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☐ NEW PROPOSAL
☐ CONTINUING PROJECT
☒ FINAL REPORT
☐ PROGRESS REPORT

F.I.R.C. 25

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL/PROJECT: Investigation to determine the cause of 'jelly disease' and 'cottonflesh' in barramundi

ORGANISATION:

PERSON(S) RESPONSIBLE: University of Queensland

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Investigation to determine the cause of 'jelly disease' and 'cottonflesh' in barramundi

Final Report (December, 1982)

This report is in two parts, an interpretative article suitable for inclusion in a 'popular' style journal, and a manuscript to be submitted for publication in a scientific journal.

The topic of the title formed the project with the author's highest priority in 1980/81, though in the same year, other parasites and diseases important to the industry were investigated, such as swimbladder proliferation in Tasmanian trevally. Since 1980/81, the barramundi project has continued, now subordinate to the work on QX Disease. Specimens are still being sought and a confirmatory experiment is underway at the moment. Thus, this 'Final Report' is not the end of our observations. However, the general picture is clear.

The popular article, if it is published, should perhaps be presented in a light-hearted tone so that it does not have a negative effect on consumer demand for barramundi. I imagine some heading such as, 'Backache in Barra' (!) with a copy of the best X-ray. I hope this would help to maintain interest in the fish rather than adversely affect sales. However, perhaps its publication, and precise form, could be at the discretion of the Committee.

Suggested text for popular article

Very occasionally barramundi are caught which, when filleted, are found to have a gelatinous mass over the backbone. The mass envelops the top and sides of the forward half of the vertebral column, and is clearly demarcated from the surrounding muscle by a layer of white fibrous tissue.

These barramundi have a spinal problem. The gelatinous mass is a long-term response to a damaged spine.

In normal barramundi, the spine consists of a series of individual bones, the vertebrae, each separated from the next by a fluid-filled sac, the intervertebral pad. This pad is equivalent to our intervertebral disc. Occasionally, for some reason the barramundi pad breaks down and starts being replaced by bone. When this happens, the rough surfaces of the new bone pinch tissues between them each time the fish flexes its body, and thus cause intermittent tissue damage, inflammation and no doubt considerable pain. Localised haemorrhages occur, and bony outgrowths develop around the vertebrae to try to stabilise the joint.

With further calcification, some vertebrae eventually become fused together (see 'F' in figure). This reduces the tissue damage but of course also reduces the flexibility of the spine. In two fish examined the loss of mobility of several vertebrae had caused the spine to become permanently curved, giving the fish a humped appearance.

In a third fish, a transverse fracture had developed across the bone fusing two vertebrae, thus allowing some flexibility, but this had merely restarted the cycle. The condition appears to be incurable.

In medical terms the disease is called 'ankylosing spondylosis', ankylosing meaning a stiffened or fused joint, and spondylosis a disease of the vertebrae.

What starts this calcification? A similar condition occurs in man and domestic animals. In some cases it is genetic, as in certain breeds of large dog where the intervertebral discs are prone to collapse. In other cases an inert foreign body is the cause. Frequently, however, a micro-organism causes the initial damage and once started the disease progresses on its own. This I think is the case here. A bacterium was recovered from one fish but was not found in two others. Cultures from intervertebral pads at an early stage of change are needed.

Fortunately, at the moment the disease is not of great economic significance. However, with the development of barramundi culture its importance will increase.

A different disease that we ought to find out much more about, that we could probably do something about, is so called 'cottonflesh', where the muscle of the fish becomes soft, spongy and opaque. Our observations indicate that it is caused either by acute starvation or by capture stress. Concomitant changes in the liver suggest the former. However, at the moment we do not know whether the fish are abnormal when they hit the net, or only after they have been struggling for 6 hours. In either case, something could be done, for example, by modifying the capture technique, leaving the fish to recover, or by fishing to decrease the population density.

Barramundi are a valuable and noble fish. Like most other animals, they have their health problems though none are of any danger to man. Consideration of them may help in the management of the fishery.

This project was carried out in collaboration with the Department of Fisheries, Northern Territory, with the help of many barramundi fishermen, particularly Ralph Carter, Jim Peady and Pedro Villegas. It was supported by a grant from the Fishing Industry Research Trust Account.

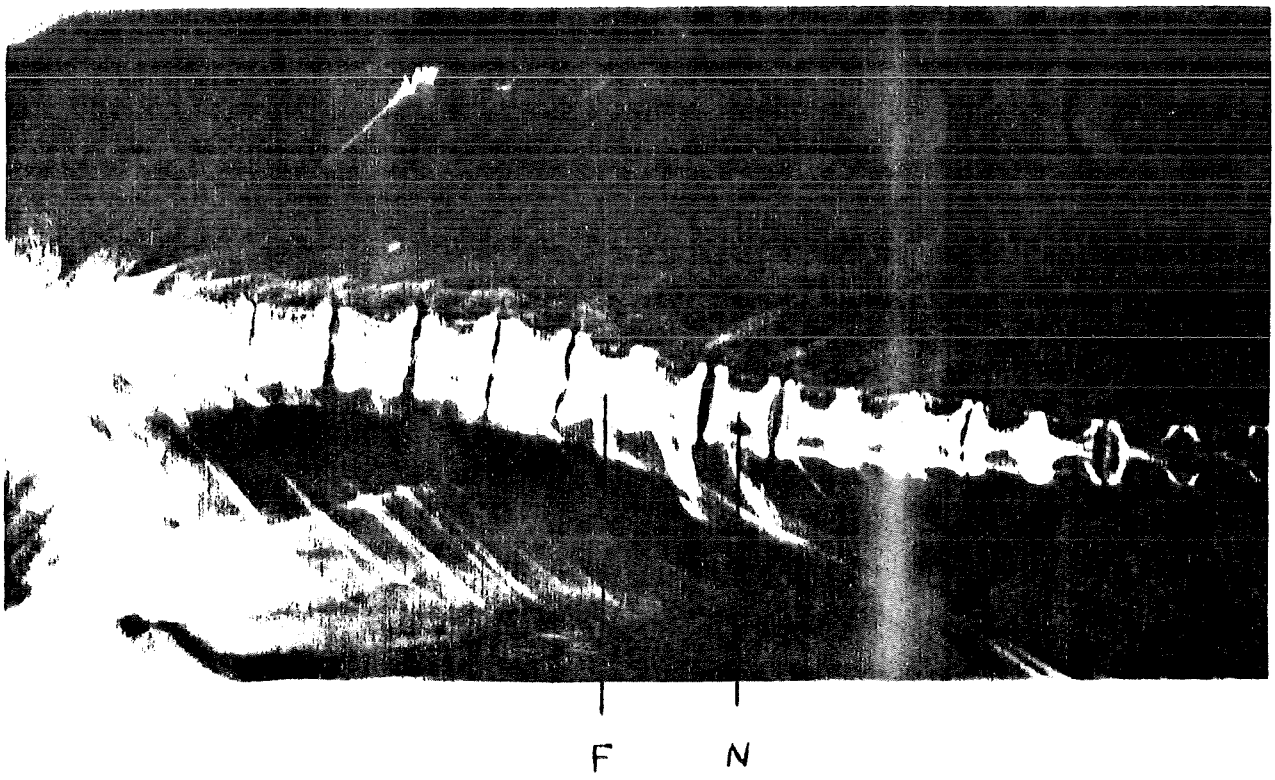

FIGURE CAPTION: Radiograph of a 57cm barramundi from the Daly River, showing fused (F) and normal (N) vertebrae.

ANKYLOSING SPONDYLOSIS IN THE GIANT PERCH

LATES CALCARIFER

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Abstract

A disease of the vertebral column is described in three adult wild-caught Lates calcarifer (Bloch) from the Northern Territory, Australia. It is characterized by the deposition of bone in the intervertebral space thus fusing the vertebrae, and the development of a thick gelatinous mass of loose connective tissue around the affected part of the vertebral column. Though a Clostridium was isolated from one fish, it was not thought to be the primary cause.

Introduction

The giant perch, Lates calcarifer is an important food fish in the tropical Indo-Pacific. In Australia, fish are taken commercially using large-mesh gill nets at the mouths of estuaries, usually in remote areas. Over the past decade there have been a number of reports from fishermen of a disease that sporadically affects fish in different areas. It has been referred to as 'jellyfish' or 'cottonflesh' and usually makes the fish unsuitable for marketing. We set out to investigate the disease and if possible to find its cause. Because of the remotness of the fishing areas, most observations were made on frozen tissue. We found that there were in fact two diseases, the one referred to in the title, and a second about which we have less information. Observations on both diseases are included below.

Materials and Methods

The three fish with spondylosis came from the mouth of the Daly River, Northern Territory. They were found by commercial fishermen when filleting their catch. Fish 1, (109cm) was caught in June, 1980 and examined immediately; Fish 2 (57cm) and Fish 3 (68cm) were caught in April, 1982 and supplied frozen. Bacteria from the first fish were grown anaerobically on TSA and Blood Agar plates. A dense suspension of spores was inoculated intraperitoneally into 2 rats, a guinea pig, and intramuscularly into 3 young barramundi.

Fish with the second disease were obtained from the Roper River, Northern Territory, and from Broad Sound, Queensland (lengths 70, 85, 89 and 63cm).

Results

The anterior half of the vertebral column in Fish 1 was enveloped on the dorsal and lateral sides by a grey gelatinous fusiform mass up to 150mm across (Fig. 1). Within the mass were 40 to 50 soft nodules 5 x 3 x 3mm which varied in colour from red to yellow, brown and black. These did not extend through the sheath of fibrous tissue 0.5mm thick which separated the mass from the musculature. A brown nodule was found in an intervebral space. There was kyphosis of the vertebral column and anterior vertebrae were hard, swollen and partly fused. Large numbers of larval trypanorhynchs were present in this fish, particularly Calliterarhynchus sp. 1 and 2, and Pterobothrium sp., in the body cavity, and Dasyrhynchus sp. in the musculature.

Fish 2 and 3 were X-rayed prior to dissection. In Fish 2 the normal trabecular pattern of many of the vertebrae was lost (compare Figs. 2 and 3). Vertebrae showed changes in shape, texture and spacing. Spondylosis had occurred in at least three places, i.e. between vertebrae 4 and 5, 8 and 9 and 14 and 15 (Fig. 2, arrows), presumably in an attempt to bridge an unstable gap. Vertebrae, particularly the end plates, had become more sclerotic as a result of bone proliferation, and there were irregular changes in the intervebral spaces, including complete loss of the space (Fig. 2). In Fish 3, the changes were less severe and consisted primarily of spondylosis and partial loss of intervertebral space between vertebrae 6 to 11. The spinal column was kyphotic like that of Fish 1.

When dissected, the gross appearance of Fish 2 was similar to Fish 1 in that there was a grey fusiform mass of gelatinous tissue enveloping the vertebral column. However, no nodules were present. Vertebral 9 to 16 were removed and split longitudinally. Vertebral interspaces 11-12 and 12-13 were apparently normal whereas space 10-11 was filled with dense bone and the two vertebrae were completely fused. Space 9-10 had similarly been ossified but a transverse fracture across the new bone had been allowed some mobility. The fracture callus at this site, and the bulky bony disc at 10-11, had resulted in enough swelling to produce a significant incursion into the vertebral canal at these points. There was also complete ankylosis of the 4 intervertebral joints behind 12-13.

In gross appearance, Fish 3 was similar to Fish 1 and 2 in having a gelatinous fusiform mass over the vertebrae. The mass did not contain any nodules.

Histologically, the gelatinous mass from Fish 1 consisted of loose connective tissue with varying numbers of inflammatory cells. Within it were large areas of caseous, eosinophilic, rather amorphous material containing cholesterol clefts. These areas were demarcated from surrounding inflammatory cell infiltrates by a thick layer of epithelioid macrophages. The caseous debris contained long filaments of relatively large slightly-beaded bacilli. They stained gram positive with Brown and Brenn stain, were PAS negative and non-acid fast. In addition, there were dense particles suggestive of endospores which were gram negative, PAS positive, and acid fast. No sections of the vertebrae themselves were made from this fish.

The histological detail in Fish 2 had been obscured by freezing, thawing and autolysis. However, sections of the vertebrae and adjacent tissue showed no evidence of acute inflammation. There were masses of woven bone with smooth surfaces suggesting maturity and cessation of active growth. The new bone had almost completely replaced the intervertebral pad as well as forming exostotic bosses on the external vertebral surfaces. There was no evidence of caseation. The histopathology in Fish 3 was similar.

Initially, no bacteria were isolated from Fish 1. Plates had been inoculated in the field using the gelatinous material as inoculation. However, on seeing the histological results, a second attempt was made in the laboratory using material from nodules which had in the meantime been stored frozen. These again failed to grow anything until the plates were cultured under anaerobic conditions. Then a Clostridium species multiplied rapidly and produced large numbers of spores of similar size, and shape and staining properties to those seen in the histological sections.

Dense suspensions of the spores inoculated intraperitoneally into rats and a guinea pig produced no ill effects in them. Spores inoculated into the dorsal muscle of young L. calcarifer were recovered 6 weeks and 5 months later. There was no sign that they had germinated or caused any inflammatory response.

In the second disease, 'cottonflesh', initially confused with the spondylosis described above, the vertebral column remained normal. The muscle, however, was soft and opaque. The one intact frozen specimen examined was 15% underweight. Histological examination of the muscles of 5 affected fish (4 of which had been frozen) showed no consistent abnormality, apart from a suggestion of oedema. No myxosporidians were present, and there was no sign of inflammation. The liver of the intact specimen showed focal hepatocellular fatty infiltration extending across several acini. Similar liver lesions were present in several apparently healthy fish caught at the same time. What appeared to be identical liver lesions

developed in a laboratory fish 46cm in length that had been starved for 3 months.

Discussion

The spondylosis was evidently a chronic condition. Whatever initially caused it may have disappeared months or years before the fish were examined. The Clostridium isolated may not have been the primary cause for two reasons. The nodules in which it was developing in Fish 1, thought to be sites of recurring haemorrhage, were absent from Fish 2 and 3; and no Clostridial spores were found in sections from Fish 2 or 3. In addition, no Clostridium has ever been associated with a similar type of disease in fish , though clostridia have been reported from the intestine and blood (Cann & Taylor 1982). It seems more likely that the Clostridium entered the bloodstream from the gut, perhaps at the time trypanorhynchs were migrating into the body cavity, was carried to areas of haemorrhage and found conditions inside the caseating clots suitable for growth and reproduction. The disposition of the organisms in the sections was not suggestive of post-mortem invasion.

Myxosporidia such as Myxosoma cerebralis have been shown to cause deformities of the vertebral column. However, no myxosporidian spores were observed in our sections. Of other factors known to produce spinal deformation, we can discount sublethal levels of pesticides as the specimens were from a remote area. Dietary deficiencies, particularly of vitamins C and E, and tryptophan, have also been related to spinal deformities in fish. Hovell (1975) f d catfish

fed a Vitamin C deficient diet developed enlarged spongy vertebrae, and many fish had haemorrhagic areas along the spinal column. However, it would be very surprising if a wild predator would suffer from a dietary deficiency, apart from straight starvation. In addition, the non-uniform changes in the column suggest foci of abnormality, consistent with the presence of a foreign agent. Fresh tissues and cultures from the invertebral spaces of fish showing the early signs of disease would be invaluable.

In the second disease ('cotton flesh'), no infectious agent seems to be, or have been present. The findings are consistent with fish starvation (Lovell 1980), but could also be associated with capture stress, as in the porcine stress syndrome of pigs (Patterson and Allen 1972). We do not know whether the fish were abnormal at the time they hit the net, or became so after they had been struggling for 6 hours, though there is slight anecdotal evidence for the former.

As the giant perch barramundi are becoming an increasingly valuable resource, it would be useful to find out more about these two diseases and what measures could be taken to reduce their effect on the industry.

Acknowledgements

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Figure captions

Fig. 1. Gross appearance of Fish 1 with fillet removed, showing the gelatinous mass enveloping the diseased vertebrae.

Fig. 2. Radiograph of Fish 2 showing spondylosis (arrows) and fused vertebrae, particularly number 10 (labelled) fused to 11.

Fig. 3. Radiograph of normal fish. All scale bars = 5cm.