish colour under the microscope by transmitted light, and bronze yellow by reflected light. They are four in number and ovoid in shape, with a pointed end where they approach each other at the apex of the spore, as in the genus *Chloromyxum*. They are invariably of markedly unequal size, the largest being usually about 3 μ in length and 2 μ in breadth, and occupying the greater part of the quadrant of the spore in which it occurs. Opposite is the smallest, about half its size, and on each side is one of intermediate size. The walls of these capsules are clear, glistening, and smooth, without differentiation; but in some cases a clear narrow line was seen to proceed from the anterior end of the capsules, being lost towards the posterior and rounded end, suggesting the existence of a suture (fig. 1, *c*, *su*). In some methylene blue preparations a clear elongate space could be seen, evidently the opening for the discharge of the filament. The wall of the capsule is not opaque, as in most Myxosporidian spores (Gurley), the filaments being visible through them when methylene blue is used as a stain. Strong ammonia water and glycerine render them slightly more transparent, but not in a marked degree. Iodine water renders them much more transparent, and the filaments, in some so treated, could be made out. Several cases were seen in preparations, in which the polar capsules were isolated from each other and with no traces of the other parts of the spore. Some of these had their filaments extruded, and, in one or two cases, single polar capsules were found completely isolated and with extruded filaments (fig. 3).

Filaments.—As stated above, the filament, coiled up in the polar capsule, can be seen in living spores stained in methylene blue. The coils are numerous and occupy the greater part of the interior of the capsule. This was usually seen in the largest capsule, less often in the smaller ones, in which the staining showed a granular appearance of the contents, perhaps indicating that the capsules mature at different periods.

The extrusion of the filaments is very uncertain. On several occasions all the spores (several hundreds) placed in water on a slide extruded their filaments with a sudden explosion on the addition of weak acid, but this did not often happen, though the same experiment was repeated several times on other spores. Any kind of acid seems to be equally efficient in securing this result. They were sometimes seen extruded in preparations fixed by heating on the slide. Strong ammonia or hot water were not found to cause extrusion of filaments, which, however, seems to be more a matter of maturity than of the exciting agent employed. The extruded filaments often stain well with methylene blue. All filaments of the four capsules are sometimes extruded, at other times three, two, or only one (fig. 3).

The completely extruded filament is very long, being almost five times the length of the capsule, which in one case was 3 μ, the filament being 15 μ; in another the capsule was also 3 μ, but its filament 20 μ measured in a bent condition, so that it was about eight times the length of the capsule.

The function performed by the filaments in the life-history of these Myxosporidia is not known from any actual observation, so that the fact

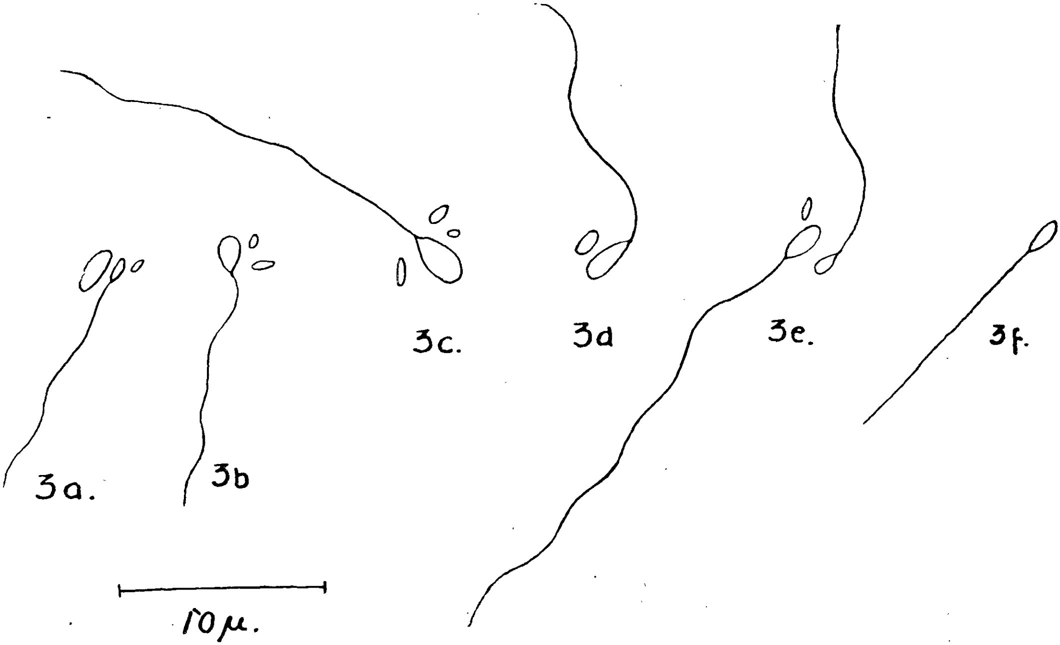

FIG. 3*a*–3*f*.—Polar capsules with extruded filaments.

noted, that the discharging filaments enable the spore to clear itself of the surrounding material in which it is embedded and float freely in the water, may be of some value. A clearly defined nucleus was always associated with each of the polar capsules. In certain lateral views, as well as by focussing downwards in other views, it was seen that these nuclei were very elongate, a characteristic of the nuclei of polar capsules when in an advanced stage of development.

Sporoplasm.—The remaining space of the cavity of the spore is occupied by a protoplasmic mass which represents the sporoplasm. It is very difficult to make out in the whole spore, as it lies between and below the polar capsules. It could, however, be seen in a lateral view of the living spore, stained in methylene blue (fig. 2, *d*), and was very distinct in cases where the spore had apparently been ruptured and the polar capsules separated from each other (fig. 1, *c*). In such cases it was often clearly marked off from the polar capsules, as it stained a bright blue and the polar capsules green in some methylene blue preparations. This sporoplasm had a vacuolated appearance, though there did not appear to be any iodine-staining vacuoles.

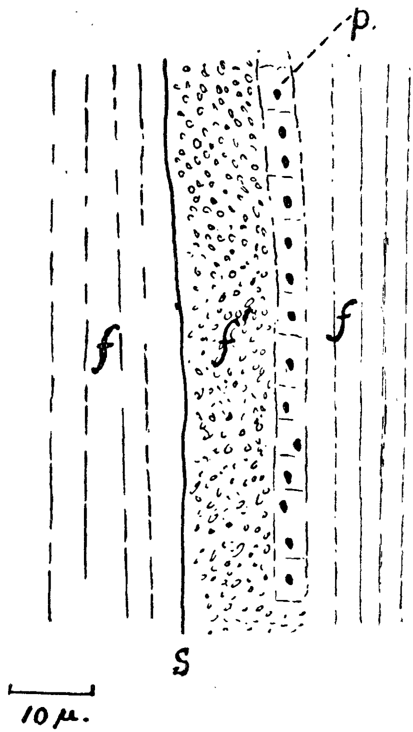

FIG. 4.—Longitudinal section of infected muscle, showing trophozite stage of parasite, *p*; Muscle fibre, *f*; Disintegrated muscle fibre, *f′*; Sarcolemma, *s*.

No trace of any nuclei were found in it by a nuclear stain in such cases, though in the unruptured spore, when treated with a nuclear stain, a number of bodies much smaller than the elongate nucleus were seen. In connection with this it may be noted that in the fresh spore, more especially if treated with a solution of potassium iodide, small dark bodies were seen in the spore outside the polar capsules. They were most clearly seen in the angular projections of the spore, and might be excretory particles.

Trophic Stages.—In some smears of the diseased muscle fixed in various ways, small nucleated bodies were to be seen along with the spores, and these appeared to be the trophozoite stages of the parasite. They each had a single well-defined nucleus and a homogeneous protoplasmic body with well-defined outlines, usually rounded. These bodies were best shown, however, in longitudinal sections of the infected muscle (fig. 4), and here their mode of multiplication and the effect which they produce on the muscular tissue of their host were clearly demonstrated. They may occur singly, but are usually in long chains of cells between the muscle fibre and the sarcolemma. These chains are readily recognised in longitudinal sections, and usually consist of about ten to twenty cells. In a single section 4 μ in thickness, as many as 50 were counted in a chain. The outline of each cell is usually well defined, except in cells apparently in the process of division. Groups of small cells also occur in the disintegrated muscle surrounded by connective-tissue (fig. 5). In this case also they were distinct from each other, and no large multinucleate bodies were found, such as occur in the corresponding stages of some other Myxosporidians. These

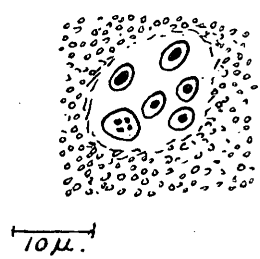

FIG. 5.—Trophozoites in groups.

small mononucleate bodies or meronts were irregular or amoeboid in outline when separate or in groups, and measured about 3 to 4 μ in diameter. When in chains they were rectangular, and measured about 5 μ in length and 4 μ in breadth. The protoplasmic body appeared quite homogeneous, and the nucleus rounded, with no marked chromatin differentiation that could be seen, or with slight indications of network. The nuclei were, however, often elongate when the meronts occurred in chains, and attained a length of 2 μ. In some a clear vesicular projection was seen, the significance of which was not apparent.

Propagative Stages or Spore Formation.—In most of the Myxosporidia the Myxosporidium or trophozoite (binucleate in its early free stages) increases greatly in size. The nuclei divide repeatedly without a division of the protoplasm, to form multinucleate apocytes which may bud. The protoplasm then becomes aggregated in spherical masses round each nucleus to form a pansporoblast or archispore, from which, by subdivision of the nucleus, several sporoblasts arise, each giving rise to a spore. In this parasite the process seems to be much simplified. Each individual is mononucleate, and that from each a single spore is formed seems to be indicated by the following observations, which, however, require further investigation with fuller material. In some of the meronts the nucleus shows indications of a differentiation in its chromatin elements, which become broken up into small parts of unequal size. Another stage observed, apparently a subsequent one, is that in which the nucleus is divided up into still smaller and more numerous parts, scattered throughout the cell. In one case seven such subdivisions were counted with certainty, and in another case eight, though there may have been more. The stage subsequent to this, and closely approaching the spore stage already described, is one in which four distinct and larger nuclei are to be seen placed at about equal distance from each other (fig 6, *a*, *b*). No aggregation of the protoplasm round these was observed in some, but in others this appeared to be the case (fig. 6, *c*), so that the whole cell appeared to be divided into four parts. This appearance, however, might quite well have been due to other causes,

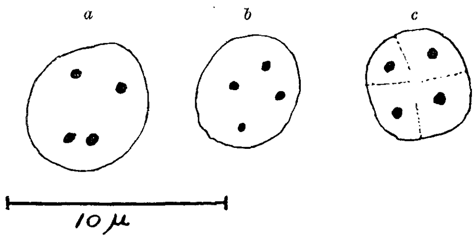

FIG. 6*a*–6c.—Later stages of Trophozite.

such as the presence of four vacuoles, destined to be the polar capsules. That these appear early may be indicated by a cell in which at least two nuclei were seen, and two large vacuoles, a solitary case, however, out of many hundreds examined, so that much importance cannot be attached to it.

The next stage observed was that of the fully formed spore, the structure of which has already been described. The early vegetative stages and the spores were found in great abundance, the intermediate stages above mentioned being seldom encountered. From this it may be inferred that the formation of spores is a very rapid process, and this is borne out by the great increase observed in the number of spores after the death of the host.

The peculiarity in the development, as compared with other species of Chloromyxum, is that the vegetative form is mononucleate and apparently becomes a sporoblast from which a single spore is formed. This, though unusual, is not unknown in other species, for in *C. cristatum*, according to Leger, each individual produces only one spore, the individuals in this case, however, being multinucleate.

The further development of the spores is unknown. They have been found in fish which have been eighteen days in salt. In fish which have been salted and sun-dried they are still recognisable, though most of them have become disintegrated. In such cases it was observed that the polar capsules had become separated from each other. That they are still alive is uncertain, though polar filaments were extruded on irritation, for it is recorded that spores which have been preserved in alcohol have extruded their filaments.

Only the above-mentioned stages (trophozite to spore formation) are as yet known. Whether infection of the fish is through the alimentary tract, blood or lymph system, is not known. That it is not through the injured epidermis, as in many other cases, is fairly obvious. If the parasite has a second host, it seems probable that this may be some large sea animal, such as the seal or the shark, which devour this fish. An observation, however, on the advanced stage of the spores suggests another and more probable second host. It was noted that after three or four days the infected flesh was invaded by the bacteria of putrefaction. In sea water the tissue was in this way gradually broken up, and the spores, still alive, were released and became free in the water. They may thus infect other fishes or even marine invertebrates. Should this second host be the food of the Snoek, the parasite would readily be conveyed again to the first host. The most probable secondary host fulfilling these conditions is the sardine or pilchard (*Sardina sagax*), on which the Snoek feeds almost exclusively, and it would be well worth while to examine the gills, alimentary tract, etc., for traces of the spores, as these can be so readily recognised by methylene blue staining. The peculiar form *Coccomyxa* occurs in the gall bladder of the European sardine, which is closely related to the Cape sardine.

Pathological Effects.—The disease is most prevalent in the "fat snoek," which appears usually in May and June. In the "poor snoek" season, when the fish are in poor condition, and often infested with characteristic parasitic worms in the flesh and elsewhere, the "pap" condition is not so often seen.

The disease causes great damage to the fishing industry, as the pap-snoek, if badly infected, which they frequently are, cannot be salted and dried. If only slightly infected, they can be cured by salting and drying; and indeed it would appear from microscopic examination of apparently healthy fish that the parasite occurs in most of the snoek caught, as on one occasion out of twelve fish taken at random, and considered perfectly healthy, spores of the parasite were found in eight.

The parasite does not form harmless cysts, nor is it an intracellular one, which, by "diffuse infiltration," destroys quickly the tissue it penetrates. It attacks the muscle fibres from the outside as a rule, and in one instance only was it found penetrating the muscle fibre. This is probably the reason why so many fish, considered to be perfectly normal, were found on microscopic examination to be infected, sometimes heavily, without seriously imparing the functional activity of the muscles, and may also explain the statement of some fishermen that any snoek, if kept for over twenty-four

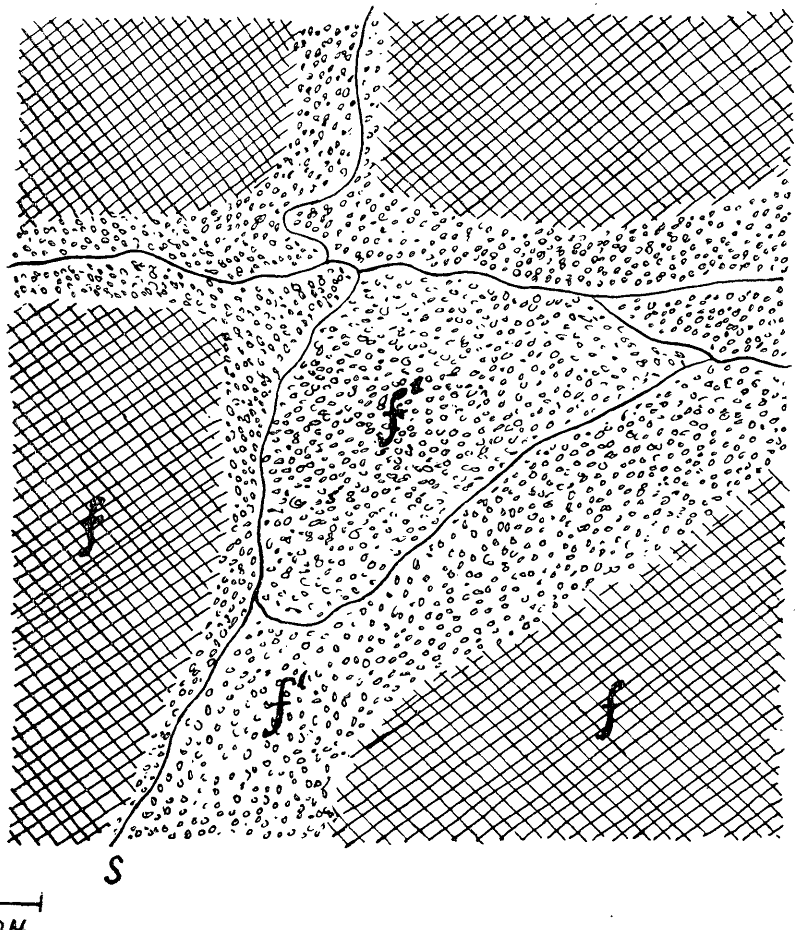

FIG. 7.—Transverse section of infected muscle. Muscle fibre, *f*; Disintegrated fibre, *f'*; Sarcolemma, *s*.

hours unpickled, will become "pap," probably owing to the very rapid formation of spores after the death of the fish. Most species of *Chloromyxum* are found in the cavities of body, such as the gall bladder, cœlom, and urinary bladder, but some are found in the muscular system, as, for instance, *C. quadratum*, in *Sygnathus acus*, *Clupea harengus*, *Abramis brama*, *Trachurus*

trachurus, where, however, they do not appear to do extensive damage. This species, however, attacks the muscle tissue in such a way that it must apparently destroy many fish in the sea. In section the destroyed muscle appears as granular or hyaline material between the muscles (fig. 7). When the badly infected fish is cut open, the muscles are somewhat soft and have a characteristic glazed or glistening appearance. Then they quickly assume a fluid condition, like thick milk. One such specimen, kept in the laboratory for two days, developed this condition, and fishermen state that on some occasions of the fish when picked up from the bottom of the boat, where they had lain a few hours after capture, nothing remained but skin and bones, the flesh flowing away in a milky stream. No bacteria are at first found in this fluid substance, and it is quite free from any odour of decay. A day or two later, however, bacteria of putrefaction attack both the diseased tissue and the parasites.

The Snoek normally appears in immense numbers at the Cape, but some years ago the fishing industry suffered a great calamity in the practical disappearance of the fish. This was put down to a variety of causes, such as change of currents, and netting operations, especially trawling. It was suggested also that the catching of immature fish might be the cause, and a law was passed imposing a size limit. It seems not improbable that the scarcity of the fish was due to this protozoal disease.

Inquiries have been made by means of circulars distributed to fishermen and others regarding the "pap snoek." Amongst other questions asked was whether or not the eating of "pap snoek" had any ill effect on the consumer? Most of those who replied to this query stated that no such effects had been observed. Others, again, said that it produced a kind of sickness. I learned also that the country people who buy these "pap snoek," on account of their cheapness, suffer from some kind of eruption or boils on the skin. The medical authorities of the city of Cape Town condemn "pap snoek" as unfit for human food, but apparently for no definite reason except its unwholesome appearance. Some of the correspondents stated that the eating of the affected fish produced leprosy.

The investigation of the life-history of the parasite seems to be of considerable zoological interest as well as of economic importance, and it is hoped that it may be followed up.

I have to express my obligations to Sir Arnold Theiler, Dr. Annie Porter, and Dr. Cleland for assistance in references and literature in carrying out these observations.