FINAL REPORT

FIRDC PROJECT 87/30 "FUNGUS DISEASE IN THE BONY BREAM"

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1. SUMMARY

The lower Murray population of bony bream is subject to an annual epidemic of the oomycete Saprolegnia (principally S. parasitica) and the bacterium Aeromonas hydrophila. The epidemic is species-specific; it affects mainly adults whose susceptibility may be increased by stress due to winter cold. Mortality rates do not appear to be high. Lesions occur on the mid-flank and are characterized by an external mycelium, epidermal erosion, scale loss, hypodermal and muscular oedema, haemorrhage, myofibral degeneration and by the presence of Saprolegnia hyphae at all stages of infection. Although A. hydrophila is common in advanced lesions there is no significant systemic bacterial infection. This appears to be a primary mycotic dermatitis and is noteworthy because Saprolegnia is best-known as a secondary pathogen.

A superficially similar disease syndrome occurs in bony bream in the North-west Branch of Cooper Creek. This syndrome differs from that described above in that it occurs in many other fish species, the principal fungal pathogen is Achlya, not Saprolegnia, outbreaks occur throughout the year but principally in the warmer months, and the disease affects both juveniles and adults.

2. INTRODUCTION

The bony bream Nematalosa erebi is caught for rock lobster bait in the lower Murray lakes (Alexandrina and Albert) and the Coorong. In the last 20 years annual catches have increased from 190 to 1000 tonnes (Rohan 1987).

The bony bream is the most abundant large fish species in the lower Murray, and yet is the only species subject to regular, widespread epidemic disease. Fishermen in the region have noticed fungal infections in late winter or early spring since at least the 1940s (L. Gray, Meningie, S. Aust., pers. comm.). Accounts of fungal disease outbreaks in bony bream (amongst other species) also have a long history in central Australia and western Queensland (Johnston 1917; Johnston & Bancroft 1921).

In the following the principal pathogens are identified, the pathology and epizootiology of the infection are described, and implications for the River Murray and rock lobster fisheries are explored.

Oomycetes of the genus Saprolegnia include a number of facultative pathogens responsible for saprolegniasis in fish. Most outbreaks follow bacterial or viral infection (Egusa & Nishikawa 1965; Willoughby 1970; Bekesi, Kovaks-Gayer, Ratz & Turkovics 1984), injury to the epidermis (White 1975; Pickering & Willoughby 1977) or conditions associated with captivity (Willoughby & Pickering 1977; Copland & Willoughby 1982). These infections normally are

single or sporadic, although there are reports of regular infections in wild salmonids subject to spawning stress (Neish 1977; Richards & Pickering 1978; Pickering & Christie 1980). S. parasitica is the dominant saprolegniacean in these infections (Willoughby 1978; Wood, Willoughby & Beakes 1988).

The bacterium Aeromonas hydrophila may be a secondary invader of lesions (Humphrey 1985; Menasveta 1985) or a primary pathogen in systemic and integumentary infections of fish subject to spawning, thermal or low-oxygen stress (Richards & Roberts 1978; Esch & Hazen 1980; Nieto, Corcobado, Toranzo & Barja 1985).

Where both A. hydrophila and Saprolegnia spp. have occurred in an infection primacy usually has been attributed to A. hydrophila (Egusa 1965; Thorpe & Roberts 1972; Inman & Bland 1981; Callinan 1985). In the one reported exception known to me -- a case where A. hydrophila was associated with an otherwise typical salmonid saprolegniasis (Richards & Pickering 1978) -- bacterial samples were not taken from integumentary lesions. This chapter reports a further exception that occurs regularly in a wild population of a non-salmonid species - the bony bream of the lower River Murray.

3. METHODS

3.1 Sampling

Bony bream were obtained from Zadows Landing on the lower Murray (34° 58'S, 138° 59'E). Sampling was monthly and continuous from September 1983 to December 1984, but thereafter confined to the epizootic and breeding seasons (June to December each year until October 1987). Seven 50-m gill nets (mesh 20-110 mm) and three seines (lengths 2, 18, 130 m; meshes 2, 12, 30/50 mm) were used. Samples comprising either the entire catch or stratified random subsamples (total length (TL) size-class interval 50 mm) were retained. Infected and healthy fish were handled separately. All fish were measured (TL to 1 mm), weighed (0.1 g), and the gonads dissected and weighed (0.01 g). Analyses employed computer-based statistical packages (BMDP Statistical Software 1987; NWA Statpak 3.1, North-Western Analytical, Washington) and our own programs in Microsoft BASIC. The Gonado-Somatic Index

$$GSI = 100\ (\text{gonad weight})/(\text{body weight})$$

and a Condition Factor

$$100\ (\text{body weight}-\text{gonad weight})/(\text{total length})$$

were routinely calculated.

Water temperature, pH, conductivity, Secchi depth, dissolved oxygen and river levels were monitored on each sampling occasion, and supplementary records of temperature, discharge, water chemistry and organo-chlorine pesticide

concentrations were obtained from the Engineering & Water Supply Dept, Adelaide.

The sampling program conducted on the North-west Branch of Cooper Creek has been described elsewhere (Puckridge & Drewien 1988). It was directed principally at monitoring incidence of fungus disease in relation to environmental factors.

3.2 Mycology

Mycelial samples from lesions were cultured on chloramphenicol-cornmeal agar (250 mg chloramphenicol per litre agar) and duplicate samples were placed in sterilized river water. The lesion was photographed, mapped on a gridded fish outline, dissected free to a depth of 1 cm and preserved in 10% buffered formalin. Hyphal tips from the agar colonies were subsampled repeatedly until bacteria-free. The mycelia kept in sterilized river water were examined before sub-culturing to check the selectivity of the agar medium (cf. Willoughby 1978). For 60 isolates the mycelial clumps from sterilized water and hyphal tips from agar cultures were transferred to sterile distilled water prior to observations of zoospore release. Fifteen isolates were maintained in the dark at 7°C on sterilized hemp seed in filtered and autoclaved Murray water to allow observations of the formation of sexual structures (cf. Willoughby 1978).

Selected isolates were also examined by Dr G.W. Beakes (University of Newcastle-upon-Tyne) under the transmission electron microscope to check their cyst coat morphology.

3.3 Bacteriology

In September 1986 bacterial sampling was included in the protocol for 34 infected and 32 uninfected fish. These samples were taken with a flamed loop, streaked on DIFCO nutrient agar in a 90-mm petri dish and spread with a sterile swab dipped in sterile saline. If mycelia were present on a lesion part of the mycelial mat was lifted with sterilized forceps and the loop touched on the exposed tissue. If mycelia were absent the ulcerated surface was sampled directly. A sample was taken also from unaffected skin on the mid-flank. The flank was then seared and opened along the swim-bladder using sterilized scissors. The liver and anterior kidney were incised with a sterilized scalpel and the incision sampled. A control plate also was streaked with the sterilized loop and spread as above.

Each infected fish was paired with a similar-sized uninfected fish caught at the same time and place; these were treated in the same way, excepting the lesion sample. The culture plates were stored for three days at about river temperature (12-18°C).

Preliminary identification of bacterial isolates was performed by C. Daley of the Institute of Medical and Veterinary Science, Adelaide. Final identification of

presumptive A. hydrophila isolates was performed by Dr Dawn Austin of the Department of Brewing & Biological Sciences, Heriot-Watt University, Edinburgh.

3.4 Virology

In September 1986 three healthy, three slightly infected and three severely infected fish were frozen on dry ice and sent to Dr J.S. Langdon of the Australian Fish Health Reference Laboratory at Benalla, Victoria, who tested the specimens for the presence of viral agents.

3.5 Histology

Skin tissue samples from nine infected and three apparently uninfected specimens were subjected to histological examination by Dr J.S. Langdon.

4 RESULTS

4.1 Epizootiology

The epizootic typically begins in June-July, when temperatures are lowest for the year (Fig. 1A) and flooding has not commenced (Fig. 1B). This is two months before the annual low in the body Condition Factor (Fig. 2A), three months before there is a significant rise in GSI and five months before spawning (Fig. 2B). Based on the 1983-87 data, the peak monthly incidence of infection is significantly negatively correlated with the mean July-August water temperature (Puckridge et al 1989). However, the 1988 results

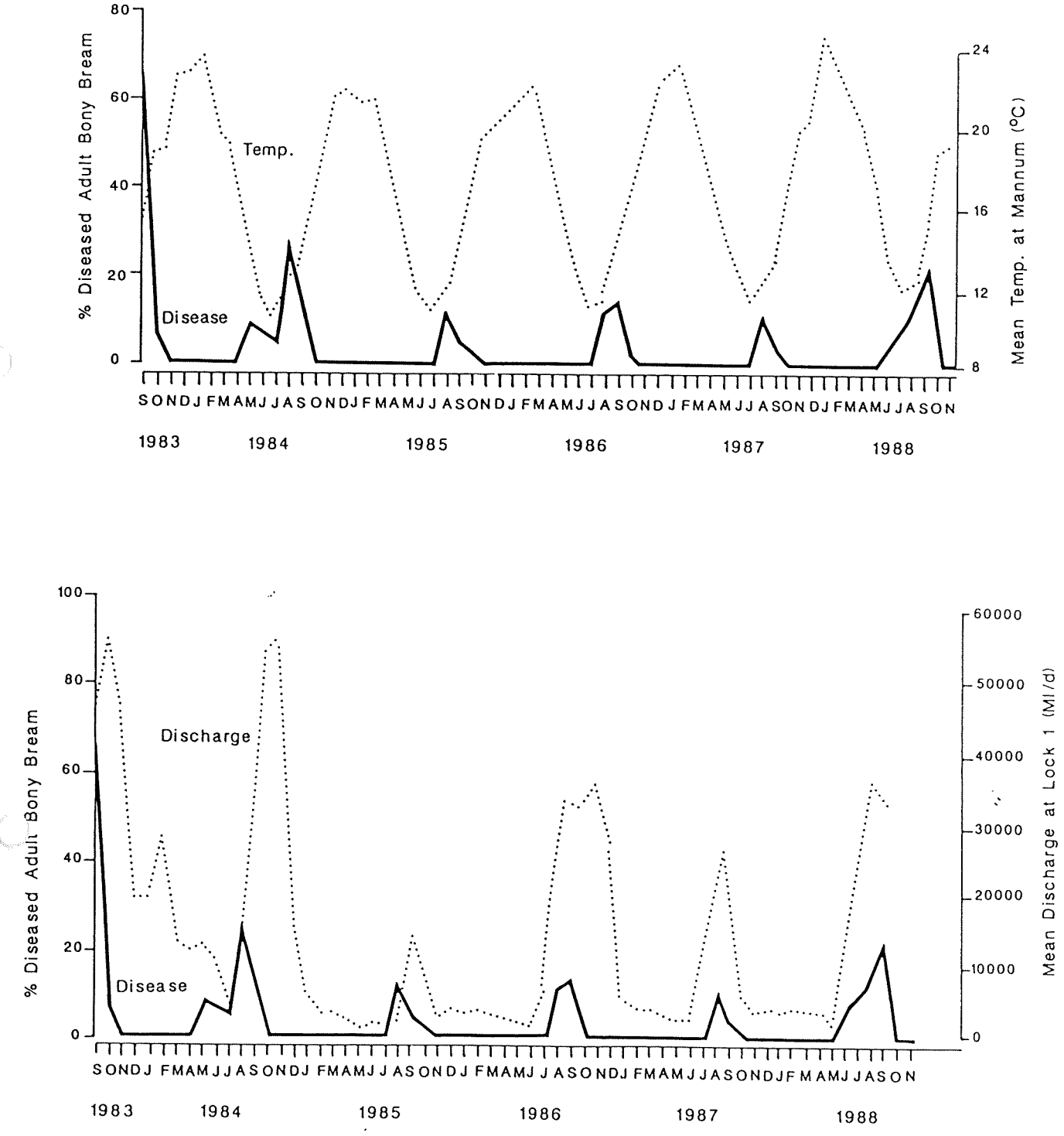

Figure 1. Percentage prevalence of dermatitis in adult bony bream 1983–1988 compared to (A) mean monthly water temperature and (B) mean daily discharge.

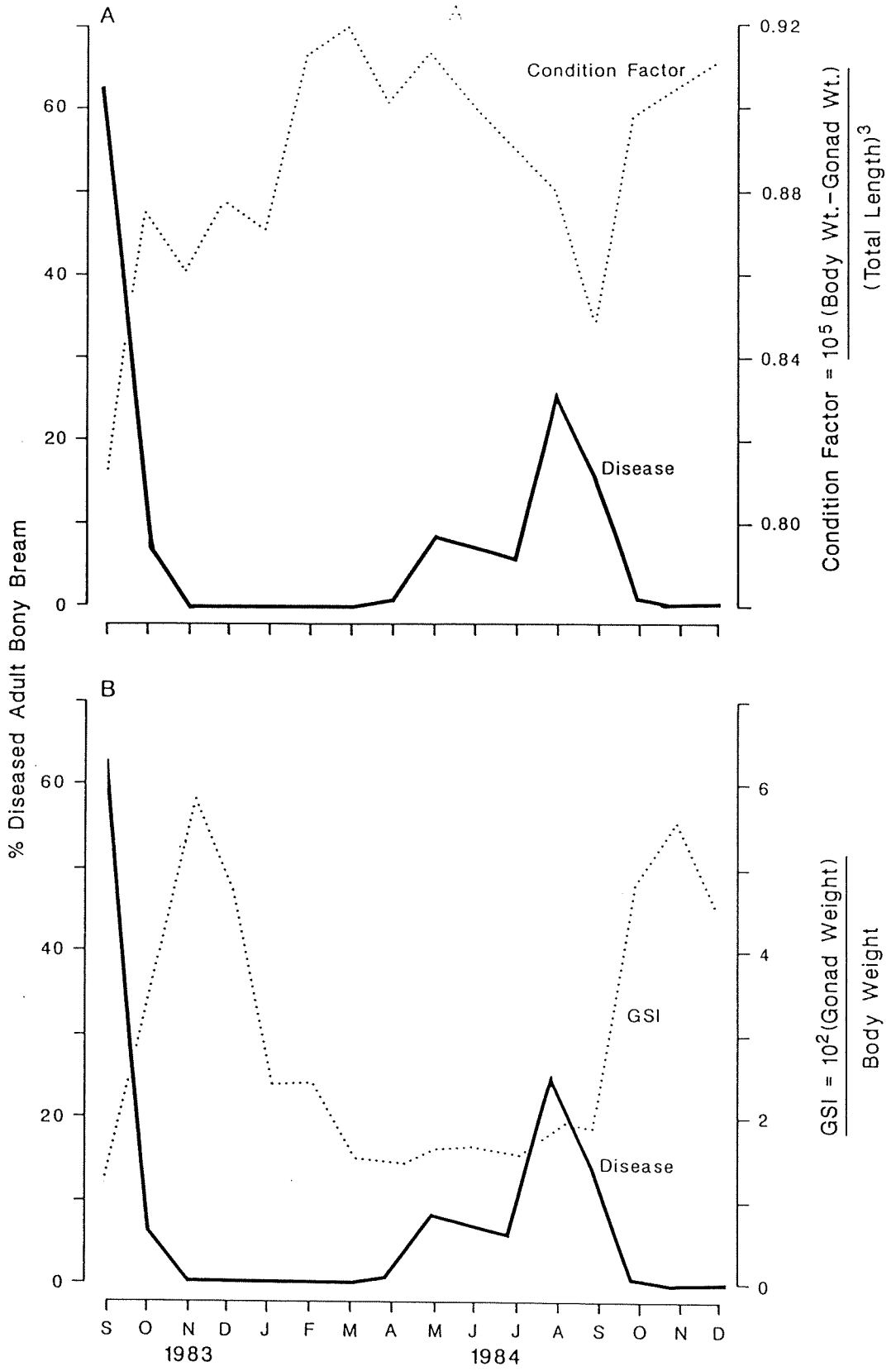

Figure 2. Percentage prevalence of dermatitis in adult bony bream in 1983–1984 compared to (A) mean monthly condition factor and (B) mean monthly GSI.

(which were analysed after publication of the above paper) do not support this correlation. No significant correlation between disease incidence and discharge was found. Incidence reaches a peak in August and September and involves 10-64% of the adult population. Juvenile fish (TL <150 mm) are rarely affected (incidence <0.4%). There is no significant difference in the incidence of infection in the two sexes corrected for population sex ratio (Chi-squared, n = 166). There are no significant differences in mean Condition Factor (log(X+1)-transformed) or mean GSI for infected and uninfected fish (paired samples t-test, n = 86 pairs), and no significant correlation between condition and area of the lesion (Spearman Rank).

The water quality data suggest that, over the sampling period, no heavy metals or organo-chlorine pesticides occurred in the lower Murray in concentrations likely to be toxic to fish. Levels of organo-chlorines were in fact below limits of detection by Engineering and Water Supply Department techniques. Nor were there changes in pH, conductivity or dissolved oxygen likely to cause stress to the bony bream. No other fish species showed signs of infection.

4.2 Gross Pathology

The proportions of infected grid cells (among 168 per fish) in four body regions (fins, head, mid-flank and posterior flank) on the left and right sides of the body

were analyzed by Stepwise Logistic Regression (BMDP Statistical Software 1987, program LR). The potential effects due to differences between individual fish, body region and side (left or right) were included as main effects in the logistic model, together with a side vs region interaction. The area of lesions is significantly higher on the anterior mid-flank (F-test, $P < 0.001$); the head and gills, fins and posterior flank are rarely affected. Curiously, there is also a significantly greater mean area of infection on the left than the right flank (F-test, $P < 0.05$). There is no significant interaction between side and region (F-test). The mean area of lesion is 5.3% of the body surface (SD = 3.5%, n = 91) and the maximum is 16%. The least severe and probably earliest lesions appear as a thin fuzz of mycelia over skin with no obvious haemorrhage or inflammation (Fig. 3A). Distinct lesions without fungus infection rarely were observed; there was occasional reddening of the skin and elevation or loss of scales, but this is typical of the reaction of bony bream to capture and handling.

Lesions progress by an increase in the density of investing mycelia, erosion of the epidermis and protrusion then loss of scales, with increasing peripheral haemorrhage and erythema (Fig. 3B). Intermyotomal haemorrhage and myomalacia occur in advanced lesions. One specimen only was found with scar tissue and pigmentation suggestive of healing, and only two moribund specimens were captured. With few exceptions

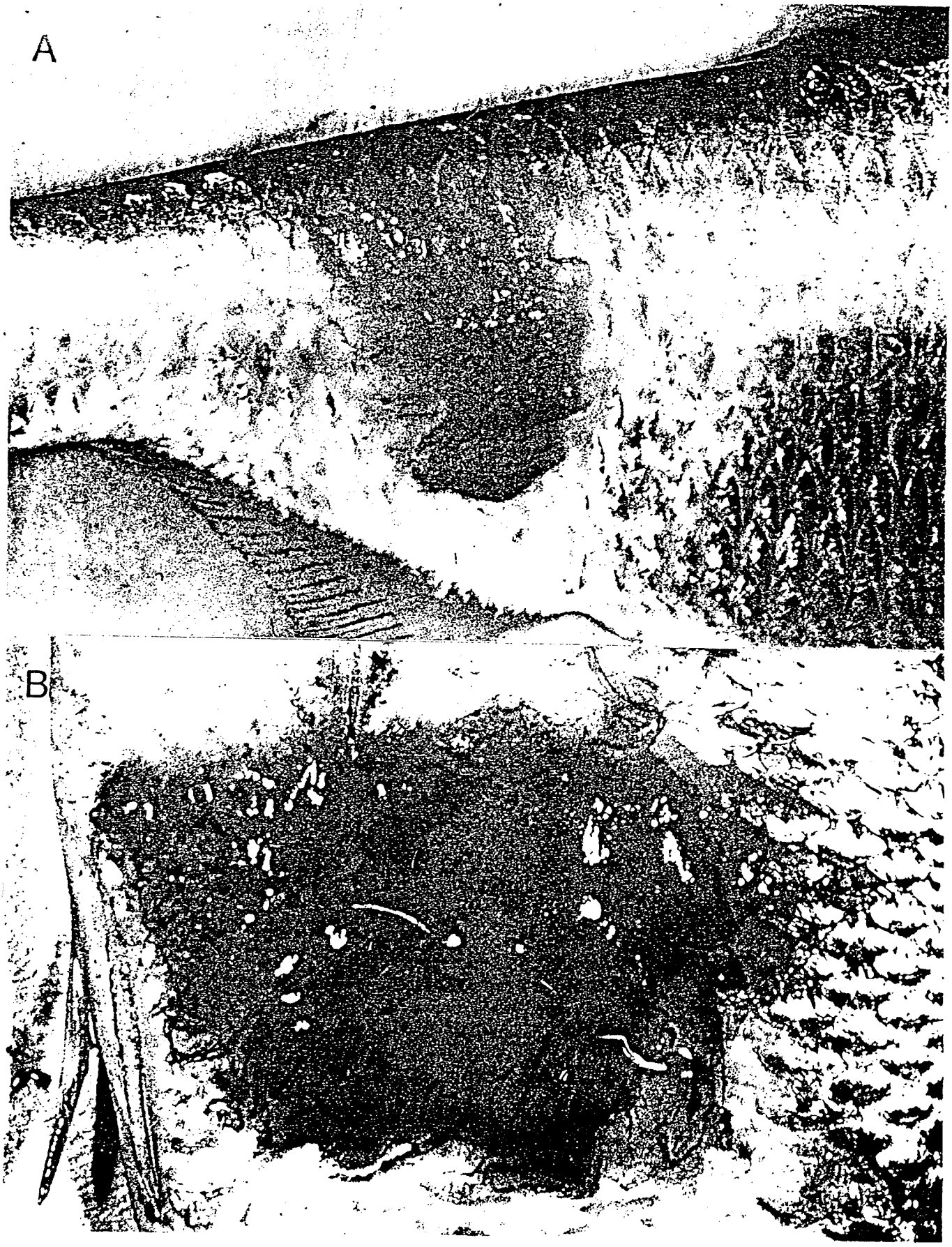

Figure 3. Mycotic lesions on skin of bony bream: (A) slight, (B) moderate.

the internal organs of fish with external lesions appeared normal.

4.3 Mycology

In mycelia transferred directly from lesions to sterile water there were occasional hyphomycetes, but the dominant organisms were non-septate oomycetes and all, apart from one specimen of Leptomitus sp., belonged to the Saprolegniales.

In 60 of 62 isolates of Saprolegniales the primary zoospores cleaved within the zoosporangium and were released terminally. Ten isolates observed at the moment of zoospore release all demonstrated active dispersal of the primary zoospores from the sporangium mouth, as is typical of Saprolegnia. In only one of 61 isolates (Achlya sp.) were primary zoospores found encysting at the sporangial opening. Of 34 fish with lesions collected in 1986, 31 yielded Saprolegnia isolates.

Six of the 15 isolates (code numbers 1289, 1356, 1376, 1431, 1494, 1570) maintained on hemp seed developed oogonia within six months. The isolate which most readily reproduced sexually (1494) was identified as S. ferax (Gruith.) Thuret and the other five as S. diclina Humphrey (after Seymour 1970).

The isolates examined by electron microscopy mostly fell into two groups. None of the first group (1323, 1358, 1372, 1380, 1408, 1428, 1504, 1512) produced oogonia and (except

for 1380, for which hair number was not determined) all had bundles of 8-16 boathook hairs 8.0-15.0 um long on their secondary cyst coats (Fig 4E, Table 1). Where measured, the primary cysts of these isolates (Fig. 4B) had unusually long spines (Table 1, c.f. Fig. 47, Beakes 1983). Isolate 4345, however, although non-sexual, had single moderately long boathook hairs on its secondary cyst coat (Table 1).

The second group (1289, 1376, 1494, 1570) produced oogonia and had single short boathook hairs on their secondary cyst cases (Fig. 4D, Table 1). In addition the simple tubular spines on their primary cyst cases were significantly shorter than those of the previous group (Table 1). Isolates 1356 and 1431 also produced oogonia and had similar primary cyst coat architecture to the second group (Fig. 4A), but differed in having a smooth secondary cyst coat (Fig. 4C).

4.4 Bacteriology

The following groups were identified tentatively in isolates from diseased and healthy fish: Aeromonas, Pseudomonas, Alcaligenes, Flavobacterium, Chromobacterium and "oxidase-negative gram-negative bacillus". Aeromonas hydrophila was identified in 38/48 suspected Aeromonas isolates.

A. hydrophila was isolated from 22/34 skin lesions and 3/31 skin samples from healthy fish. Only 4/34 fish with lesions yielded A. hydrophila from liver and/or kidney. Seven samples from uninfected skin areas of 34 diseased fish gave A. hydrophila isolates (Fig. 5). There was no difference between the incidence of Saprolegnia and A. hydrophila in

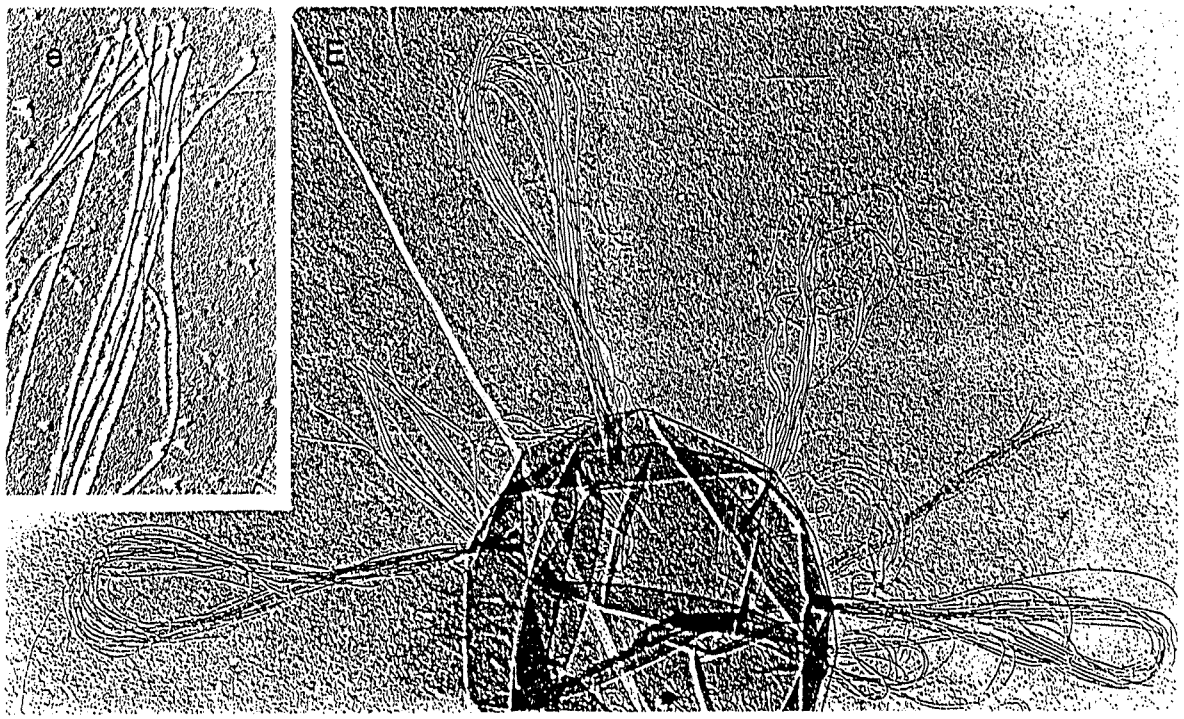

Figure 4. All figures of gold palladium shadowed whole mounts of fungi on formvar coated grids. (A) Part of a primary cyst coat of isolate 1356, showing tufts of short primary spines, typical of oogonium-forming species (× 15 800). (B) Part of a primary cyst coat of isolate 1372, showing groups of much longer primary spines typical of the non-oogonium-producing fish lesion isolates (× 15 800). (C) Secondary cyst case of isolate 1356, showing smooth wall, without hooked hairs (× 9600). (D). Detail of part of secondary cyst case of isolate 1376, showing short single boathook hairs typical of oogonium-producing isolates (*S. ferax* and *S. diclina*) (× 20 000). (E, e) Part of secondary cyst of isolate 1372, showing characteristic bundles of hooped boathook hairs which distinguish the non-oogonium-producing isolates (*S. parasitica*) (× 5920).

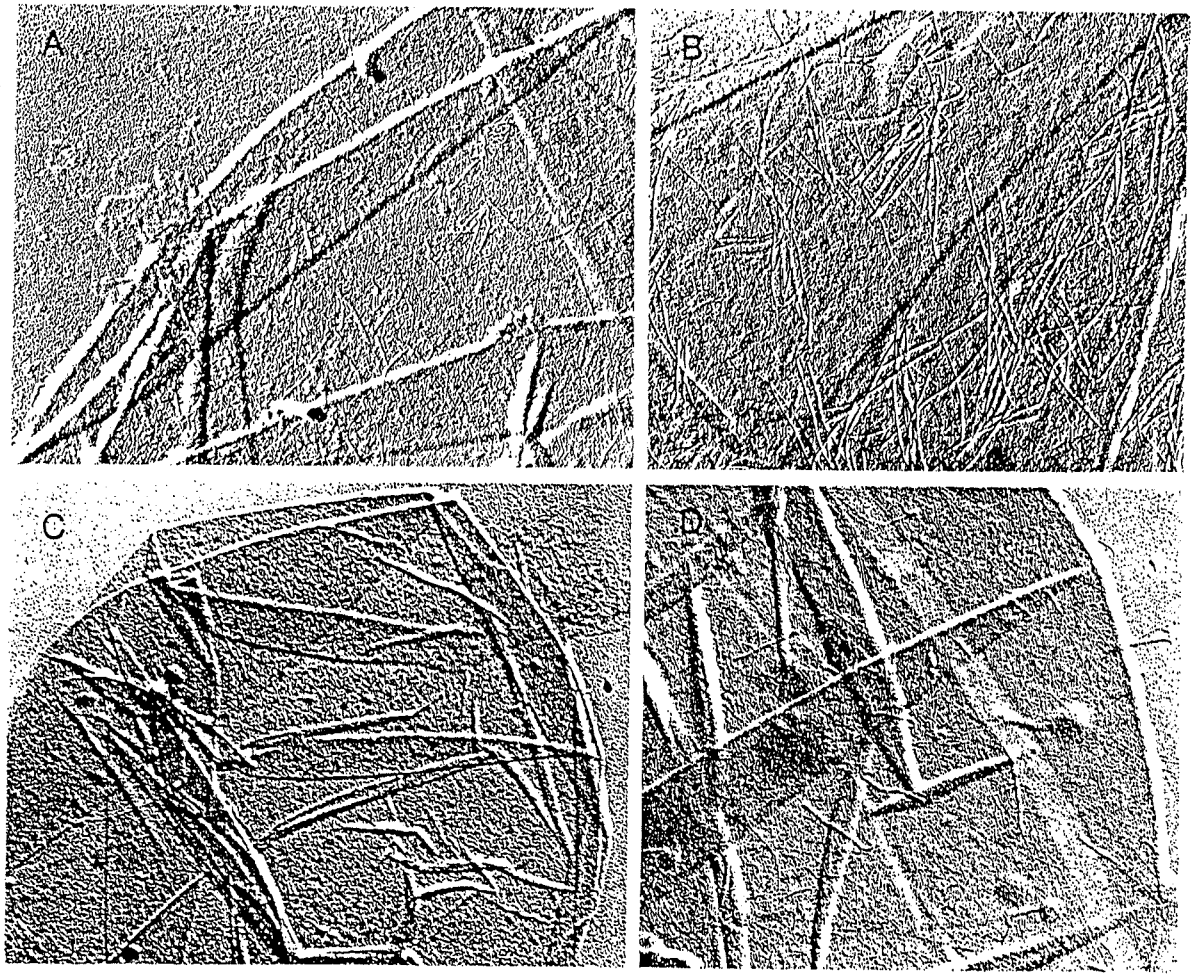
A
B
C
D

Table 1. Summary of cyst coat morphology for isolates selected for whole mount examination in the TEM

Isolate no.	Primary cyst coat mean spine length (SE) µm	Secondary cyst coat Bundle/hair length (SE) µm	Mean no. hairs/bundle (SE) µm
1289	0·34 (0·20)	0·41*	1
1323	1·84 (0·53)	9·18*	14*
1356	0·35 (0·09)	smooth	
1358	1·56 (0·42)	9·44 (0·84)	9 (0·58)
1372	1·61 (0·17)	9·46 (0·60)	12 (0·70)
1376	0·28 (0·02)	0·39 (0·04)	1
1380	2·01 (0·30)	10·00*	ND
1408	1·61 (0·10)	12·80 (0·85)	16
1428	2·14 (0·31)	11·20 (1·51)	14 (1·05)
1431	c. 0·30*	smooth	
1494	c. 0·30*	0·40*	1
1504	ND	13·30*	12
1512	ND	8·41 (0·44)	12 (1·07)
1570	c. 0·30	0·40*	1
4345	ND	3·40*	1

Where the standard error of the mean (SE) is given each measurement represents the mean of at least 20 measurements. Dimensions which are followed by a * are from data sets of less than 10 measurements or from poor preparations where accurate measurements were not possible. In some samples, some of the spore stages were not observed and these are indicated as not determined (ND).

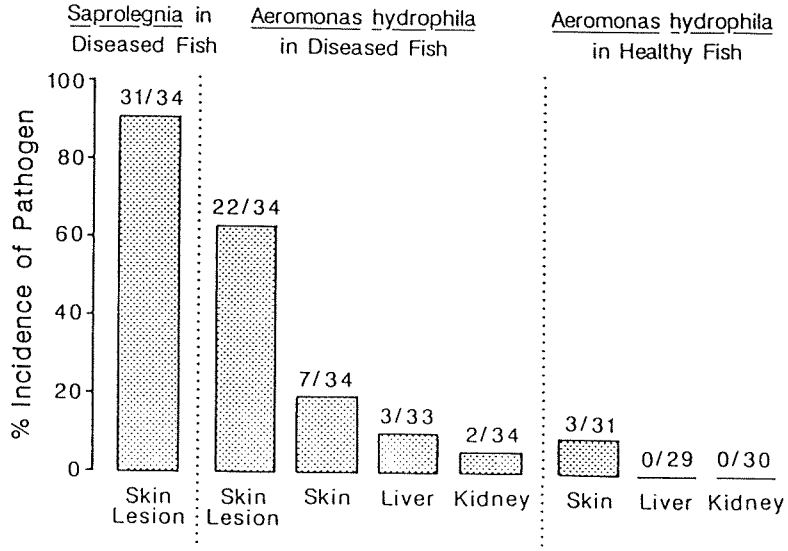

Figure 5. Prevalence of *Saprolegnia* and *Aeromonas hydrophila* isolates in infected and healthy tissues of bony bream.

lesions (Chi-squared, P >0.05), but of the 3/34 fish that did not yield Saprolegnia, one did not yield A. hydrophila, one showed an integumentary infection of A. hydrophila and the third had obvious Aeromonas septicaemia.

4.5 Virology

No cytopathic agents were isolated from the kidney, liver, spleen or skin lesions of diseased fish.

4.6 Histopathology

The principal lesions involved erosion of the epidermis and loss of scales, hypodermal and muscular oedema, haemorrhage and myofibral degeneration. The earliest obvious infections consisted solely of fungal hyphae penetrating the epidermis and dermis, often in scale pockets (Fig. 6A). This progressed to erosion of the epithelium and hypodermal oedema, as previously described for saprolegniasis (Copland & Willoughby 1982). Bacterial invasion also was commonly observed at this stage in development of the lesion.

Extensions to the underlying musculature occurred in severe cases, inducing haemorrhage and oedema, particularly in the intermyotomal connective tissue. Fungal hyphae were not visible in sections stained with haematoxylin and eosin, but methenamine silver staining revealed one or more hyphae, but never numerous hyphae, within each haemorrhagic focus (Figs. 6B, 6C). Individual myofibres often contained a single hypha within the sarcoplasm, with or without sarcoplasmic

Figure 6 (A) Fungal hyphae (arrow) in scale pocket in earliest stage of infection (methenamine silver, ×130). (B) Haemorrhage and oedema of intermyotomal connective tissues at junction of pink lateral and white muscle, and white myofibril degeneration (H & E, ×100). (C) Section similar to Fig. 5B but silver-stained to reveal fungal hyphae (arrows) within haemorrhagic foci and myofibres (methenamine silver, ×130).

degeneration. Bacteria were rarely visible in these deeper lesions.

No stage of the lesions showed leucocytic inflammatory response to the fungal elements. Lesions with substantial bacterial involvement displayed mononuclear inflammatory infiltration at the sites of invasion.

4.7 Cooper Creek fungus disease

Neither analysis nor sampling are complete in this project, but preliminary findings can be outlined.

Outbreaks of fungus disease may occur at any time of the year, but incidence is higher in the warmer months. The syndrome involves shallow ulceration infested with fungal mycelium, and appears in adult and juvenile bony bream, juvenile callop and juvenile goldfish. Other species are also affected, but at much lower frequency. Lesions most commonly occur on the head and fins. The epidemics persist through summer and autumn, and subside in winter. Causal factors are not obvious, but low oxygen levels brought about by cessation of flow and high temperatures may be important.

The principal fungal pathogen isolated from the lesions was Achlya sp. Histopathology on the lesions by Dr J.S. Langdon of the Fish Health Reference Laboratory, Benalla, Victoria, revealed a fungal dermatitis extending to myositis, with invasion of the epidermis, dermis and muscle by fungal hyphae. The inflammatory reaction was moderate.

5 DISCUSSION

Irruptions of Saprolegnia and Aeromonas hydrophila commonly occur in late winter and spring (Jester & Jensen 1972; De Figueiredo & Plumb 1977; Porak & Tranquilli 1981). It appears that low winter temperatures may act directly to lower antibody production and blood proteins and thereby immunity, or indirectly through inhibition of feeding (Roberts 1975; Cipriano, Bullock & Pyle 1984; Brenden & Huizinga 1986a). The rise of temperatures in spring also may lower immunity through stress, particularly in the case of A. hydrophila infections (Esch & Hazen 1980; Nieto, Corcobado, Toranzo & Barja 1985). Sexual maturation may contribute but the mechanism is unclear (Pickering & Pottinger 1985, Pickering 1986).

The general preference of bony bream for warmer waters is consistent with the winter-stress model; the lower Murray lies near the southern extreme of the species' range. Bony bream possibly are sensitive to low temperatures and low oxygen concentrations (Cadwallader 1977; Allen 1982), as these conditions are implicated in reported infections by bacteria and Saprolegnia (Johnston & Bancroft 1921), Chilodonella (Langdon, Gudkovs, Humphrey & Saxon 1985) and rhabdovirus and Aeromonas (Department of Ports & Fisheries 1986) in this species. An exception to this pattern is the mycosis (involving Achlya sp.) of bony bream in the Northwest Branch of Cooper Creek, which occurs under a

variety of conditions, including both high temperatures and high dissolved oxygen concentrations (Puckridge, unpub. data).

The timing of the epizootics in the lower Murray also is consistent with the winter-stress model. Peak incidence occurs after the July winter minimum. However, in 1984 the incidence of the lower Murray disease peaked before body condition reached its minimum. There was no difference between the condition of healthy and diseased fish, and condition was not significantly correlated with the area of lesions. If winter-cold stress is acting as an initiating factor in the disease cycle, it seems likely that it is doing so directly through suppression of immunity rather than by lowering body condition through inhibition of feeding.

Several authors have commented on the lack of a leucocytic inflammatory response to invading hyphae, and the presence of haemorrhage in the inflammation induced by the hyphae of Saprolegnia sp., particularly in deeper lesions (Bootsma 1973; Wolke 1975; Neish 1977; Copland & Willoughby 1982). The lack of such a response in bony bream could arise from temperature-mediated inhibition, or it may reflect the fungal species involved (there is some cellular inflammatory response to bacterial invasion). This contrasts with the marked granulomatous inflammatory response shown by fish infected by Aphanomyces sp. (Noga & Dykstra 1986), Phoma herbarium (Ross, Yasutake & Leek 1975), Aphanomyces

piscicida (Hatai, Takahashi & Egusa 1984) and Exophiala pisciphila (Langdon & McDonald 1987).

The fact that adults, rather than juveniles, are affected may arise from differences in the feeding habitats of the two (Atkins, 1984), which could cause differential exposure to the pathogen. It also suggests that hormonal changes associated with sexual maturation may increase susceptibility to the disease. In fact pre-maturational changes are indicated because the infection becomes intense two months before the onset of vitellogenesis. Similarly, the annual flooding of the Murray usually occurs too late to affect the initiation of infection. However, it may be instrumental in the cessation of the epidemic.

Captive salmonids and eels succumb rapidly to saprolegniasis (Copland & Willoughby 1982; Pickering & Willoughby 1982), and it is possible that wild populations also suffer high mortalities (White 1975). In the present case the scarcity of healing or dead bony bream is difficult to explain. However, the absence of large numbers of dead or moribund fish suggests that mortality rates due to the disease are low. Both actual mortality rates and effects on reproductive output, however, should be examined experimentally, perhaps in river enclosures.

The specificity of the lower Murray disease for bony bream contrasts with the generality of a dermatitis attributed to Achlya sp. in fish of the Coongie Lakes region of Cooper

Creek in central Australia, where bony bream, callop, desert rainbowfish (Melanotaenia splendida tatei) and goldfish (Carassius auratus) are affected (Puckridge & Drewien 1988).

It seems likely that S. parasitica is the principal fungal pathogen involved in bony bream dermatitis, as it is in British salmonid saprolegniasis. But in Japan S. diclina also has been implicated as a salmonid pathogen (Hatai, Willoughby & Beakes, in press), so a definite answer must await a study of the pathogenicity of both taxa.

In saprolegniasis the localization of surface lesions is distinctive for particular host-pathogen relationships. The pattern seen in bony bream, favouring the mid-flank region, differs from that in salmonids (White 1975; Neish 1977; Richards & Pickering 1978) where head, dorsal surface and fins are affected. It differs also in being not consistent with initiation of infection by injury. The significant difference in infection area between the two sides of the fish is not simply explained, and may be an artifact of large sample size. However, there is evidence of assymetry in parasitic infections (Moser, Sakanari, Wellings & Lindstrom 1984; Rohde 1984), and bony bream have significant gonad assymmetry, with the larger gonad on the more severely infected side (see 5.4).

The Chi-square analyses reported by Richards & Pickering (1978) are at best marginal indicators of environmental and biological effects, whereas the method employed here is

multivariate in nature and has potential for comprehensive studies of the relations between such effects and the distribution and intensity of infection.

The task of establishing primacy in a complex disease process is difficult, and conflicting evidence is common. This is well-shown by work on Japanese "fungus disease" of eels (Egusa 1965), UDN of salmonids (Carbery 1968) and the Asia-Pacific ulcer disease (Roberts, MacIntosh, Tonguthai, Bronyaratpalin, Tayapatch, Phillips & Millar 1986). In the present situation, it is possible that inconspicuous prefungal A. hydrophila lesions occur initially in the lower Murray disease, and that Saprolegnia invades these before they become obvious. However, there are several lines of evidence to suggest that this is a case of primary saprolegniasis:

(1) Saprolegnia occurs in the earliest detectable lesions.

(2) Gross haemorrhage and inflammation are absent in early lesions.

(3) External and internal symptoms of systemic bacteraemia are absent.

(4) The yield of A. hydrophila cells from internal organs is low (this is not uncommon (Thorpe & Roberts 1972; Snieszko 1974) and the slightly enhanced levels that occur in fish weakened by Saprolegnia lesions are to be expected).

(5) About a third of lesions (11/34) yielded Saprolegnia but not A. hydrophila.

(6) Few lesions (2/34) yielded A. hydrophila but not

Saprolegnia.

(7) Saprolegnia hyphae occur in the scale pockets in early lesions, and deep in the muscle in advanced lesions.

(8) Viral agents were not apparent, at least in preliminary investigations.

The evidence presented here suggests that the lower Murray bony bream epizootic is an addition to the comparatively few known instances in fish of primary pathogenicity among aquatic fungi (Ross & Yasutake 1973; Ross, Yasutake & Leek 1975; Hatai, Takahashi & Egusa 1984; Noga & Dykstra 1986). In addition, this appears to be the first reported case of primary saprolegniasis in a wild, non-salmonid fish population.

The Cooper Creek disease syndrome differs markedly from the River Murray disease.

1. Incidence does not follow a well-defined pattern. Outbreaks may persist for six months, and although they are more common in the warmer months, they may occur at all times of the year.

2. The disease is most common in bony bream, callop and goldfish, but may occur in all species.

3. Both juveniles and adults are affected.

4. Lesions commonly occur on the head and fins, not the mid-flank. Serious erosion of bone and cartilage is common.

5. The incidence of seriously affected and even moribund fish - particularly juvenile callop - is higher than in the

Murray syndrome.

6. The principal fungal pathogen is Achlya, not Saprolegnia.

7. The inflammatory reaction is intermediate between the haemorrhagic Saprolegnia lesions in the River Murray fish, and the profound granulomatous reactions in the Northern Territory and Queensland "red spot" fish.

IMPLICATIONS AND RECOMMENDATIONS

1. Mycotic dermatitis may be an expression of stresses experienced by a species at the margin of its range. Marginal changes in the lower Murray environment could precipitate major changes in the severity of the epidemics. The possible susceptibility of bony bream to low temperatures and the association of the mycotic dermatitis epidemics with winter temperature minima suggests that change in the thermal regime could be critical. For example, the projected Greenhouse temperature rise of 2-4°C by the year 2030 (Graetz, Walker & Walker, 1988), could eliminate the outbreaks entirely.

2. The disease syndrome seems to be a natural phenomenon, dating back to the pre-regulation era. Human intervention - from pollution, increasing salinity or hydrological changes - does not seem to be implicated.

3. There is no danger of spread of the disease to other species, nor of the infection being transmitted to rock lobsters. The specificity of the disease is particularly striking in contrast with the generalist pattern of the Cooper Creek syndrome.

4. The Aeromonas bacteria in the lesions have been known to cause infection in man. However, the incidence is low, and need not be considered a hazard to fishermen. The implications of the disease for other uses of bony bream - for example as pet food - would have to be determined experimentally.

5. Mortality rates from the disease should be determined, perhaps by the use of in-river enclosures during the disease season. However, the scarcity of dead or moribund individuals during the outbreaks suggests that mortalities are low.

6. Further elucidation of the environmental factors involved in the disease outbreaks would require more intensive sampling (at least weekly) during late winter and early spring, multivariate analysis of results, and experimental work on the correlated factors. This was beyond the scope of the present study.

7. Because of its specificity, the River Murray disease does not present a problem for use of bony bream as a forage species in aquaculture. However, in Queensland and the Northern Territory, the Cooper Creek syndrome could present problems. Bony bream appear to be major disease carriers, and both percichthyids and teraponids appear to be susceptible.

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