RED SPOT DISEASE OF SEA MULLET
(Mugil cephalus)

FINAL REPORT TO FRDC

Project No. 86/53

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BACKGROUND

Red spot disease (RSD), a cutaneous ulcerative disease of estuarine fish, was first reported from the Burnett River in Queensland in 1972. By 1985, when the present application was submitted, seasonally recurrent RSD outbreaks were adversely affecting commercial fisheries in many east coast estuaries. For example, in 1983, commercial fishers on the Clarence River, which supports the most productive estuarine fishery in NSW, expressed concern that RSD was causing substantial economic losses and possible long term damage to the fishery. A preliminary study, conducted in 1985 in response to these concerns, showed that approximately 20% of the commercial sea mullet catch in that year on the Clarence was discarded due to RSD (Virgona, 1992).

A previous FRDC-funded study in Queensland had suggested that RSD ulcers were initiated by the bacterial agent, *Vibrio anguillarum*, after susceptible fish were stressed by exposure to rapid salinity and temperature changes following rain events (Burke and Rodgers, 1981; Rodgers and Burke, 1981). The subsequent preliminary Clarence River study failed to confirm the association of *V. anguillarum* with early ulcers (Callinan and Keep, unpublished data).

The present project was intended to examine the impact of RSD on the fishery and to identify causes of the disease.

OBJECTIVES

Project objectives were:

* To examine the status of the Clarence River finfish fishery.
* To assess the impact of RSD on the commercial fishery.
* To identify the causes of RSD.

RESULTS

Status of the fishery

Historic commercial catch data from both NSW Fisheries and the Clarence River Fishermen's Cooperative were collated. Sea mullet dominated the catches, comprising 70.4% of the total catch (by weight) followed by much smaller catches of flat-tail mullet (9.7%), bream (4.6%), luderick (3.8%), dusky flathead (2.7%) and other fish (8.8%).

Total catch increased from around 650 tonnes during the late 50's to around 1000 tonnes during the early 70's, returning to around 700 tonnes in the early 80's. This pattern was influenced mainly by fluctuations in the sea mullet catch. Catch composition has generally remained the same during this period, except that eels became a significant part of the catch during the early to mid 80's (around 10% in 85).

Surveys of recreational fishers' catches were conducted during July 1984 and the summer of 1984/85. Although sampling intensity was low compared to similar NSW Fisheries surveys conducted in other estuaries, results suggested that catch rates were comparable and that the fishery is much smaller, in terms of fisher hours, than other estuaries such as Lake Macquarie and Botany Bay.

NSW Fisheries catch data were not available during the grant period due to the introduction of a new computer database system. The data for this period and until the present are now available. Although there can be considerable fish loss due to RSD in any year (see next section), there do not appear to be any significant long term declines in catches for important commercial species which are affected by RSD, such as sea mullet, yellowfin bream and sand whiting (Figure 1). Dusky flathead are an exception, but the observed decline in catches for this species commenced well before the first reportings of RSD (Figure 1) and therefore a causal association is not indicated.

Impact of RSD on the fishery

Six RSD outbreaks were reported on the Clarence River between 1972 and 1984. A further 3 outbreaks were observed during the present project, between 1985 and 1988. In 1985, 20% of the entire commercial sea mullet catch for the year was discarded due to RSD. Discard rates for other commercially important susceptible species were not determined, but were probably similar; subsequent studies have indicated that sand whiting *Sillago ciliata* and yellowfin bream *Acanthopagrus australis* are at least as susceptible as sea mullet to RSD (West, unpublished data). The total annual value of the commercial catch of RSD-susceptible species on the Clarence is approximately $1 million (Heyen, unpublished data). If RSD outbreaks occur, on average, once every two years (ie. 9 outbreaks were observed in the 17 years from 1972 to 1988), then 10% of this catch, worth $100,000, is discarded annually due to RSD.

Other consequences of RSD outbreaks may also damage the fishery. Fish populations are probably stressed by exposure to adverse environmental conditions prior to, and during, outbreaks. Ulceration *per se* is probably stressful and ulcerated fish have a lower condition score than those without ulcers (Virgona, unpublished data). These stressful and injurious occurrences may result in reduced fecundity and growth rates. Mortality rates due to RSD could not be measured during the project, but were likely to have been substantial. Definitive measurement of the cumulative effects on the fishery of the above insults was beyond the scope and resources of the project, but reduced recruitment is one possible outcome. Mathematical models developed in North America for estuarine stocks have suggested that quite small declines in fishery recruitment over several decades may result in large reductions in populations. Such dramatic declines in biomass may not be noticed until serious and possibly irreversible damage has occurred in the fishery (Merriner and Vaughan, 1987; Vaughan, Merriner and Schaff, 1987).

Fortunately, some of the main commercial fish species which are affected by RSD belong to larger coastal stocks. This is the case for sea mullet and yellowfin bream which are recruited to

estuaries from ocean waters at larval and adult stages of life. As a consequence, it is likely that the impact of mortality due to RSD for these species is ameliorated by recruitment from other estuaries. However, if there is an increase in the frequency and geographic extent of RSD outbreaks, then this buffering capacity would be reduced.

Causes of RSD

Investigations were conducted in the following areas:

Water quality

RSD outbreak occurrences were matched with rainfall and river flow records for the period 1972 to 1988. Detailed disease prevalence, rainfall, river flow and water quality data were compared for the period 1985 to 1988. Significant correlations between weekly rainfall in the lower catchment and the prevalence of early stage lesions were found. Progression to later stages of the disease occurred after rainfall and high river flows, which also caused rapid changes in various water quality characteristics such as salinity, temperature, dissolved oxygen, pH and turbidity. Results are reported fully in Virgona (1992); see Appendix.

In a supplementary study, selected water quality parameters were measured daily in representative tributary and main channel sites on the lower Richmond River in the weeks immediately following major rain events in 1987 and 1988. A similar study was conducted on the Clarence River in 1988. Results showed that dissolved oxygen concentrations fell from normal levels of >6 mg/l to <1 mg/l within 7-10 days of the rain events. Concentrations remained below 1 mg/l for a further 8-10 days as flood waters receded (Callinan, Fraser and Virgona, unpublished data).

Pathology

Lesions present on sea mullet during the first 3 weeks of RSD outbreaks were identified. Necrotising dermatitis was a severe, locally extensive granulomatous lesion associated with invasion

of dermis and underlying skeletal muscle by numerous, non-septate, fungal hyphae 12-18 µm in diameter. Erythematous dermatitis was a mild to severe, focal, chronic active dermatitis without fungal involvement. Lesions intermediate between these two forms, with small to moderate numbers of fungal hyphae in dermis and skeletal muscle also occurred. Findings indicated that erythematous dermatitis lesions and intermediate-type lesions subsequently resolved, while necrotising dermatitis lesions consistently developed into dermal ulcers, with associated severe necrotising granulomatous myositis. Generally, dermal ulcers occurred more often on posterior and dorsal areas of the body surface than on anterior and ventral areas. Lesions healed by combinations of epidermal and dermal repair, fibroplasia, destruction of fungi, removal of necrotic skeletal muscle and regeneration of myofibres. Atrophy of exocrine pancreas occurred in both diseased and clinically normal fish, but was generally more severe in diseased fish. Results are reported fully in Callinan, Fraser and Virgona (1989); see Appendix.

Bacteriology

Bacteria associated with RSD in sea mullet from the Clarence and Richmond Rivers were identified. Various bacteria, including Aeromonas spp., *Alcaligenes* spp., *Pseudomonas* spp. and *Vibrio* spp. were recovered from cutaneous lesions designated erythematous dermatitis, necrotising dermatitis and dermal ulcer, but no genus was consistently dominant in cultures from any of the lesion types. *Vibrio anguillarum*, previously proposed as the cause of RSD, was recovered from 4 of 47 lesions of erythematous dermatitis, none of 46 lesions of necrotising dermatitis and 8 of 36 dermal ulcers. Bacteria were recovered only rarely from internal organs (liver and posterior kidney) of diseased fish. These results suggest that none of the bacteria isolated is the primary cause of RSD. Results are reported fully in Callinan and Keep (1989); see Appendix.

Parasitology

Cutaneous ectoparasites associated with RSD in sea mullet from the Clarence River were identified. There was no evidence that these agents, including the digenean *Prototransversotrema steeri*, are important in the pathogenesis of RSD in sea mullet. None were found on 8 normal fish collected in the month preceding a major RSD outbreak. In the first 2 months of the outbreak, none were found on 18 fish with erythematous dermatitis, 8 with dermal ulcers or 11 of 12 normal fish. A single digenean, morphologically consistent with *P. steeri*, was recovered from a normal fish collected during this latter period.

Virology

Frerichs, Millar and Roberts (1986) recovered a rhabdovirus from 5 of 70 Southeast Asian fish with Epizootic Ulcerative Syndrome (EUS), a condition now recognised as indistinguishable from RSD. They suggested the rhabdovirus was a possible cause of EUS. A rhabdovirus was recovered from a blackfish, *Girella tricuspidata*, from NSW in 1985 (Roberts, unpublished data). However, using similar techniques and 5 cell lines, we were unable to recover virus from necrotising dermatitis lesions and internal organs from 30 sea mullet with typical RSD (Callinan, Fraser, Calder, Humphrey and Langdon, unpublished data).

CONCLUSIONS

Status of the fishery

There was evidence of a slight decline in the total river fish catch since 1973. However, no long term decline was indicated when data prior to 1973 (back to 1955) were examined. There do not appear to be any significant long term declines in catches for most of the important commercial species which are affected by RSD. The decline in catches of dusky flathead does not appear to be associated with RSD reportings.

Impact of RSD on the fishery

There can be substantial fish loss due to RSD in some years, as shown during the grant period. It is estimated that a minimum of $100,000 per year (the value of 10% of the commercial catch of susceptible species) may be lost due to RSD. This figure is based on discards from the commercial fishery, it does not include other sources of mortality such as inflated natural mortality. However, it is likely that the impact of RSD on fish in the Clarence River is buffered by recruitment from ocean waters.

Other consequences of RSD outbreaks may also damage the fishery. Fish populations are probably stressed by events preceding, and during, outbreaks. Definitive measurement of the cumulative effects on the fishery of these insults was beyond the scope and resources of the project, but reduced recruitment is one possible outcome. Overseas studies have suggested that large reductions in populations may result in the long term.

Causes of RSD

Findings indicated that lower catchment rainfall, probably via subsequent rapid and severe changes in one or more water quality parameters (including salinity, dissolved oxygen, pH and temperature) is an important determinant of outbreak occurrence. Findings also showed that RSD ulcers are a consequence of massive invasion of skin and underlying muscle by morphologically uniform, non-septate fungal hyphae, and that these hyphae provoke a characteristic granulomatous inflammatory response. There was no evidence that bacteria, cutaneous ectoparasites or viruses are essential in induction of lesions.

These findings represent significant advances in our understanding of the epidemiology and pathogenesis of RSD. However, further research is required, particularly regarding identification of specific water quality changes associated with outbreak occurrence, isolation and identification of the pathogenic fungi, and experimental disease reproduction (See Project 89/81).

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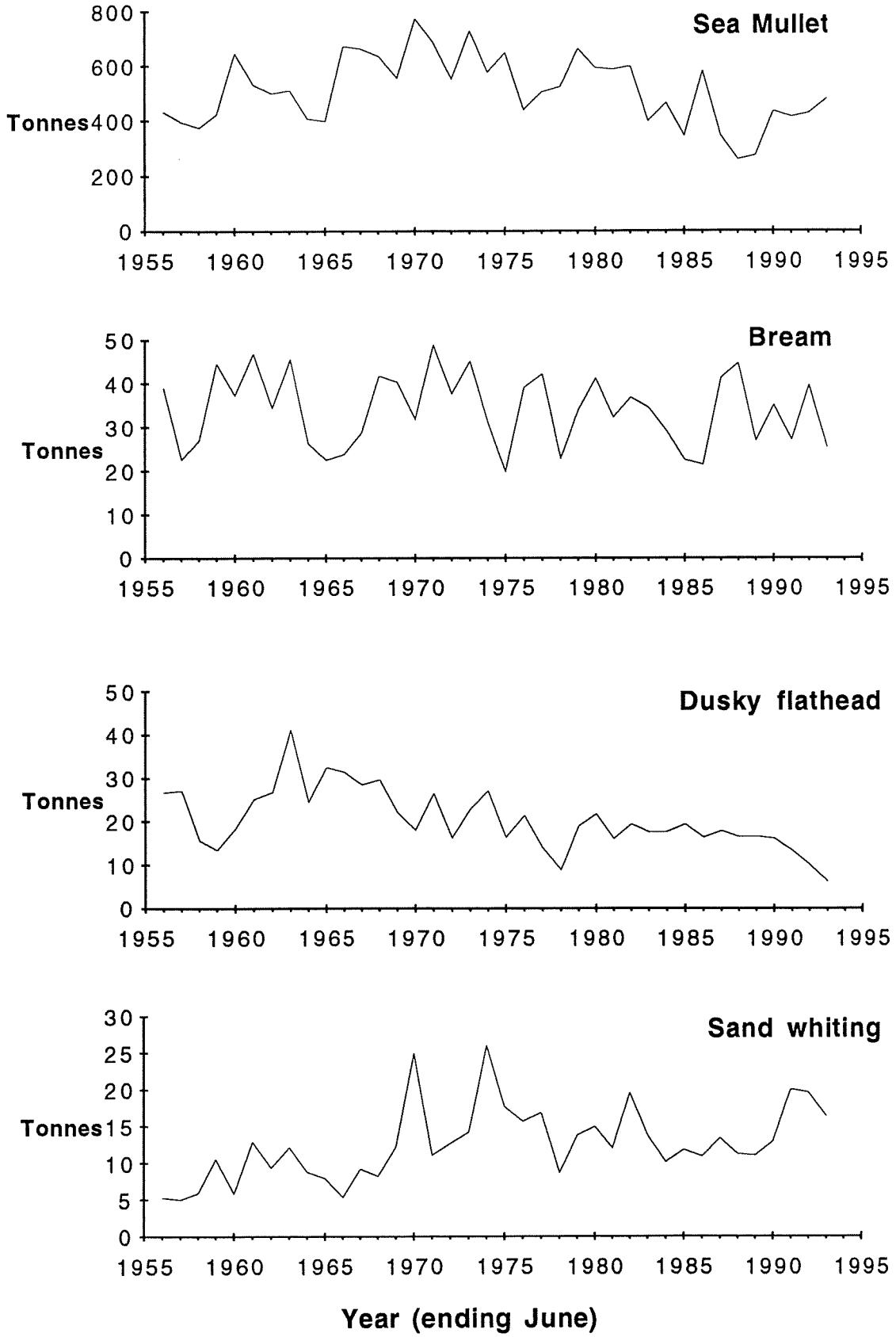

Figure 1

Commercial catches of sea mullet, bream, dusky flathead and sand whiting from the Clarence River from 1995-6 to 1994-95.

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Environmental factors influencing the prevalence of a cutaneous ulcerative disease (red spot) in the sea mullet, *Mugil cephalus* L., in the Clarence River, New South Wales, Australia

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Abstract. A cutaneous ulcerative disease (red spot) in sea mullet, *Mugil cephalus* L., from the Clarence River, New South Wales, Australia, was first reported in 1972. In this study, reports of disease outbreaks have been compared with rainfall and river flow records for the period from 1972 to 1988. Detailed disease prevalence, rainfall, river flow and water quality data were compared for the period from 1985 to 1988. Significant correlations between weekly rainfall in the lower catchment and the prevalence of early stage lesions have been found. Progression to later stages of the disease occurred after rainfall and high river flows, which also caused rapid changes in various water-quality characteristics such as salinity, temperature, dissolved oxygen, pH and turbidity. Organochlorine insecticide residues were not found to be associated with the disease in this area. The rainfall record since 1902 does not explain the absence of earlier reports of the disease. It is postulated that extensive structural developments for flood mitigation purposes and the increase in agricultural cultivation in the lower Clarence catchment during the last 20 years may be factors associated with the onset of the early stage of red spot disease. Fish in this early stage of the disease may then develop dermal ulcers under the stressful river conditions typically present during the autumn (high rainfall) season.

Introduction

Syndromes of cutaneous necrotic ulcer disease in fishes occur in many coastal areas around the world, including Papua New Guinea (Rodgers & Burke 1981), Southeast Asia (Roberts, Macintosh, Tonguthai, Boonyaratpalin, Tayaputch, Phillips & Millar 1986, cited in Humphrey & Langdon 1986), the Philippines (Alcestis & Gacutan 1987), the southeastern United States (Noga & Dykstra 1986; Hazen, Raker, Esch & Fliermans 1978) and Finland (Larsen, Jensen & Christensen 1987). In some cases, aetiological agents and/or environmental factors have been proposed.

Over the past 15 or so years, cutaneous fish ulcer disease has been reported in estuaries in most states in Australia. Munday (1985) observed ulcer disease in red cod, *Pseudophycis barbatus*, from the Tamar River, Tasmania, in November 1980 and 1981, and attributed the cause to environmental pullution. An outbreak of fish ulcer disease in Port Philip Bay, Victoria, in February 1984 was attributed to an environmental toxin possibly resulting from a phytoplankton bloom (Gwyther & McShane 1984). This bloom followed a period of high rainfall that had been preceded by drought. Ulcer disease was reported in fish from a wide range of freshwater and brackish locations in the Northern Territory in mid-1986, coinciding with unseasonal rain. Humphrey & Langdon (1986) suggested that a virus may have been involved in the cause of this disease. In Queensland, studies of red spot disease (previously known as 'Bundaberg Fish Disease', McKenzie & Hall 1976), focused on sea mullet, *Mugil cephalus* L., in the Noosa River

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system. Here, Rodgers & Burke (1981) proposed that red spot disease was related to low or rapidly changing temperatures and rapid or prolonged depressions of salinity induced by heavy rain. In concurrent microbiological investigations, they suggested that *Vibrio anguillarum* was the causative agent (Burke & Rodgers 1981). In a later paper (Rodgers & Burke 1988), they included the suggestion that the ectoparasitic digenean *Prototransversotrema steeri* might be a mechanical vector for bacterial pathogens.

The present study examines the relationship between various environmental factors and red spot disease in sea mullet from the lower Clarence River, NSW. Reports of disease outbreaks are compared with rainfall and river flow records for the period from 1972 to 1988. Detailed disease prevalence, rainfall, river flow and water-quality data are compared for the period from July 1984 to June 1985. In this paper, the word 'stress' is defined as, 'those harmful factors which influence biological systems' (Pickering 1981). Some authors including Huizinga, Esch & Hazen (1979), who are cited in this paper, equate stress with the response of the biological system.

The Clarence River supports the largest estuarine fin-fish fishery in New South Wales with its catch comprising ~20% by weight of the State's total estuarine catch. The sea mullet catch from the Clarence River averages around 700 tonnes per annum, accounting for ~75% of the total finfish catch from this river estuary. During the financial year 1984/1985, an estimated 20% of the commercial sea mullet catch was discarded because of the red spot disease outbreak in early 1985. The consensus of opinion amongst commercial fishermen was that such outbreaks of red spot disease had not occurred prior to 1972 and that extensive structural developments for flood mitigation in the lower Clarence River, which had been constructed in the late 1960s, were implicated in these outbreaks. Six outbreaks were reported during the period from 1972 to 1984, generally after periods of high rainfall. During the present study (1984–1988), outbreaks of red spot disease were observed early in each of the years 1985, 1987 and 1988.

Study area

The Clarence River is the largest coastal river system in New South Wales, rising near the Queensland border, some 115 km north-west of its ocean mouth at 29°25′S, 153°21′E (Fig. 1b). The total area of the catchment is ~21 900 km^2, and there are many smaller rivers and creeks which drain into it. Approximately one-third of the catchment landform is rugged or mountainous, another third is hilly to steep, one-sixth is undulating to hilly, and the remaining sixth is mainly flat (Anon. 1968). Most of the flat land is in the fertile and intensively cultivated area between Copmanhurst and the coast, where sugar cane is the principal crop. During the past 20 years, this lowland has been altered by considerable structural development for flood mitigation, involving the extensive construction of drainage channels. Steel flood gates have been installed at the river ends of these artificial channels and also of many natural creeks. These gates are top-hinged and swing toward the river, inhibiting regress of saline water into the channels and creeks. The bottom sill and weight of these gates retain water for extended periods and only allow minimal exchange of river water with channel water. These developments have resulted in a change from predominantly brackish to predominantly freshwater wetlands, and the exposure of more land, with an accompanying increase in terrestrial vegetation including species of agricultural importance (Soros-Longworth and McKenzie Consulting Engineers 1980). Exposure of sulphidic subsoils has led to acidification of run-off water in some areas.

The area of the river investigated in this study was within the tidal zone from Copmanhurst to the river mouth. This tidal zone supports important estuarine/coastal finfish and crustacean

fisheries. During high flow periods, fresh water can extend to Yamba near the river mouth. During low flow periods, salinity can be detected at Grafton (80 km from the river mouth).

Materials and methods

Sea mullet from the Clarence River system were sampled at the Clarence River Fishermen's Cooperative from August 1984 to November 1985 and from September 1986 to June 1988. Approximately 100 fish were examined from each of 249 catches and lesion type and number were recorded for individual fish. Classifications of lesions described by Callinan, Fraser & Virgona (1989) were used. Necrotizing dermatitis, dermal ulcer and healing lesions are referred to as stages 1, 2 and 3, respectively, in this study. Stage 1 was not identified and recorded until April 1985; erythematous dermatitis lesions and intermediate-type dermatitis lesions were excluded. Prevalence estimates were based on the number of fish with one or more lesions for each lesion stage. During outbreaks, fishermen were asked to bring in for examination diseased fish that normally would have been discarded and details for marketed and discarded fish were recorded separately. In order to estimate prevalence for the whole catch, records were culled randomly from the over-represented component of the catch (usually the discarded fish) so that the sample weights were in the same proportions as the total weights of marketed and discarded fish in the catch. The sites of capture of these samples were dictated by movements of fishermen, probably reflecting movements of fish; however, regular samples were usually obtained from both the upper and lower estuarine sections of the river. In this paper, the upper and lower estuarine sections of the river refer, respectively, to the areas above and below the junction of the north arm and the main channel of the Clarence River (located between Maclean and Harwood, Fig. 1).

While information available on when red spot disease first occurred in the Clarence River is inconclusive, it is felt that there has been a significant increase since 1972 because the prevalence levels recorded during this study are likely to have been noted and to have led to a public outcry had they occurred before 1972. Therefore, river flow and rainfall data from 1972 to 1988 were compared with historic data from 1929 to 1972. Daily river flow records from gauging stations at Lilydale on the Clarence River and Bawden Bridge on the Orara River were obtained from the Water Resources Commission of New South Wales (Fig. 1b). To obtain an index of flow from the upper catchment, records from Lilydale gauging station alone were used because (1) its catchment includes most (93%) of the catchment area above Grafton, and (2) because of a complicating backing-up effect of the Orara River during large river discharges (Glaister 1978). To obtain an index of rainfall in the lower catchment, daily rainfall records for seven gauging stations were obtained from the National Climate Centre, Victoria. These stations were Copmanhurst, Grafton Post Office, Ulmarra, Brushgrove, Lawrence Post Office, Maclean and Yamba Pilot Station (Fig. 1). As there were some discontinuities in these data, statistical analyses (Kendall coefficient of concordance W, corrected for ties) were done to determine whether rainfall patterns amongst stations were correlated. There was a significant positive correlation ($P < 0{\cdot}001$) for weekly (Sunday to Saturday) rainfall between these rain stations for the years 1904 to 1988, indicating that the stations with continuous data therefore could be used to represent rainfall in the lower catchment, at least at the weekly interval level.

Analyses of variance were done on available annual and seasonal records of river flow at Lilydale and rainfall in the lower catchment (using an average of the stations at Grafton Post Office, Maclean and Yamba Pilot Station) to examine whether the last 17-year period differed from previous periods for these factors. The annual and seasonal mean values for both river

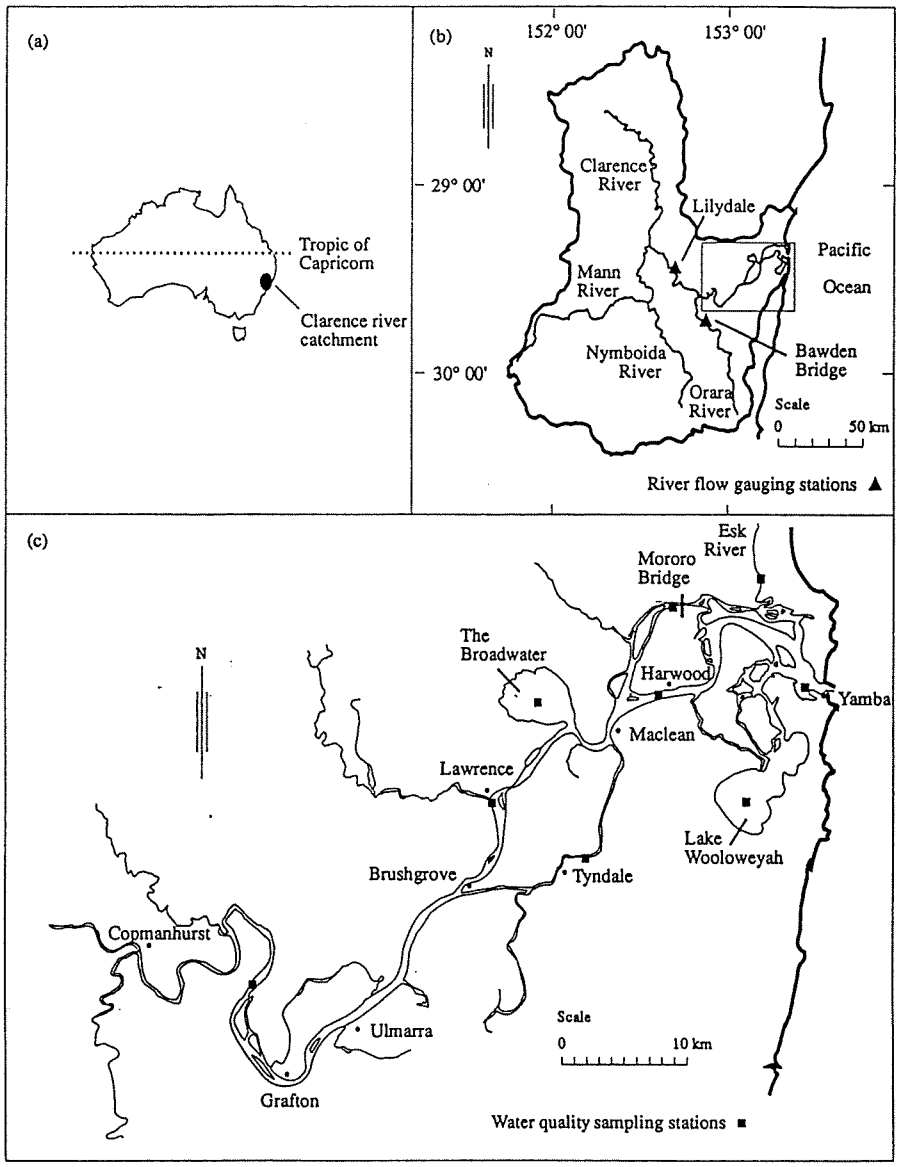


Figure 1. The Clarence River: (a) catchment; (b) river flow gauging stations; and (c) water-quality sampling stations.

flow and rainfall between the periods 1929–1945, 1950–1966 and 1971–1987 (the latter being that during which disease outbreaks have been reported) were compared. Year groups were separated by 5 years because auto-correlation was detected ($P < 0{\cdot}05$) for rainfall at a lag of 4 years for annual values and at a lag of 3 years for summer values. Log(10)-transformed data were used to better approximate normal distributions and to stabilize variances (Bartlett's test — $P > 0{\cdot}05$). Variances for autumn river flow data were homogeneous at a lower level of significance ($P > 0{\cdot}025$). To identify atypical rainfall or river flow at the monthly level, the data

for each month since 1972 were ranked with equivalent months for the years 1904 to 1988 for rainfall and 1922 to 1988 for river flow.

Spearman's rank correlation was used to test for correlations between prevalence of stage 1 lesions recorded in this study and both river flow and rainfall. Stage 1 was chosen because it was the earliest stage of the disease recorded, and therefore was the closest in time to the occurrence of any causal factors. Correlation matrices were compiled to relate river flow and rainfall in the lower catchment, for durations ranging from 1 to 4 weeks, with prevalence at this stage of the disease in fish from the upper and lower estuarine sections of the river at lags ranging from 1 to 12 weeks.

Characteristics of surface water [including salinity ($mg\,l^{-1}$), temperature (°C), dissolved oxygen ($mg\,l^{-1}$), pH and turbidity (NTU)] at a representative range of locations in the lower catchment (Table 1, Fig. 1c) were obtained from surveys done by the NSW State Pollution Control Commission from November 1983 to March 1986 and from September 1986 to February 1988. These surveys were at 2–3-weekly intervals during the high rainfall priods of autumn 1985 and 1987, and at 6-weekly intervals at other times. Usually, the same order of sampling was followed on survey days to keep sampling times consistent. The measured characteristics of surface water were plotted against time to examine the patterns associated with red spot disease outbreaks.

To test whether red spot disease could be related to the presence of organochlorine insecticide residues, samples of sea mullet from the Clarence River were frozen and sent to the Department of Agriculture and Fisheries' chemical laboratory at Lismore for analysis. At 6-weekly intervals from June 1984 to May 1985, approximately six each of diseased (one or more stage 2 lesions) and non-diseased fish were analysed. McDougall (1987) did the following laboratory and statistical analyses: whole fish were minced, 10 g of tissue from each fish was ground with 80 g of sodium sulphate, the fish fat was eluted with petroleum ether and a portion of the fat cleaned using florisil. Extracts were then evaluated by GLC/ECD for organochlorine residues. For the residue types detected, differences in the mean levels between diseased and non-diseased fish were examined using *t*-tests on natural logarithms of the data. Means and variances for the data were estimated by the method of maximum likelihood to allow for levels below the minimum detectable levels of the testing equipment.

Table 1. Details of water quality sampling sites

Site	Distance upstream (km)	Salinity ($mg\,l^{-1}$) recorded during the present study		Location in relation to flood mitigation area	Water body type
		Average	Range		
Above Grafton	80·5	0·2	0–4	Above	Main channel
Tyndale	34·6	1·5	0–11	In	North arm
Lawrence	34·4	2·9	0–15	In	Main channel
Broadwater	32·0	4·3	0–16	In	Lagoon
Harwood	16·0	10·6	0–25	In	Main channel
Mororo Bridge	16·4	9·9	0–27	In	North arm
Lake Wooloweyah	16·7	18·9	0–30	In	Lagoon
Esk River	8·5	14·7	0–32	Above	Tributary
Yamba	3·0	22·3	0–36	In	Main channel

Results

Six outbreaks of red spot disease were reported by the Clarence River Fishermen's Cooperative during the years 1972–1984 and a further three observed during this study, usually occurring during autumn and being preceded by above average summer and/or autumn rainfall in the lower catchment (Fig. 2a). There was no clear relationship between outbreaks of red spot disease and seasonal river flow (Fig. 2b). During this study, the latter part of the outbreak in early 1984 was observed, followed by a further three outbreaks in the autumns of 1985, 1987 and 1988 (Fig. 3b,c). There was a suspension of disease monitoring from December 1986 to August 1987; however, no outbreaks involving stage 2 or greater were reported by the Fishermen's Cooperative during this period. The prevalence of stage 1 was low compared to that of stages 2 and 3 (Fig. 3a,b). Stages 1, 2 and 3 were involved in each of the observed outbreaks since 1984, except for that in November 1986, when the increase in prevalence of stage 1 lesions did not result in a subsequent increase in the prevalence of stage 2 (Fig. 3a,b). Increases in the prevalence of stage 1 lesions usually occurred after peaks in rainfall in the lower catchment (Fig. 3a,d).

There were no significant differences (ANOVA, $P < 0{\cdot}05$) in mean annual or any seasonal river flow or rainfall values between the year groups 1929–1945, 1950–1966 and 1972–1987 (Fig. 4a,b). Examination of monthly data revealed some atypical values in the period since the beginning of 1972 (Tables 2 & 3). At the time of the first reported outbreak of red spot disease, late in 1972, October rainfall in the lower catchment was not only the highest for the 85-year period, but also 2·5 times greater than the closest value, which was recorded in 1914. River flow from the upper catchment for the same months was also the highest recorded, but only 1·2 times greater than the closest value, which was recorded in 1950. Record highs for rainfall and river flow also occurred in March 1974 and April 1988. Although outbreaks of red spot disease have been reported after each of these atypical months, other outbreaks were reported during less extreme monthly rainfall and river flow conditions.

There were strong correlations between the prevalence of stage 1 lesions and both rainfall and river flow at the weekly level, but their patterns differed markedly (Tables 4 & 5). Stage 1

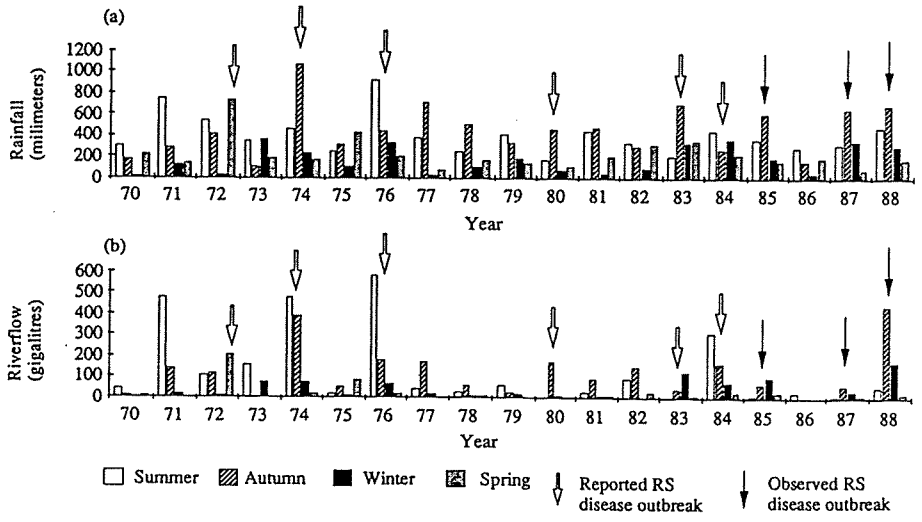


Figure 2. Outbreaks of red spot disease from 1970 to 1988 compared with (a) rainfall and (b) river flow.

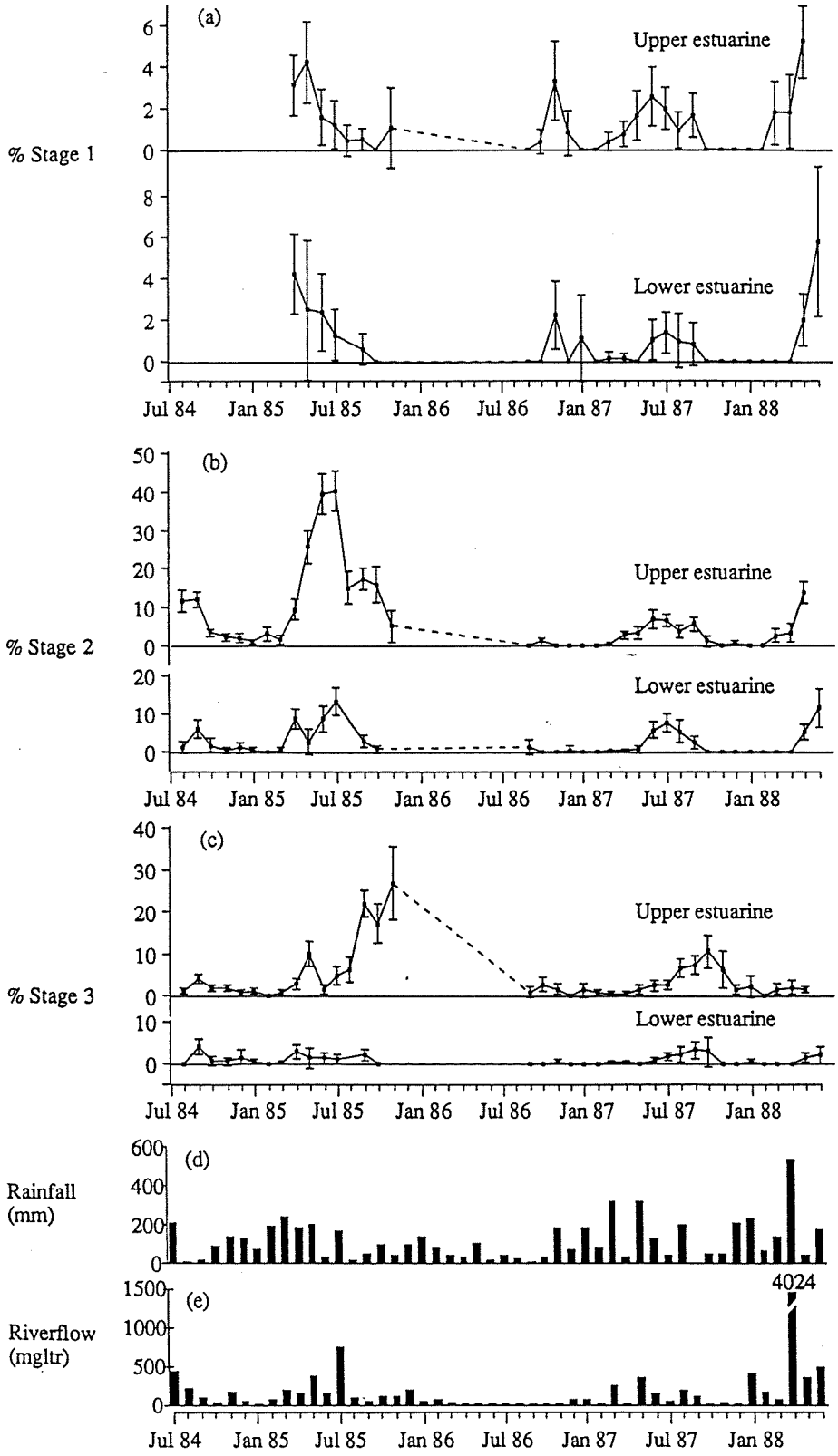


Figure 3. Prevalence (with 95% CI) of red spot disease stages (a) 1, (b) 2 and (c) 3, and relative (d) rainfall at Maclean and (e) river flow at Lilydale from 1984 to 1988.

Table 2. Ranks of monthly rainfall in the lower catchment. Rainfall for each month of the year was ranked using data from 1904 to 1988. Only the years since 1972 which had a monthly rank less than 5 are included

	Month											
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1972*	9	18	21	53	22	44	83	74	54	1‡	11	77
1974*	7	63	1‡	6	58	14	73	32	65	69	12	56
1978	38	64	4	51	46	65	49	21	18	9	74	20
1980*	36	62	74	73	2	50	54	71	85	73	37	35
1982	16	50	33	26	70	67	34	28	3	4	63	33
1988†	13	52	28	1‡	72	17	14	63	4	83	79	–

* Year during which red spot disease was reported by the Fishermen's Cooperative.
† Year during which red spot disease was observed in this study.
‡ Highest record for month of the year.

Table 3. Ranks of monthly river flow from the upper catchment. River flow for each month of the year was ranked using data from 1922 to 1988. Only the years which had a monthly rank less than 5 are included

	Month											
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1972*	21	15	19	20	22	32	47	44	51	1‡	5	13
1974*	1‡	18	1‡	9	10	9	24	19	21	31	15	33
1975	39	42	23	35	46	45	36	46	36	10	3	1‡
1976*	7	3	8	25	20	12	21	29	29	24	21	49
1980*	57	58	61	63	2	35	51	52	60	60	54	52
1988†	22	38	49	1‡	13	11	4	17	7	30	–	–

* Year during which red spot disease was reported by the Fishermen's Cooperative.
† Year during which red spot disease was observed during this study.
‡ Highest record for month of the year.

was associated with river flow over a broad range of time lags commencing with zero lag (Table 5), while associations with rainfall in the lower catchment occurred at discrete lags (Table 4). In the upper estuarine section of the river, the lag in stage 1 was 3–4 weeks, while in the lower estuarine section it was 6–9 weeks (Table 4).

Figure 5 illustrates the patterns of changes in various water-quality factors at the sites detailed in Table 1, and show the times when outbreaks of red spot disease commenced in terms of the prevalence of stage 1 and stage 2 lesions. The period monitored includes the outbreaks of 1985, 1986 and 1987, but not that of 1988. Prior to the outbreaks of 1985 and 1987, which both progressed from stage 1 to stage 2, there were similar changes in most factors. There were substantial declines in salinity (Fig. 5a) at most sites except at the Grafton site where salinities were already close to zero. Temperature (Fig. 5b) fluctuated in the upper range (24–30°C) at all sites. Dissolved oxygen (Fig. 5c) decreased to the low range (4–6 $mg\,l^{-1}$) at the upper estuarine sites. pH (Fig. 5d) decreased at most sites and generally remained above 6. There were peaks in turbidity (Fig. 5e) at most sites and large fluctuations in Lake Wooloweyah in 1985, coinciding with (and possibly due to) the prawn trawling season (October to May). However, prior to the 1986 outbreak, which did not progress from stage 1 to stage 2, these

Table 4. Correlation matrix showing significant associations (Spearman's Rank) between accumulated rainfall, for varying durations, and the prevalence of necrotizing dermatitis lesions (stage 1), at varying time lags, in the upper and lower estuarine sections of the river*

Weeks of accumulated rainfall	Time lag (weeks)											
	0	1	2	3	4	5	6	7	8	9	10	11	12
Upper estuarine													
4		+	++	+	+								
3			+	+	+								
2			+	++	+								
1				+	+								
Lower estuarine													
4							+++	++	+				
3							+	++	+	+			
2									++	+			
1							+			+			

* Significant positive correlation: +, $P<0{\cdot}05$; ++, $P<0{\cdot}01$; +++, $P<0{\cdot}001$.

Table 5. Correlation matrix showing significant associations (Spearman's Rank) between river flow volume, for varying durations, and the prevalence of necrotizing dermatitis lesions (stage 1), at varying time lags, in the upper and lower estuarine sections of river*

Weeks of accumulated rainfall	Time lag (weeks)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Upper estuarine													
4	+++	+++	+++	++	++		+	+					+
3	++	+++	+++	++	++	+		+					
2	+++	+++	+++	+++	++	+		+	+	+			
1	+++	+++	++	+++	++	+				+			
Lower estuarine													
4	++	++	++	+++	++	+++	+++	+					
3	++	++	++	++	++	+++	+++	++					
2	++	++	+	++	++	+++	+++	++	+				
1	++	++	+	+	++	++	++	++	+				

* Significant positive correlation: +, $P<0{\cdot}05$; ++, $P<0{\cdot}01$; +++, $P<0{\cdot}001$.

factors were relatively stable, with different trends than those above for most factors. Salinity was in the upper range ($>20\,mg\,l^{-1}$) at the lower estuarine sites and the mid to low range ($0-20\,mg\,l^{-1}$) at the upper estuarine sites. Temperature was increasing in the mid (18–24°C) to upper range at all sites. Dissolved oxygen decreased slightly, within the $6-10\,mg\,l^{-1}$ range at most sites. pH was within the 7–9 range at the upper estuarine sites and was stable at approximately 8 at the lower estuarine sites. Turbidity remained low at upper estuarine sites and increased slightly at most of the lower estuarine sites. Stage 1 fish recorded in October

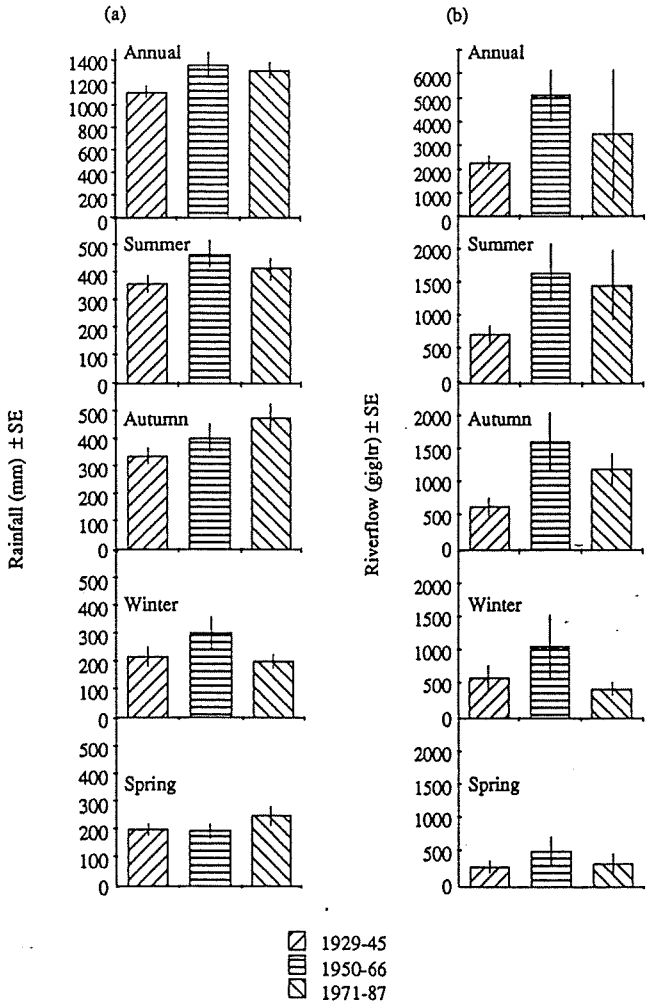


Figure 4. Mean annual and seasonal values for (a) rainfall and (b) river flow between the year groups 1929–1945, 1950–1966 and 1971–1987.

and early November 1986 were preceded only by 2–3 isolated days of rainfall in the lower catchment, in an otherwise very dry period.

Low levels of dieldrin (average of 0·011 mg kg^{-1}) and DDT (average of 0·023 mg kg^{-1}) were found in the sea mullet samples but no significant differences in the levels of these organochlorines were found between diseased and non-diseased fish ($P > 0{\cdot}05$) (McDougall 1987).

Discussion

The findings of a concurrent study in the Clarence River regarding disease agents (Callinan *et al.* 1989; Callinan & Keep 1989) suggested that there were two stages leading to dermal ulceration. Necrotizing dermatitis lesions (stage 1) result from invasion of the dermis and underlying skeletal muscle by fungi. Lesions then develop into dermal ulcers (stage 2) with associated severe necrotizing granulomatous myositis. There has been a world-wide increase

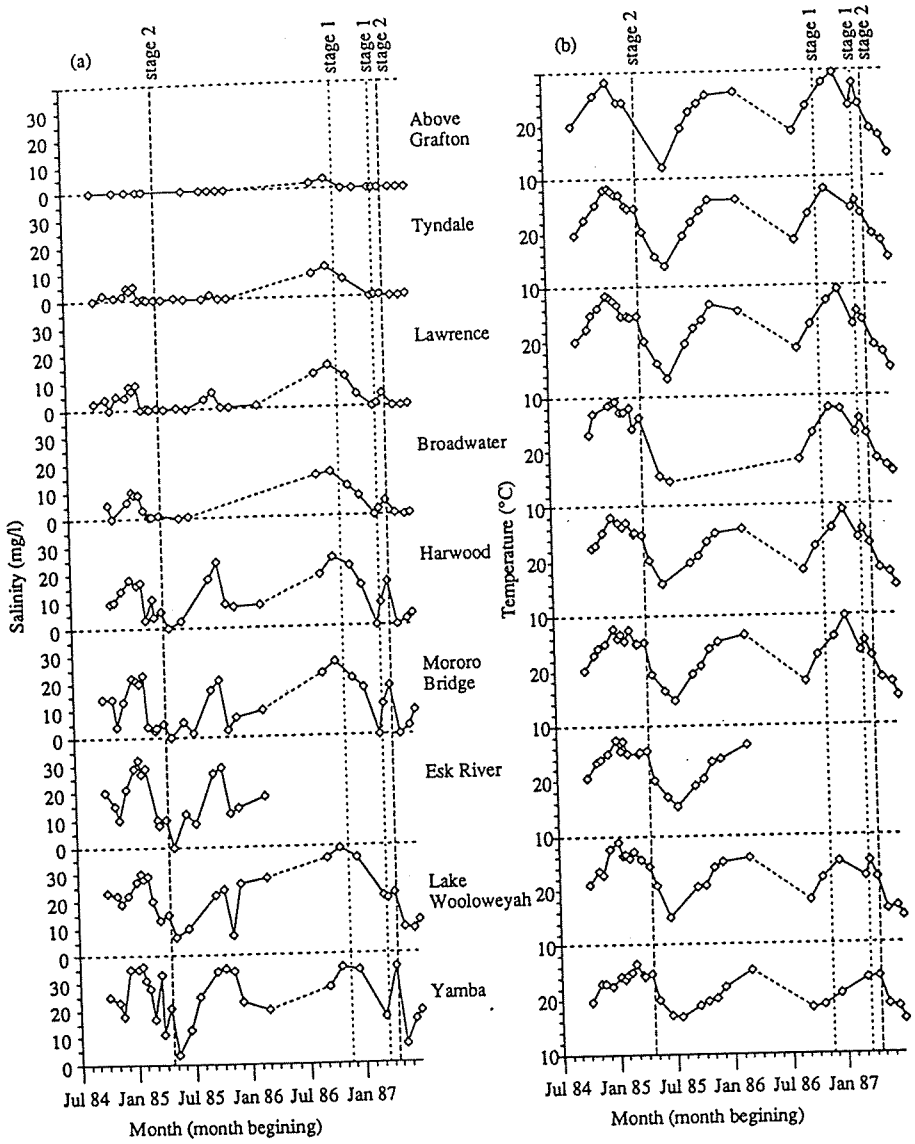


Figure 5. Patterns of changes in (a) salinity, (b) temperature, (c) dissolved oxygen, (d) pH and (e) turbidity at the water-quality sampling sites in Table 1.

in the reporting of ulcerative diseases in estuarine fish over the last 2 decades. These cases could involve man-made or natural environmental changes, the spread of exotic disease agents and/or a greater awareness of the presence of these diseases. A basic assumption in this paper is that the reporting of red spot disease from the Clarence River since 1972 reflects a true increase in its prevalence and is not due to increased awareness.

Since 1972, when outbreaks of red spot disease began being reported in the Clarence River, rainfall in the lower catchment and river flow from the upper catchment were shown to be typical of the last 60 years, therefore discounting the notion that changes in rainfall or river flow alone are responsible for the disease. However, strong associations between rainfall, river

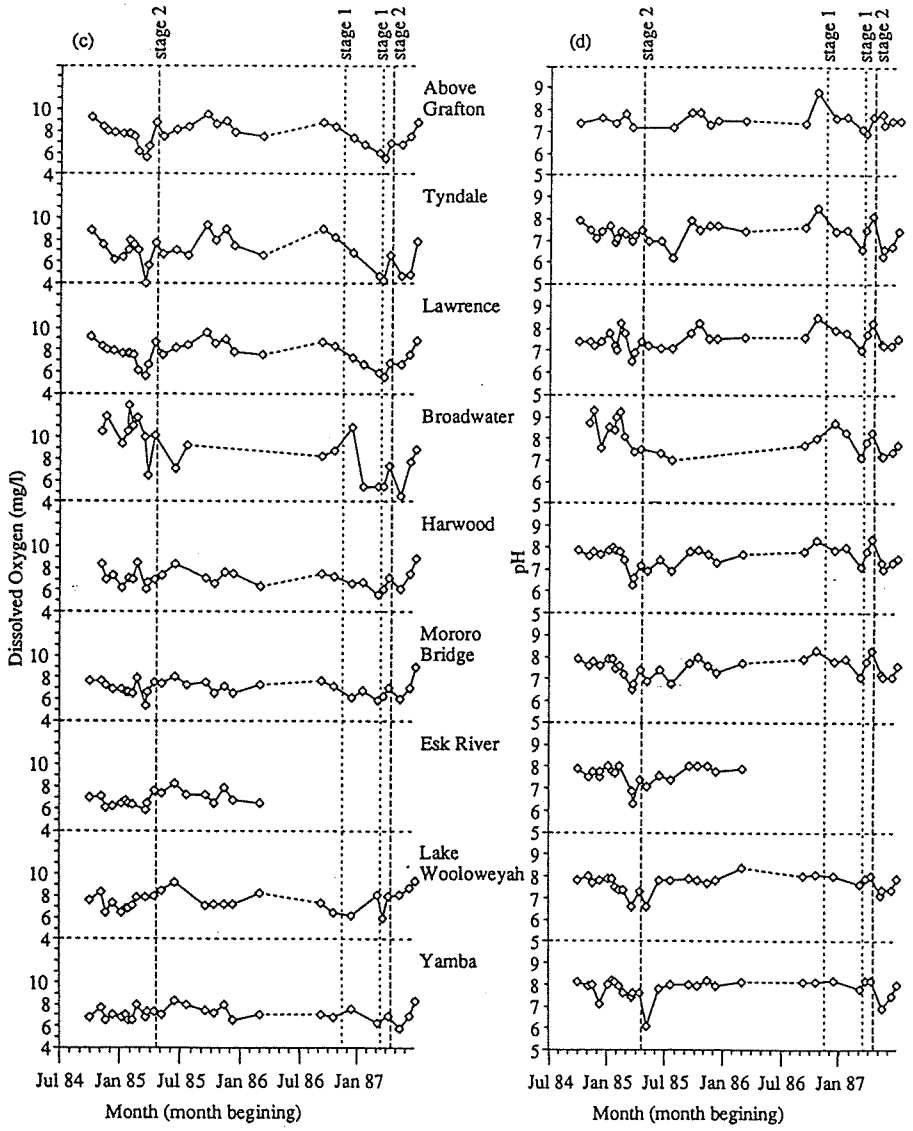
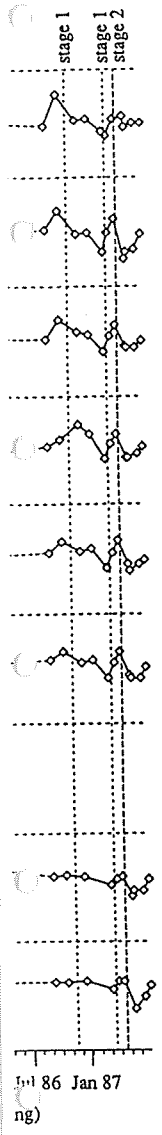


Figure 5. (Continued.)

flow and outbreaks of red spot disease monitored in this study strongly suggest that other factors have exacerbated the effects of, or changed the nature of, the run-off water and so facilitated outbreaks of the disease. In particular, high rainfall in the lower catchment is strongly associated with stage 1 of red spot disease. Examination of water-quality data indicated that rapid changes in the characteristics of river water occurred after high rainfall and river flow prior to the observed autumn outbreaks of red spot disease. In contrast, the characteristics of river water were relatively stable in November 1986, though stage 1 of the disease still occurred. This suggests that potentially stressful conditions in the river are not necessary for stage 1 lesions to occur initially.

Analysis of rainfall and river flow data at the annual and seasonal levels showed that the last 17 years were not very different from previous years. Pittock (1975) reported a secular



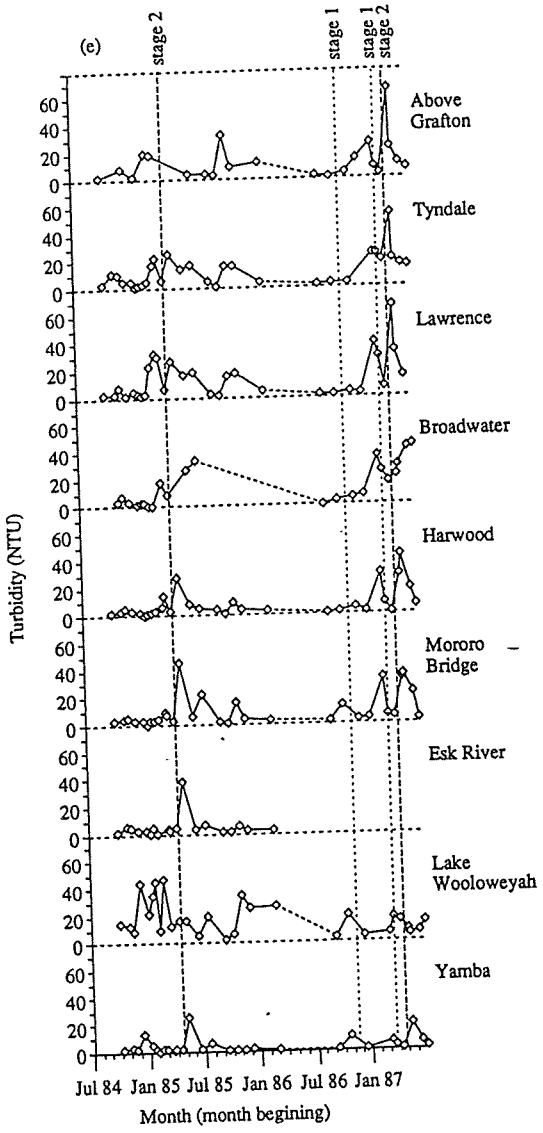


Figure 5. (Continued.)

increase in annual rainfall over much of eastern Australia around 1945–1946. In the Clarence River, mean annual river flow and rainfall in the lower catchment were higher in the period 1950–1966 than in 1929–1945, but the difference was not statistically significant. In any case, the time of this reported secular increase was well before the commencement of reporting of red spot disease. However, at the monthly level, there were some atypical records; in particular, the record highs of rainfall in the lower catchment in October 1972 and March 1974. A possible scenario is that this atypically high rainfall may have triggered off a normally latent disease, but more likely, this may have been the first occasion when exacerbating factors (either a pathogen and/or a non-infectious agent) came into effect.

The November 1986 outbreak was very different from three other observed outbreaks. Those in 1985, 1987 and 1988 progressed through stages 1, 2 and 3, and followed the typically

high rainfall periods in autumn with high rainfall and high river flow. However, the November 1986 outbreak, which did not progress beyond stage 1, commenced after a few isolated days of high rainfall in the lower catchment and in an otherwise very dry period in the whole catchment, implying that amongst these factors stage 1 may be related only to rainfall in the lower catchment. While there were significant positive correlations between both rainfall and river flow, for various durations, and stage 1 lesions, at various lags in time, the patterns of these associations were different. The pattern between river flow and stage 1 was general, whereas that between rainfall in the lower catchment and stage 1 involved specific timing, supporting the idea that the relationship with rainfall in the lower catchment may be related to causal factors. An association between high local rainfall and disease outbreaks has been noted in several Australian cases of cutaneous fish ulcer disease (Rodgers & Burke 1981; Humphrey & Langdon 1986; Gwyther & McShane 1984). The general pattern between river flow and stage 1 could be due to (1) the persistent nature of river flow and its coincidence with rainfall, or (2) the high rainfall in the lower catchment and low river flow conditions of November 1986 being under-represented in the correlations.

The low proportion of fish infected with stage 1 of the disease compared to stages 2 and 3 suggests that stage 1 has a shorter duration than stages 2 or 3 (Fig. 3a,b). The presence of stage 3 (Fig. 3c) provides evidence of recovery from red spot disease. However, it cannot be directly compared with stage 2 because it is based on population size after any mortality due to the disease, the difference in persistence and the relative ease of identification of stages 2 and 3. The difference in the rainfall/stage 1 lag between the upper and lower estuarine sections of the river (approximately 4 weeks) implied that (1) red spot disease was initiated in the upper estuarine section of the river, with subsequent dispersion of stage 1 infected fish into the lower estuarine areas, or (2) there is a lag in initiation in the lower estuarine section of the river. This is contrary to the findings of Rodgers & Burke (1981) who noted that sea mullet in the Noosa River, Queensland, which exhibited early lesions were located initially near the river mouth.

Snieszko (1974) provides many examples showing the coincidence of infectious diseases with environmental stress. In the present study, there was evidence of possible environmental stress to fish in the main water compartments of the lower catchment during the autumn periods of 1985 and 1987, prior to outbreaks of red spot disease. Temperature fluctuated in an upper range (24 to 30°C) at all sites. Huizinga *et al.* (1979) postulated that the 'red sore disease' in largemouth bass, *Micropterus salmoides* (Lacépède), was linked to elevated water temperatures stimulating increased metabolism and decreased body condition, leading to the increased production of corticosteroids and a concomitant rise in susceptibility to infection. Studies in Albemarle Sound, North Carolina, USA, found a positive correlation between temperature and the abundance of *Aeromonas hydrophila* (the proposed aetiological agent for red-sore in that system — Hazen 1983, and which is also associated with red spot disease in the Clarence River, Callinan & Keep 1989). Rodgers & Burke (1981) reported that outbreaks were associated with rapidly changing and decreasing temperatures. A temperature decrease was also observed during the present study, but only after the commencement of both stages 1 and 2, and therefore, was not indicated as a necessary condition for the initiation of red spot disease.

The patterns of other water quality factors prior to the 1985 and 1987 outbreaks also may have been stressful to fish. The sharp decreases in salinity at most sites indicated that osmoregulatory stress may be implicated, supporting the findings of Rodgers & Burke (1981) to this effect. Dissolved oxygen decreased in the upper estuarine sites. A reduction in dissolved oxygen has been associated with ulceration in channel catfish (Plumb, Grizzle & Defigueiredo 1976). Hazen (1983) also found that declining levels of dissolved oxygen were associated with

an increase in abundance of *A. hydrophila*. The decline in pH and increase in turbidity were also possible stressful factors.

In contrast to the above, the patterns of water quality factors were different and relatively stable prior to the November 1986 outbreak of stage 1 lesions. Temperatures were in the mid to upper range, dissolved oxygen and pH were not at stressful levels, and turbidity was low. The high salinity in December 1986 indicated that water discharge into the river from rainfall in the lower catchment during November 1986 (Fig. 5a) was insufficient to cause significant long-term changes in water quality.

There was no appreciable difference in the patterns of water quality factors between stations above the flood mitigation area (Grafton and Esk River) and those below, indicating that the trends in water quality factors measured were dictated largely by the quality of water from above the flood mitigation area.

The findings of this study suggest that the dermal ulcers (stage 2) typical of red spot disease in the lower Clarence River are the result of the presence of predisposing necrotizing dermatitis and are associated with rapid changes in environmental conditions. The cause of necrotizing dermatitis is the key to the aetiology of red spot disease but this is not clear. It appears to be related to rainfall in the lower catchment and may occur without otherwise stressful conditions occurring in the main compartments of the lower catchment. This suggests that a noxious stimulus (either a pathogen and/or a non-infectious agent) may be introduced into the river after rain flushes in the lower catchment. The occurrence of an exotic pathogen is possible; however, the historical coincidence of red spot disease with flood mitigation would suggest that changes in land use may have favoured particular pathogens. Organochlorine insecticides do not appear to be involved in red spot disease (McDougall 1987).

Regardless of the precise mechanism, this study has indicated that causal factors of red spot disease may be related to the quality or some characteristic of run-off water in the lower catchment. The principal factor that has influenced this run-off water over the last 20 years has been the development of structural flood mitigation and the subsequent increase in cultivation in this area.

It is suggested that occasions with high rainfall in the lower catchment and low river flow occurring together should be examined further to confirm the relationship between rainfall in the lower catchment and stage 1 of the disease. If confirmed, studies of red spot disease and water quality on such occasions may help elucidate causal factors without the complicating effects of river flow.

The public reportings of fish with red spot type lesions from many NSW estuaries suggests that disease agents may be widespread. There could be an association between disease agents and land use in river estuaries, and/or the stresses in highly variable river environments may allow outbreaks of red spot disease to occur. Comparative studies of land use, water quality and the prevalence of red spot disease between estuary types would be of great value in understanding the aetiology of red spot disease.

Acknowledgments

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Pathology of red spot disease in sea mullet, *Mugil cephalus* L., from eastern Australia

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Abstract. Lesions present on sea mullet, *Mugil cephalus* L., during the first 3 weeks of red spot disease outbreaks are described. Necrotizing dermatitis is a severe, locally extensive, granulomatous lesion associated with invasion of dermis and underlying skeletal muscle by numerous, non-septate, fungal hyphae 12–18 μm in diameter. Erythematous dermatitis is a mild to severe, focal, chronic active dermatitis without fungal involvement. Lesions intermediate between these two forms, with small to moderate numbers of fungal hyphae in dermis and skeletal muscle also occur. Findings indicate that erythematous dermatitis lesions and intermediate-type lesions subsequently resolve, while necrotizing dermatitis lesions consistently develop into dermal ulcers, with associated severe necrotizing granulomatous myositis. Generally, dermal ulcers occur significantly more often on posterior and dorsal areas of the body surface than on anterior and ventral areas. Lesions heal by combinations of epidermal and dermal repair, fibroplasia, destruction of fungi, removal of necrotic skeletal muscle and regeneration of myofibres. Atrophy of exocrine pancreas occurs in both diseased and clinically normal fish, but is generally more severe in diseased fish. The possible roles of suspected disease determinants, including falls in dissolved oxygen concentrations to sub-lethal levels prior to red spot disease outbreaks, are discussed.

Introduction

Red spot disease (RSD) is a syndrome of cutaneous ulceration affecting several species of estuarine fish in New South Wales and Queensland, Australia. Although the cause of the disease is unknown, outbreaks occur after periods of heavy rainfall in the lower catchment areas of coastal river systems. Burke & Rodgers (1981) suggested that the ulcers of RSD are initiated by *Vibrio anguillarum* on the skin of fish stressed by salinity and temperature changes. They also suggested (Rodgers & Burke 1988) that the ectoparasitic digenean *Prototransversotrema steeri* initiates some lesions of RSD in sea mullet, *Mugil cephalus* L., by breaching the epidermis during feeding, thereby providing sites for infection by *V. anguillarum* and other *Vibrio* spp. However, Callinan & Keep (1989) found no evidence of significant cutaneous ectoparasite infestations on diseased and normal sea mullet examined before and during a RSD outbreak. They were also unable to demonstrate significant infection by *Vibrio* spp. in early ulcers on sea mullet. Viruses have been associated with syndromes of cutaneous ulceration in fish from Europe (Jensen & Larsen 1982), south-east Asia (Frerichs, Millar & Roberts 1986; Hedrick, Eaton, Fryer, Groberg & Boonyaratapalin 1986) and the Northern Territory, Australia (Humphrey & Langdon 1986). These findings suggest that viruses may be involved in the pathogenesis of RSD.

Limited descriptions of cutaneous lesions of RSD in sea mullet have been presented by McKenzie & Hall (1976) and Rodgers & Burke (1981). The aims of the present study were to

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describe lesions associated with the disease in sea mullet and to relate findings to possible causative agents.

Materials and methods

Between 1984 and 1988, clinically normal sea mullet and sea mullet with cutaneous lesions consistent with RSD were collected by net from the Clarence River (29° 25′S; 153° 21′E) and Richmond River (28° 52′S, 153° 35′E) in north-east New South Wales. During this period, six outbreaks of RSD were observed. Each was preceded by several days of heavy rainfall over the lower river catchment area, defined in this study as a significant rain event, which caused moderate to severe local flooding. Estimates of the prevalence of the various lesion types described in this study were based on observations of commercial catches made during these six outbreaks. Fish were not available for examination in the 2 weeks immediately following significant rain events, as difficult field conditions prevented commercial fishing operations.

Characterization of lesions

Fish were held alive in tanks containing river water for no more than 4 h and were killed by decapitation. Abdominal and cranial cavities were opened and cutaneous or other lesions described. After fixation of each whole fish in 10% neutral buffered formalin, representative samples of cutaneous lesions or normal skin were decalcified using Gooding and Stewart's fluid (Culling 1974). These, and samples of other major organs, were embedded in paraffin wax, sectioned at 4 μm and stained with haematoxylin and eosin (H&E), Gomori methenamine silver and haematoxylin and eosin (GMS-H&E), Giemsa, periodic acid-Schiff, or Ziehl–Nielson methods. Alternatively, fish were sealed individually in plastic bags, held on ice and processed within 4 h of collection. Cutaneous lesions were described and, together with underlying skeletal muscle, were excised, fixed and processed as above. No other organs were examined from these fish.

Distribution of dermal ulcers

Diseased fish, from commercial catches on the Clarence River, were held on ice and examined within 18 h of capture. The occurrence of dermal ulcers within defined areas on the body surface, excluding fins, was recorded. Left and right sides of each fish were distinguished and then subdivided, using the margins of the operculum and various fins as landmarks (Fig. 1). Subdivisions were designated head (H), anterodorsal (AD), anteroventral (AV), posterodorsal (PD), posteroventral (PV) and caudal peduncle (C). Where an ulcer overlapped the boundary between subdivisions, it was attributed to that subdivision containing most of the lesion.

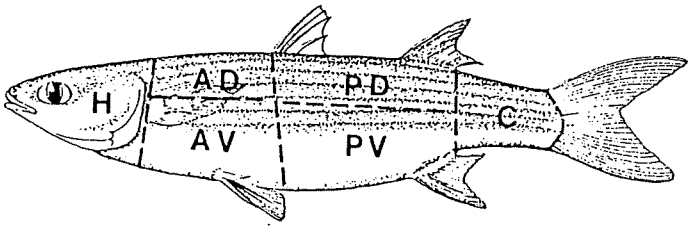

Figure 1. Sea mullet showing the surface subdivisions used for recording dermal ulcer distribution.

Surface area estimation

In order to establish mathematical relationships between subdivision surface areas and length to caudal fork (LCF), a total of 70 normal fish, comprising seven fish for each 1 cm length class between 27 and 36 cm LCF, was collected as above and the skin of each fish coated with latex. After drying, the latex over each subdivision was removed and its surface area measured with an electronic planimeter (Paton Scientific Pty Ltd, Victor Harbour, South Australia). Contingency tables were developed to test these measurements for allometric growth effects amongst the 70 fish; such effects were not significant ($P<0{\cdot}05$). The following relationships between surface area of each subdivision and LCF were then developed using least squares regressions:

$$H = 0{\cdot}0910 \times LCF^{2{\cdot}0071}$$
$$AD = 0{\cdot}0290 \times LCF^{2{\cdot}1666}$$
$$AV = 0{\cdot}0363 \times LCF^{2{\cdot}2463}$$
$$PD = 0{\cdot}0346 \times LCF^{2{\cdot}1761}$$
$$PV = 0{\cdot}0119 \times LCF^{2{\cdot}5462}$$
$$C = 0{\cdot}1212 \times LCF^{1{\cdot}7005}$$

These relationships predicted the total surface area of each subdivision in the sample of 70 fish with less than 2·1% error in any instance. They were then used to estimate total surface area of each subdivision in the sample of diseased fish. The ratios of these total surface areas of subdivisions in the diseased fish sample were used to predict the ratios of dermal ulcers in the subdivisions. The predicted and observed ratios of dermal ulcer numbers in these subdivisions were then compared using a chi-square test for homogeneity.

Results

Characterization of lesions

Gross and microscopic examinations were carried out on skin and other major organs from 73 diseased and 44 clinically normal fish. Cutaneous lesions only were examined from a further 79 fish. A total of 185 cutaneous lesions was examined. All fish measured 20–35 cm LCF.

Skin. Most diseased fish had a single lesion, although some had multiple lesions which were usually of similar type but often of different size. Lesions rarely occurred on fins. The lesions were classified as follows:

Necrotizing dermatitis. These lesions occurred 2–5 weeks after significant rain events; 27 were examined. In the first week of this period, yellowish-grey to red, ovoid domed areas, 1–4 cm in diameter, were found (Fig. 2). Dermal tissue was swollen with a macerated surface. Scales were usually absent from the lesion centre. When the lesion was immersed in water, small numbers of fungal hyphae trailed from the surface; no 'cotton wool'-like fungal growths were seen. On microscopic examination, there was moderate to severe, locally extensive, necrotizing, granulomatous dermatitis (Fig. 6). Epidermis was absent over affected areas; no cutaneous ectoparasites were seen. Haemorrhage and cells resembling osteoclasts were associated with fractures in scales at the edges of the lesions. Large numbers of sparsely branching, non-septate, fungal hyphae 12–18 μm in diameter were present, usually within granulomas, throughout the *stratum spongiosum* (Fig. 7), *stratum compactum*, and underlying subcutaneous tissue and skeletal muscle (Fig. 8). There was severe, locally extensive, floccular degeneration and

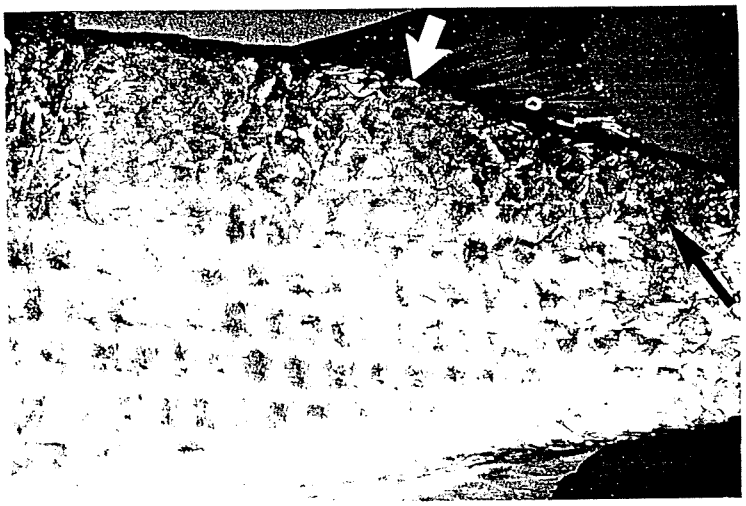

Figure 2. Necrotizing dermatitis. Three discrete lesions are present, with dermal swelling (thick arrow) and early central sloughing of necrotic dermis (thin arrow).

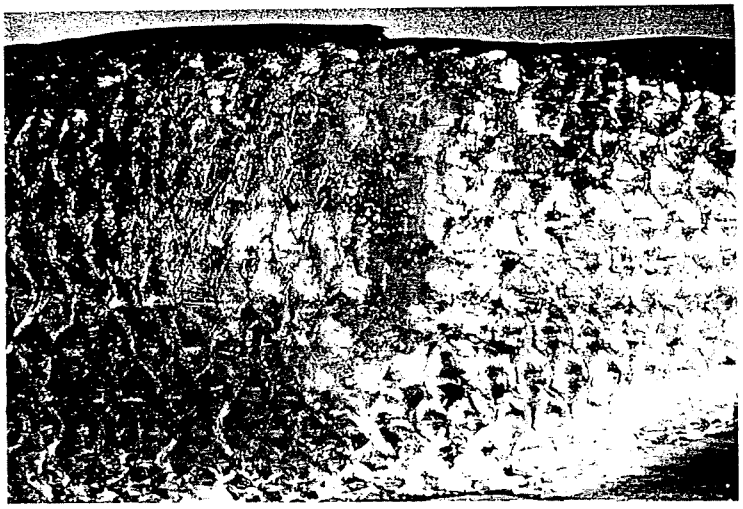

Figure 3. Necrotizing dermatitis. Sloughing of necrotic dermis from the lesion centre has exposed underlying skeletal muscle.

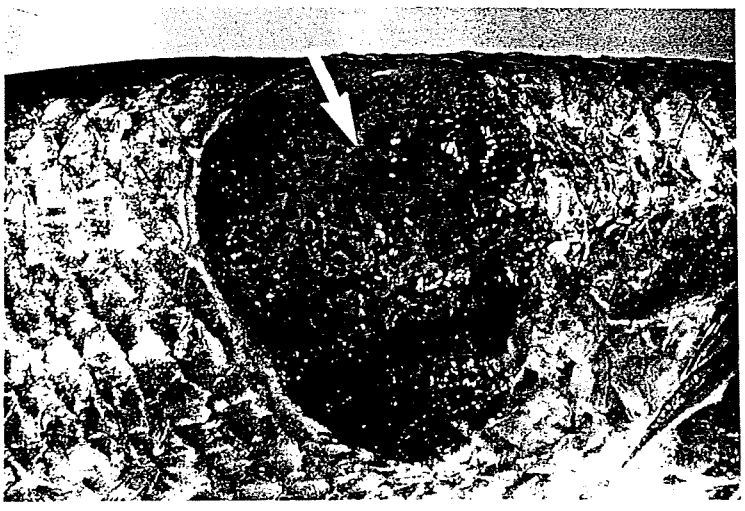

Figure 4. Dermal ulcer. Granulomas (arrow) are a response to fungal invasion of skeletal muscle.

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Figure 5. Erythematous dermatitis. The posterior margin of the affected scale is eroded (arrow).

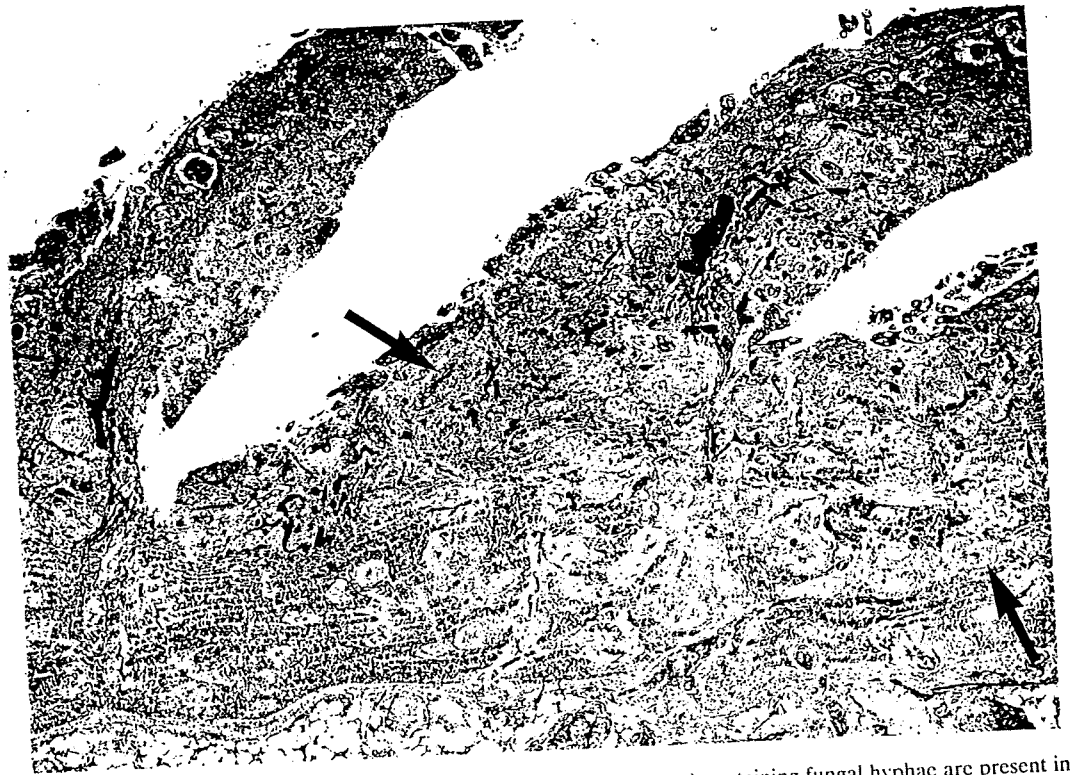
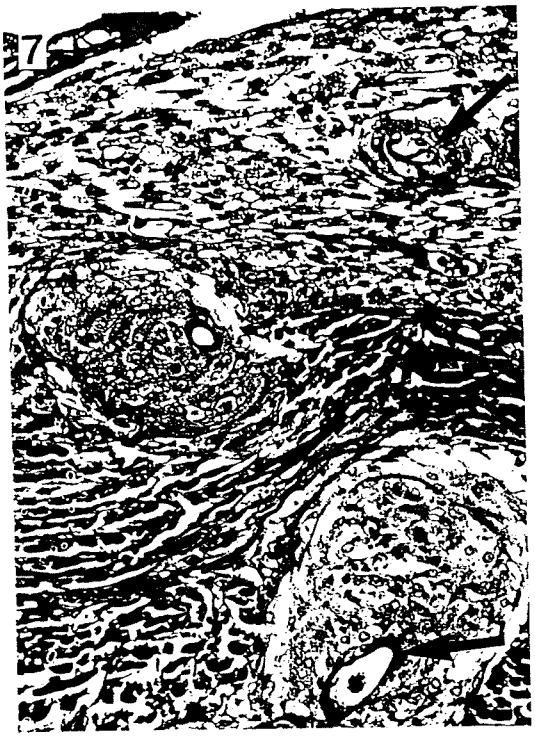


Figure 6. Necrotizing dermatitis. Large numbers of granulomas (arrows) containing fungal hyphae are present in swollen *stratum spongiosum*, *stratum compactum* and subcutaneous tissue. Epidermis and scales are absent (GMS-H&E, × 36).



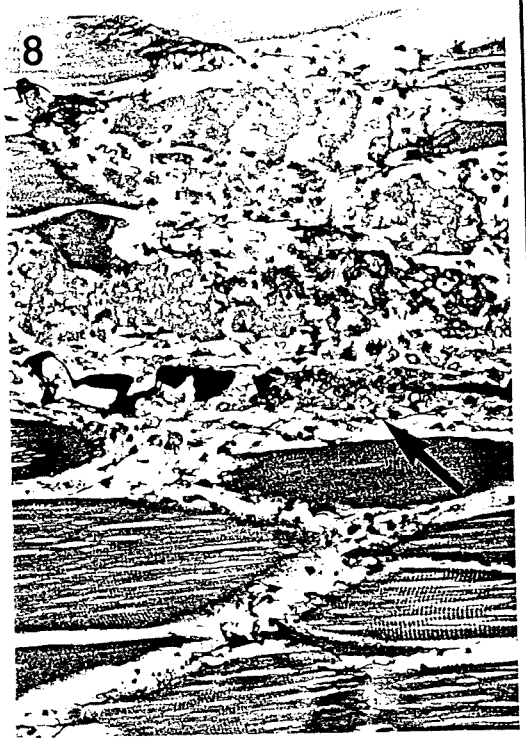


Figure 7. Necrotizing dermatitis. Fungal hyphae (arrows) within granulomas in *stratum spongiosum* and *stratum compactum* (GMS-H&E, × 300).
Figure 8. Necrotizing dermatitis. There is necrosis of skeletal muscle fibres and early granuloma formation (arrow) associated with invading fungal hyphae (GMS-H&E, × 280).

necrosis of skeletal muscle fibres. The changes were segmental, involving all elements in affected parts of fibres; endomysial connective tissue and capillaries remained intact.

Many necrotizing dermatitis lesions examined 2 weeks after significant rain events showed evidence of central sloughing of necrotic dermal tissue (Figs 2 & 3). This sloughing process apparently extended peripherally and was completed in all necrotizing dermatitis lesions within 5 weeks of significant rain events; the residual lesions were classified as dermal ulcers. Microscopically, the *stratum spongiosum* had sloughed from the centres of early, ulcerating lesions. In the centres of more advanced lesions, the *stratum compactum* had sloughed, exposing underlying skeletal muscle with severe myonecrosis and large numbers of fungal hyphae within granulomas.

Erythematous dermatitis. These lesions occurred 2–8 weeks after significant rain events; 84 were examined. Skin in affected areas was yellow with irregular reddening and scales were fractured, absent or small and fragile. Most lesions were < 1 cm in diameter, involving only single scales (Fig. 5) or small numbers of adjacent scales. Microscopically, in lesions collected 2–3 weeks after significant rain events, epidermis was present at the margins and irregularly distributed over the lesion itself. It was often hyperplastic or attenuated, oedematous and infiltrated by mononuclear cells. In the *stratum spongiosum* there was mild to severe locally extensive congestion, oedema, and mononuclear cell infiltration. Fungal hyphae and granulomas were not demonstrable and cutaneous ectoparasites were never seen. Mature scales often had

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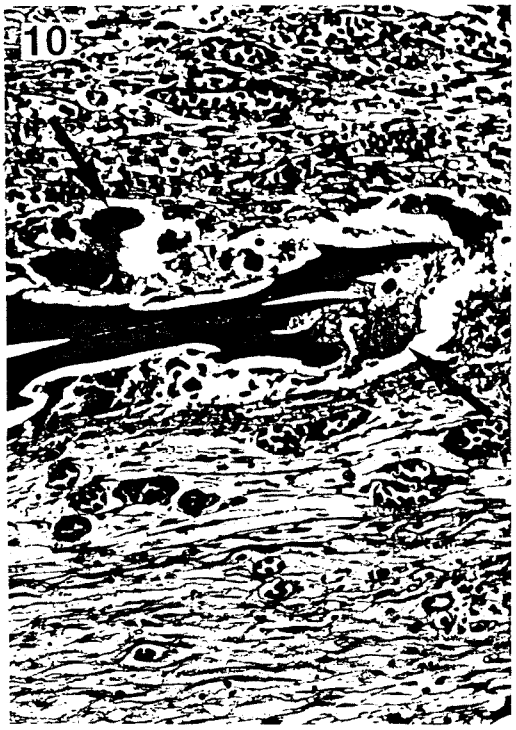
irregular surfaces and fractures (Fig. 9) and cells resembling osteoclasts were usually associated with fragments or damaged areas of scales (Fig. 10). Many scale pockets were empty. *Stratum compactum*, subcutaneous tissue and skeletal muscle were normal. In lesions collected subsequently, there was consistent evidence of repair, including re-establishment of epidermal continuity, regeneration of lost scales (Fig. 11) and remodelling of damaged scales.

Intermediate-type dermatitis. Thirty skin lesions classified as intermediate in type between necrotizing dermatitis and erythematous dermatitis were examined. Such lesions occurred 2–12 weeks after significant rain events and were approximately 1 cm in diameter. On gross examination they resembled lesions of necrotizing dermatitis except that they were smaller and scales were often retained. Microscopically, in lesions collected early in this period, epidermis was absent over the lesion and irregular fractured mature scales were often present. There was mild to moderate chronic active dermatitis with small to moderate numbers of fungal hyphae and associated granulomas in the *stratum spongiosum*, *stratum compactum* and underlying skeletal muscle. Degenerative and necrotic changes in skeletal muscle were present in many cases, but were always less extensive than those seen in necrotizing dermatitis lesions. In intermediate-type lesions collected later in the period, the *stratum spongiosum* and *stratum compactum* were intact. There was evidence of epidermal and scale regeneration as well as destruction of hyphae and necrotic muscle within granulomas.

Figure 9. Erythematous dermatitis. Epidermis (arrow) is absent over the lesion. There is diffuse congestion, oedema and cellular infiltration of *stratum spongiosum*. Mature scales are fractured and have irregular surfaces (H&E, × 38).



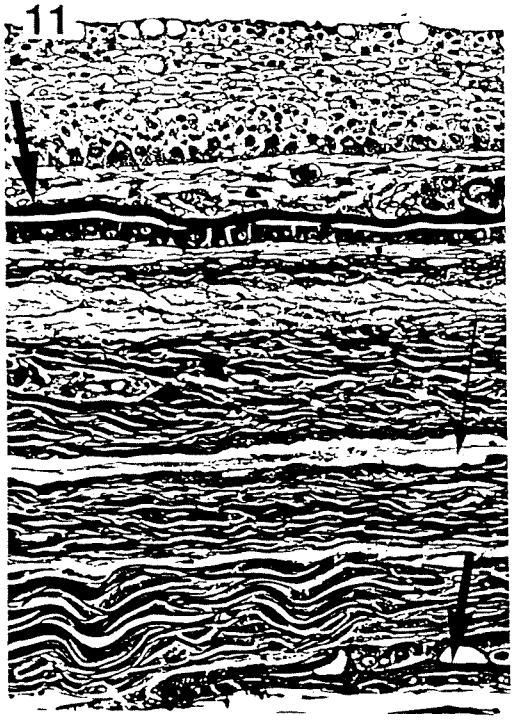


Figure 10. Erythematous dermatitis. There is diffuse vascular congestion, oedema and mononuclear cell infiltration in the *stratum spongiosum*. Cells resembling osteoclasts (arrows) are associated with a scale fragment (H&E, × 280).

Figure 11. Erythematous dermatitis. There is evidence of healing with re-establishment of epidermal continuity and scale regeneration (short arrows). One scale pocket (long arrow) lacks a scale (H&E, × 280).

Dermal ulcer. Forty-four lesions classified as dermal ulcers were examined. Most were 1–4 cm in diameter. Typically, the margins were sharply defined and skeletal muscle was exposed up to 1 cm below the skin surface. There were usually large numbers of discrete, firm, pale, circular or linear areas, up to 1 mm in diameter and 4 mm in length, on the ulcerated surface (Fig. 4) and extending into underlying soft tissues. In some cases, bone or viscera were exposed. Microscopically, epidermis and dermis were present only on the periphery of the lesion. There was moderate to severe diffuse granulomatous myositis (Fig. 12). Hyphae, and in many cases necrotic muscle fibres, were present within granulomas (Fig. 13). Fungi rarely penetrated beyond skeletal muscle to involve internal organs.

Dermal ulcers, many with evidence of healing, were most common 4–16 weeks after significant rain events. Smooth-surfaced, pale, greyish-yellow areas with irregular reddening were present on the margins of healing ulcers. Microscopically, proliferating, often hyperplastic, epidermis extended over the surface of the ulcer from the periphery, covering granulation tissue and granulomas. Scale regeneration and limited skeletal muscle regeneration accompanied these changes.

Pancreas. No lesions were seen on gross examination. Microscopically, there was mild to severe atrophy of exocrine pancreas. Atrophy was generally most severe in fish with dermal ulcers, although normal fish and those with dermatitis were also affected. There was an

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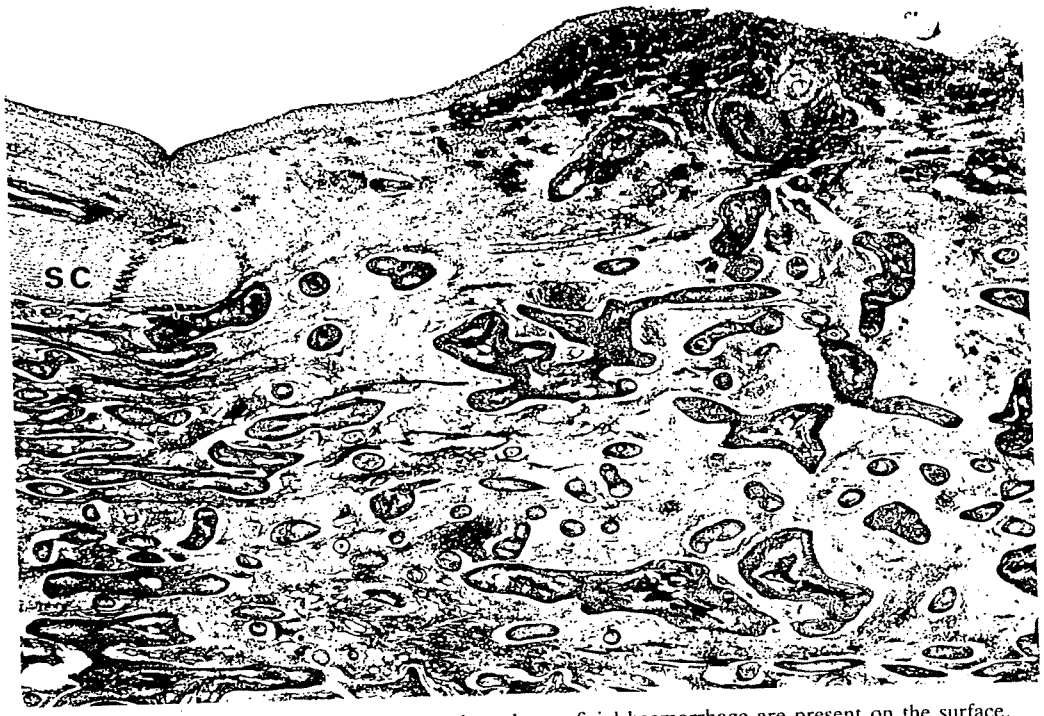
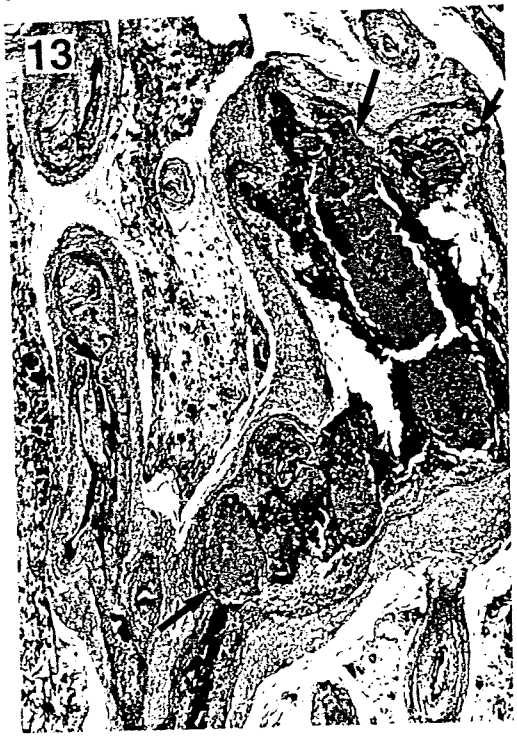


Figure 12. Dermal ulcer. Regenerating epidermis and superficial haemorrhage are present on the surface. Oedematous fibrovascular connective tissue and large numbers of granulomas have replaced much of the dermis, subcutaneous tissue and skeletal muscle (SC: *stratum compactum*) (GMS-H&E, × 36).



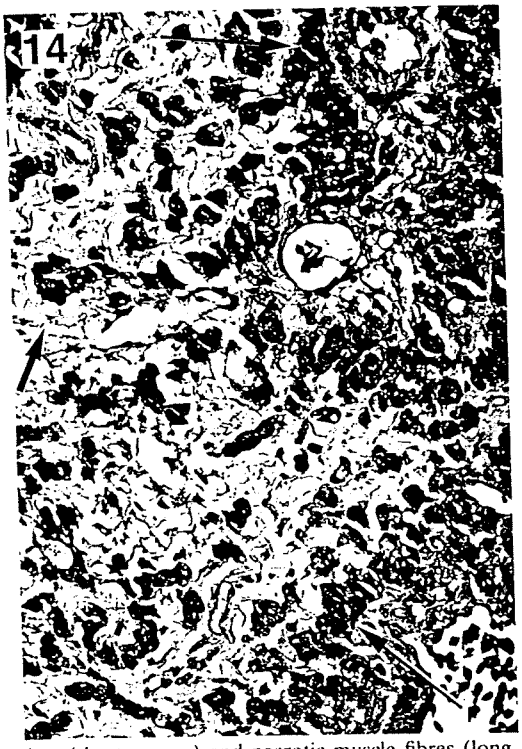


Figure 13. Dermal ulcer. Granulomas surround fungal hyphae (short arrows) and necrotic muscle fibres (long arrows) (GMS-H&E, × 140).

Figure 14. Atrophy of exocrine pancreas. Atrophic acinar cells have been replaced by fibrous tissue (short arrow); acinar cells around blood vessels (long arrows) have been spared (H&E, × 300).

increased amount of fibrous tissue and diffuse reduction in numbers of acinar cells, excepting those cells adjacent to blood vessels (Fig. 14). Moderate numbers of eosinophilic granule cells were often present in interstitial tissue in affected areas. Pancreatic ducts, islet cells and adipose tissue appeared unaffected.

No significant lesions were seen in gills, heart, liver, spleen, anterior kidney, posterior kidney, brain, stomach, intestine or gonads of diseased or clinically normal fish.

Distribution of dermal ulcers

A total of 2560 sea mullet, with 3948 dermal ulcers, was examined. Of these fish, 94·3% were within the LCF range used to estimate the logarithmic relationships between subdivision areas and LCF. There was no significant difference ($P>0·05$) between numbers of ulcers recorded in matching subdivisions on left and right sides of the fish and results for each were combined. For each subdivision, Table 1 shows total surface area, total number of dermal ulcers recorded and number of dermal ulcers per square meter.

Generally, dermal ulcers occurred significantly more often in the posterior and dorsal subdivisions than in the anterior and ventral subdivisions (Table 2).

Discussion

Cutaneous lesions which occurred on sea mullet 2–3 weeks after significant rain events, at the beginning of red spot disease outbreaks, were classified as necrotizing dermatitis, erythematous dermatitis and an intermediate form of dermatitis. Large numbers of fungal hyphae, morphologically consistent with Oomycetes (Neish & Hughes 1980), were present in necrotizing dermatitis lesions. Lesser numbers were present in intermediate-type lesions and none were seen in erythematous dermatitis lesions.

The present authors' findings suggested that, during the course of outbreaks, each lesion type evolved in a predictable manner. Intact necrotizing dermatitis lesions, without evidence of dermal sloughing, were found only in the second week after significant rain events; transitional forms, consistent with evolution of these lesions to dermal ulcers, were common 2–5 weeks

Table 1. Total area of subdivisions on sampled sea mullet body surface, total numbers and density of dermal ulcers in each. Left and right sides of the body are combined

	Subdivision					
	H[1]	AD[2]	AV[3]	PD[4]	PV[5]	C[6]
Total surface area (m^2)	23·01	12·70	20·92	15·66	19·29	10·66
Total numbers of dermal ulcers	164	703	634	928	828	691
Dermal ulcers/m^2	7·12	55·35	30·30	59·25	42·92	64·82

[1] Head.
[2] Anterodorsal.
[3] Anteroventral.
[4] Posterodorsal.
[5] Posteroventral.
[6] Caudal peduncle.

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Table 2. Comparisons between subdivisions of sea mullet body surface. For each comparison, subdivision code denotes the subdivision with a significantly higher than expected ($P<0{\cdot}05$) occurrence of dermal ulceration

	Subdivision					
	H[1]	AD[2]	AV[3]	PD[4]	PV[5]	C[6]
H						
AD	AD					
AV	AV	AD				
PD	PD	*	PD			
PV	PV	AD	PV	PD		
C	C	C	C	*	C	

* Denotes no significant difference.
[1] Head.
[2] Anterodorsal.
[3] Anteroventral.
[4] Posterodorsal.
[5] Posteroventral.
[6] Caudal peduncle.

after significant rain events. Lesions classified in our study as erythematous dermatitis are consistent with the 'early developing' lesions proposed as precursors of dermal ulcers by Rodgers & Burke (1981, 1988). However, the present authors saw no evidence that dermal ulcers were derived from either erythematous dermatitis lesions or intermediate-type lesions; transitional forms were not seen and both these lesion types appeared to heal progressively during the course of outbreaks. Nor was there evidence in this study or elsewhere (Callinan & Keep 1989) to support the suggestion that cutaneous ectoparasites, such as the digenean *P. steeri*, initiate some cutaneous lesions (Rodgers & Burke 1988).

Intense granulomatous responses to invading fungi, morphologically consistent with Oomycetes, have also been described in sea mullet with RSD by McKenzie & Hall (1976). Noga & Dykstra (1986) and Noga, Levine, Dykstra & Hawkins (1988), described similar responses in ulcerative mycosis of menhaden, *Brevoortia tyrannus* (Latrobe), and recovered *Aphanomyces* sp. and *Saprolegnia* spp. from lesions. *Saprolegnia* spp. and *Pythium* spp. were among fungi isolated from RSD lesions in a preliminary study (Fraser & Callinan, unpublished data) but the identity of the invasive fungus remains to be established. *Saprolegnia* spp. are usually associated with relatively superficial skin lesions in freshwater fishes, although hyphae may invade skeletal muscles. They usually provoke only a mild inflammatory response. *Pythium* spp. have been associated with clinically similar lesions (Wolke 1975; Richards 1978).

Damage to fish skin may be necessary before infection by oomycete fungi or bacteria can become established (Pickering & Willoughby 1982; Singhal, Jeet & Davies 1987; Crouse-Eisnor, Cone & Odense 1985). The occurrence, on fish with RSD, of chronic active dermatitis lesions 2–3 weeks after significant rain events suggests that a primary insult occurs early in this period and is followed by localized invasion of the skin by opportunistic fungi and/or bacteria (Callinan & Keep 1989). Viral infection of the skin may provide this primary insult. Rhabdoviruses have been proposed as a possible cause of a cutaneous ulcerative syndrome in south-east Asian fishes (Frerichs *et al.* 1986) and have been associated with a similar syndrome in the Northern Territory, Australia (Humphrey & Langdon 1986). A rhabdovirus has been recovered from a luderick, *Girella tricuspidata* (Quoy & Gaimard), with RSD from New South Wales (Roberts, personal communication). However, preliminary studies on 30 sea mullet with

necrotizing dermatitis lesions have failed to demonstrate virus infection (Callinan, Fraser, Calder, Humphrey & Langdon, unpublished data).

Environmental studies, to be published separately, have demonstrated changes which may damage skin and underlying skeletal muscle of sea mullet and other fishes. Dissolved oxygen concentrations fell from normal levels of > 6 mg/l to < 1 mg/l within 7–10 days of significant rain events in representative tributaries of the lower Clarence and Richmond Rivers. Concentrations remained below 1 mg/l for a further 8–12 days as flood waters receded (Virgona, Williams, Callinan & Fraser, unpublished data). Plumb, Grizzle & Defigueiredo (1976) described apparently sterile haemorrhagic and necrotic lesions in skin and skeletal muscle of channel catfish, *Ictalurus punctatus* (Rafinesque), exposed to dissolved oxygen concentrations of < 1 mg/l for several days. They suggested that loss of epidermal integrity allowed subsequent bacterial invasion and septicaemia. Similar degenerative and necrotic changes may have occurred in skin and skeletal muscle of sea mullet exposed to these low dissolved oxygen concentrations. Variations in severity and extent of tissue damage, as well as severity of subsequent fungal or bacterial invasion, may determine which forms of dermatitis result.

Dermal ulcers occurred significantly more often on the posterior/dorsal subdivisions of the body surface than elsewhere. Factors determining this distribution were not identified, but an attractive hypothesis is that these areas are at the periphery of circulatory fields relatively distant from the heart and hence most compromised under hypoxic conditions. Similarly, the lower than expected frequency of dermal ulcers on the head may be due to its relative proximity to the heart and/or the small proportion of scaled skin with underlying muscle in the region.

Atrophy of exocrine pancreas was most severe in fish with dermal ulcers and may have been caused by protein depletion associated with malnutrition (Jubb, Kennedy & Palmer 1985). Fish with dermal ulcers had significantly poorer body condition than clinically normal fish (Virgona, unpublished data), possibly as a result of debilitation by disease and reduced food intake associated with habitat disturbances after flooding. Alternatively, hypoxia may have caused the atrophy, selectively affecting those acinar cells remote from blood vessels.

Further studies of the aetiology and pathogenesis of RSD are required. The significance of decreased dissolved oxygen concentrations remains to be established, the possible role of viruses needs to be fully examined and the invasive fungus must be identified. The significance of changes in other water quality parameters, acting either indirectly as stressors (Wedemeyer & Goodyear 1984) or as direct insults, also remains to be determined.

Acknowledgments

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Bacteriology and parasitology of red spot disease in sea mullet, *Mugil cephalus* L., from eastern Australia

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Abstract. Bacteria and cutaneous ectoparasites associated with red spot disease (RSD) in *sea mullet, Mugil cephalus* L., from the Clarence and Richmond Rivers in north-east New South Wales were identified. Various bacteria, including *Aeromonas* spp., *Alcaligenes* spp., *Pseudomonas* spp. and *Vibrio* spp. were recovered from lesions designated erythematous dermatitis, necrotizing dermatitis and dermal ulcer, but no genus was consistently dominant in cultures from any of the lesion types. *Vibrio anguillarum*, previously proposed as the cause of RSD, was recovered from four of 37 lesions of erythematous dermatitis, none of 46 lesions of necrotizing dermatitis and eight of 36 dermal ulcers. Bacteria were recovered only rarely from liver and posterior kidney of diseased fish. These results suggest that none of the bacteria isolated is the primary cause of RSD. There was no evidence that cutaneous ectoparasites, including the digenean *Prototransversotrema steeri*, are significant in the pathogenesis of RSD in sea mullet. None were found on eight normal fish collected in the month preceding a major RSD outbreak. In the first 2 months of the outbreak, none were found on 18 fish with erythematous dermatitis, eight with dermal ulcers or 11 of 12 normal fish. A single digenean, morphologically consistent with *P. steeri*, was recovered from a normal fish collected during this latter period.

Introduction

Red spot disease (RSD) affects several species of estuarine fish in Queensland and New South Wales, Australia. Outbreaks of RSD occur after prolonged periods of heavy rainfall and affected fish develop cutaneous lesions including circular or oval, red ulcers up to 4 cm in diameter, usually extending into underlying muscle. There are no other gross lesions (McKenzie & Hall 1976; Rodgers & Burke 1981; Callinan, Fraser & Virgona 1989). Following a study of RSD in sea mullet, *Mugil cephalus* L. in Queensland, Rodgers & Burke (1981, 1988) suggested that highest disease prevalence occurred in seasonal aggregations of fish stressed by low or rapidly changing water temperatures and rapid or prolonged depressions of salinity. They consistently recovered large numbers of *Vibrio anguillarum* from 'early' lesions on sea mullet from marine or brackish water and suggested it was the causative infectious agent (Burke & Rodgers 1981; Rodgers & Burke 1981). They also suggested that some of these 'early' lesions were initiated by the ectoparasitic digenean *Prototransversotrema steeri* (Rodgers & Burke 1988). They proposed that, during feeding, the parasite damaged small epidermal areas which were subsequently infected by *V. anguillarum* and other *Vibrio* spp.

The aim of this study was to identify bacteria and cutaneous ectoparasites associated with RSD in sea mullet from the Clarence and Richmond Rivers, New South Wales.

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Materials and methods

Disease outbreaks

Major outbreaks of RSD in sea mullet occurred on the lower Clarence River (29°25'S, 153°21'E) in March 1985, and on the lower Richmond River (28°52'S, 153°35'E) in May 1987. Results of epidemiological and environmental studies of these outbreaks will be published separately. Both outbreaks followed prolonged heavy rain, with flooding, in the lower catchment areas. Minor outbreaks, apparently in response to more localized rainfall, also occurred on the Clarence River during 1985 (Virgona, Williams, Callinan & Fraser, unpublished data).

Specimen collections

Bacteriology. Normal sea mullet and those with one or more examples of a single lesion type were collected by gill net from sites on the rivers dictated by commercial fishing activities. All measured between 20 to 35 cm in length. On the Clarence River, collections were made monthly between December 1984 and November 1985. Fish were held alive in tanks containing river water and were processed within 4 h of collection. A single collection of live sea mullet was made on the Richmond River in May 1987. Some fish were maintained in tanks containing river water. Others were sealed individually in new plastic bags and held on ice. All were processed within 4 h of collection.

Parasitology. Sea mullet were collected as above from the Clarence River between February and May 1985. Fish were carefully removed from the net, immediately sealed in new plastic bags and held at 4°C for up to 9 h. All measured between 25 and 37 cm in length.

Lesion classification

Lesions of RSD were classified according to gross and histological criteria (Callinan *et al.* 1989); briefly, the types examined in this study were:
Erythematous dermatitis. Mild to severe chronic active dermatitis without fungal involvement. Affected areas were < 1 cm in diameter. Skin was yellow with irregular reddening and scales were fragile, damaged or absent.

Necrotizing dermatitis. Severe necrotizing granulomatous dermatitis associated with extensive fungal invasion. The central ulcerated area was red and surrounded by pale grey to red swollen dermal tissue. The lesion was usually oval to circular and 1–4 cm in diameter.

Dermal ulcer. Severe necrotizing granulomatous myositis. Typically there was a circular to oval ulcer 1–4 cm in diameter extending into skeletal muscle. Edges were sharply defined and numerous pale granulomas were often visible on the red ulcerated surface. There was no evidence of healing.

Bacteriology

Clarence River collections. A sterile cotton swab was rubbed over the entire surface of each lesion, or a representative area of large lesions. Occasionally, more than one lesion was

cultured from individual fish with well separated lesions. On each fish, one area of normal skin 3 × 3 cm adjacent to the lesion(s) was swabbed separately. Half the surface of each swab was used to inoculate Medium B agar (Simidu & Hasuo 1968; modified according to Burke & Rodgers 1981), and the remaining half was inoculated on to Cytophaga agar (Anacker & Ordal 1958). After immersion in 70% ethanol for 2 min, fish were removed and the body cavity opened using sterile instruments. The surfaces of liver and posterior kidney were seared, tissue samples were collected in sterile Pasteur pipettes and inoculated onto Medium B agar.

Agar plates were incubated at 22°C for 48 h. Using a colony counter, growth was classified as light (< 35 colonies), moderate (35–75 colonies) or heavy (> 75 colonies). A typical example of each dominant colony type from lesions or internal organs was subcultured and identified. Where growth was mixed, up to three colony types were selected. Colony types recovered from normal skin were identified if they resembled dominant colonies from the adjacent lesion(s).

Conventional tests were used to characterize isolates (Cowan 1974; Baumann, Furniss & Lee 1984; Popoff 1984). All media for biochemical tests contained 1% (w/v) NaCl, except for growth at selected concentrations of NaCl. An incubation temperature of 22°C was used throughout, except for growth at selected temperatures. Representative *Vibrio* spp. were submitted to reference laboratories (School of Public Health and Tropical Medicine, University of Sydney and/or Department of Microbiology, University of Queensland) for confirmation of identification.

Richmond River collection. Material from the single skin lesion on each fish was collected as above and inoculated onto Medium B agar. Incubation conditions and criteria for classifying growth were as described above. Three typical examples of each dominant colony type were subcultured and examined for Gram reaction, oxidase and catalase production, motility and sensitivity to 0129. They were then further characterized using the API20E system (API System S. A. — Montalieu Vercieu, France). Additional conventional tests were used to identify *Aeromonas* spp. (Popoff 1984).

Parasitology

Fish were removed from plastic bags and the entire skin surface thoroughly scraped with the edge of a glass slide. The scrapings were placed in a sterile Petri dish. The inside of the plastic bag was thoroughly rinsed with distilled water which was then poured into the Petri dish. The contents of the dish were examined by dissecting microscope.

Conductivity measurements

Conductivity at sites representing major lower river areas (Williams 1987a, b) was measured fortnightly or monthly, depending on rainfall, between December 1984 and November 1985 on the Clarence River and twice weekly in the 3 weeks following the May 1987 rainfall on the Richmond River. It was measured by an Extech digital water analyser (PT 1/20 unit, CHK Engineering, Sydney) at 25°C. Conductivity at each fish collection was taken to be that measured, on the date nearest the collection, at the representative site in the river area from which the fish were collected.

Results

Numbers of fish examined and types of lesions present varied with catch size and dise prevalence.

Bacteriology

Clarence River 1984/1985. Seventy-seven fish were examined. Table 1 shows numbers of e lesion type examined, distance from the river mouth and conductivity at each collection.

Erythematous dermatitis: Thirty-seven lesions were cultured yielding light to heavy, usu mixed, growth. Eight lesions yielded *Alcaligenes* spp., *Aeromonas sobria* was recovered fr six lesions and six lesions yielded *Acinetobacter* spp. Other genera were recovered less oft *Vibrio anguillarum* was recovered from four lesions, all of which were examined at the June July collections. No other *Vibrio* spp. were recovered.

Necrotizing dermatitis: Seven lesions were cultured. Growth was light to heavy and usu mixed. *Aeromonas sobria* was recovered from four lesions, *Alcaligenes* spp. from three, *Acinetobacter* spp. from three. Two lesions yielded *A. hydrophila*, two yielded *Bacillus* s and *V. cholerae* non 01 was recovered from one lesion. *Vibrio anguillarum* was not found

Dermal ulcer: Thirty-six lesions were cultured, yielding moderate to heavy, usually mix growth. *Aeromonas sobria* was recovered from 21 lesions, *A. hydrophila* from 14, *Alcalige* spp. from nine and *V. anguillarum* from eight. One other *Vibrio* spp., *V. campbellii*, recovered from one lesion. Other genera were recovered less often. *Vibrio anguillarum* recovered only from lesions examined at the June, July and August collections.

Table 1. Collection sites and lesions examined at monthly bacteriology collections, Clarence River

Month	Distance from river mouth (km)	Conductivity (μS. cm^{-1})	Numbers of lesions examined		
			ED*	ND**	DU***
December	75	165	4	0	3
January	75	747	1	0	2
February	34	176	1	1	1
March	31	931	5	6	0
April	31	196	7	0	4
May	31	845	4	0	4
June	16	7580	4	0	4
July	16	19300	5	0	4
August	16	28500	2	0	3
September	31	9100	0	0	3
October	50	2610	4	0	4
November	34	258	0	0	4
Total			37	7	36

* Erythematous dermatitis. ** Necrotising dermatitis. *** Dermal ulcer.

Normal skin: Seventy-seven sites were cultured. In almost all cases, bacteria which were dominant in lesions were either not recovered or occurred in smaller numbers on adjacent skin.

Internal organs: Bacteria were recovered from liver and/or posterior kidney of only three diseased fish which were collected between June and August. *Vibrio anguillarum*, *V. furnissii*, and *V. pelagius* I were recovered from liver and kidney of a fish with a dermal ulcer, while *V. anguillarum* and *V. campbellii* were recovered from the lesion. *Aeromonas hydrophila* was recovered from kidney of two fish with dermal ulcers. It was also recovered from lesion and adjacent skin in both cases. Bacteria were not recovered from liver or kidney of normal fish.

Richmond River 1987. Thirty-nine diseased fish were collected in May at a site 15 km from the river mouth. Conductivity was 180 $\mu S.cm^{-1}$

Necrotizing dermatitis: Thirty-nine lesions were cultured. Moderate to heavy mixed growth, usually with one or two dominant colony types, was recovered. *Aeromonas hydrophila* was dominant in 25 of the 39 cultures. *Pseudomonas putrifaciens*, *A. caviae* and *A. sobria* occurred less often. Various other species occurred rarely. No *Vibrio* spp. were found.

No other lesion types were examined.

Parasitology

Forty-six fish were examined. Table 2 shows numbers of normal and diseased fish collected, distance from the river mouth and conductivity at each collection. Eight normal fish were collected in late February and early March, before the major RSD outbreak began. The remaining diseased and normal fish were collected during the outbreak between late March and mid-May. Only one cutaneous ectoparasite was found; a digenean, morphologically consistent with *P. steeri*, was recovered from a normal fish collected during May.

Table 2. Collection sites and fish examined at parasitology collections, Clarence River

Month	Distance from river mouth (km)	Conductivity ($\mu S.cm^{-1}$)	Numbers of fish examined		
			ED*	DU**	N***
February	34	176	0	0	4
March[1]	31	530	0	0	4
March[2]	31	931	5	0	4
April	31	196	8	4	4
May	31	845	5	4	4
Total			18	8	20

[1] Two weeks before start of outbreak.
[2] One week after start of outbreak.
* Erythematous dermatitis.
** Dermal ulcer.
*** Normal.

Discussion

During this study of RSD, a wide variety of bacteria was recovered from skin lesions of sea mullet from the Clarence and Richmond Rivers. No single bacterial genus or species was consistently dominant in any of the lesion types examined. *Vibrio anguillarum*, which Rodgers & Burke (1981) considered the causative agent of RSD, was recovered from lesions on sea mullet from brackish water only. It was recovered from four of 37 lesions of erythematous dermatitis, from none of 46 lesions of necrotizing dermatitis and from eight of 36 dermal ulcers.

Vibrio anguillarum is halophilic, indigenous to coastal waters and may be a component of normal fish microflora. It is also an opportunist pathogen of fish, typically causing acute haemorrhagic septicaemia or a more chronic systemic infection in which dermal ulcers predominate (Watkins, Wolke & Cabelli 1981; Colwell & Grimes 1984; Larsen & Mellergaard 1984). Burke & Rodgers (1981) failed to recover *V. anguillarum* from posterior kidney of sea mullet with RSD and the organism was recovered from internal organs in only one of 77 diseased sea mullet in our study. These findings indicate that systemic invasion does not precede ulcer development in RSD.

Histopathological studies of RSD in sea mullet (Callinan *et al.* 1989) suggested skin lesions develop after focal to locally extensive epidermal injury, with the following consequences. Where injured areas are > 1 cm in diameter, the skin is invaded by large numbers of fungi, resulting in lesions designated necrotizing dermatitis. These lesions progress to dermal ulcers as necrotic dermal tissues are sloughed. By contrast, skin in areas of epidermal damage < 1 cm in diameter is rarely invaded by fungi and lesions of chronic active dermatitis develop, presumably in response to opportunistic infection by various bacteria. Such lesions, designated erythematous dermatitis, heal without developing into dermal ulcers. Rogers & Burke (1981, 1988) described lesions consistent with erythematous dermatitis as 'early' and considered them precursors of dermal ulcers. This apparently erroneous assumption, combined with the consistent recovery of large numbers of *V. anguillarum* from such lesions on sea mullet taken from marine to brackish water (Burke & Rodgers 1981), led to their suggestion that dermal ulcers were initiated by *V. anguillarum* invading skin from the external surface. In this study, 46 lesions of necrotizing dermatitis, the putative precursors of dermal ulcers, were cultured and *V. anguillarum* was recovered from none of them. These findings indicate that *V. anguillarum* is not the cause of RSD.

Crouse-Eisnor, Cone & Odense (1985) have suggested that bacteria can readily attach to cutaneous lesions on fish but are unable to colonize normal skin. They proposed that bacteria isolated from normal skin are in suspension near the skin surface, but are not capable of maintaining prolonged contact with it. This might explain the recovery of a variety of bacteria, including opportunist pathogens such as *Aeromonas* spp. and *V. anguillarum*, from skin lesions and normal skin in our study and that of Burke & Rodgers (1981). The results of an epidemiological and environmental study of RSD, to be published separately, suggest that lesions of RSD are initiated in fresh water (Virgona, Williams, Callinan & Fraser, unpublished data). *Vibrio anguillarum* probably colonizes many established skin lesions on sea mullet which subsequently move into brackish water.

No cutaneous ectoparasites, including the digenean *P. steeri*, were found on eight normal sea mullet collected in late February and early March 1985, less than 1 month before the beginning of an RSD outbreak. Nor were they found on 18 fish with erythematous dermatitis, eight fish with dermal ulcers or 11 of 12 normal fish examined during the first 2 months of the outbreak. Only one cutaneous ectoparasite, a digenean morphologically consistent with *P.*

steeri, was found on a normal fish collected during the outbreak. Prevalence of dermal ulceration in commercial catches of sea mullet collected at the 31 km parasitology sampling site rose from < 5% to 30% between March and May. These sea mullet, in similar length classes to those examined in the parasitology study, were moving downstream prior to the annual spawning migration (Virgona, Williams, Callinan & Fraser, unpublished data). Therefore it is unlikely that fish collected at this site during this period had been recently exposed to *P. steeri* infestation in the brackish estuarine zone (Rodgers & Burke 1988). Taken together, these findings do not support the suggestion by Rodgers & Burke (1988) that cutaneous ectoparasites, particularly the digenean *P. steeri*, play a significant role in the pathogenesis of RSD.

Fungi, morphologically consistent with Oomycetes, are important in the pathogenesis of dermal ulceration in RSD (Callinan *et al.* 1989). Other microbial agents may also be involved. Nutritionally fastidious bacteria, such as atypical strains of *A. salmonicida* (Ishiguro, Ainsworth, Kay & Trust 1986), may not have been detected by methods used in this study. Moreover, any primary bacterial agent occurring in relatively small numbers in lesions may have been obscured on culture by more dominant isolates. Viruses, including rhabdoviruses, have been associated with cutaneous ulcerative syndromes in fishes from Europe (Jensen & Larsen 1982), South-East Asia (Frerichs, Millar & Roberts 1986; Hedrick, Eaton, Fryer, Groberg & Boonyaratapalin 1986) and the Northern Territory, Australia (Humphrey & Langdon 1986). A rhabdovirus has been recovered from a luderick, *Girella tricuspidata* (Quoy & Gaimard), with RSD from New South Wales (Roberts, unpublished data). Further studies into the possible role of these microbial agents in RSD are in progress.

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