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13 March, 1990

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Dear Mike:

FIRC 81/20

The final report for the above project follows (11 pages plus this covering page). The original is being mailed to you today.

Yours sincerely,

R.J.G. Lester.

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

FIRC 81/20

FINAL REPORT, 1981 to 1989

Project Title:

Consultancy service on industry problems associated with marine parasites and diseases, and investigation into QX disease of oysters.

Report:

This long-running FIRTA grant supported a centre in Australia for research, consultancy and training on parasites and diseases important to the fishing industry. The Centre was established by FIRTA in 1977. During the life of FIRC 81/20 many industry problems were successfully investigated, industry queries answered and training programs completed. To keep this Final Report to a manageable size I have briefly summarised many projects and given references to publications in which the findings are described in more detail.

The consultancy service handled 174 enquiries about problems in fish and fish products (Appendix 1 lists those from September, 1988, to June, 1989). Several were from members of the press concerned about public health as this has become an issue in some seafood overseas such as Canadian cod and West German herring. Fortunately there is no record of human disease as a result of any parasitic infection in Australian seafood. (I do not include here microbial infections acquired from shellfish growing in polluted waters.)

Many enquiries requested identification of specimens. Five of these resulted in published descriptions (Appendix 2, refs. 2,4,5 and 8). Some required specific research projects to solve the problem. In the following sections I deal first with QX Disease and then summarise the results of other projects.

QX DISEASE

The organism that causes QX Disease in oysters, the protozoan Marteilia sydneyi, was described from Queensland oysters by Perkins and Wolf in 1976. It is harmless to man but kills oysters within a few weeks in the summertime. By placing uninfected oysters on leases at regular intervals, we showed that oysters became infected only during the summer, particularly after heavy rain. At one field site in Pummicestone Passage,

Queensland, oyters were infected several times in one season. As the site was remote from cane farms and other agriculture, the high levels of QX infection were not associated evidently with chemicals released from farms upstream. This was contrary to the view held by growers in Northern New South Wales. I presented my findings at meetings of the Australian Oyster Farmers and Producers Association, Northern Rivers Branch (see Appendix 2), and expanded the field study area to include the Tweed River and Terranora Lakes. In southeast Queensland and northern New South Wales few new infections occurred after the second week of April and little development occurred during the winter. Some oysters infected in April survived and carried the infection through the winter (Ref. 31).

Oysters could not be infected experimentally in the laboratory, a phenomenon also experienced by researchers in France working with the closely related parasite Marteilia refringens. I transplanted pieces of infected tissue to other oysters and 11 days later found the transplants which seemed to be still healthy but no spread of infection had occurred. No infection developed in 12 oysters inoculated with parasites and kept for at least 4 weeks under various environmental conditions. I also found that early infections in oysters in the field ceased to develop when the oysters were brought into the laboratory. This may be because the parasite, which develops within the cells of the digestive gland, requires the cells to be in an excellent nitritional condition before it can develop; normally the digestive gland cells of oysters brought into the laboratory regress as a result of the non-optimal diet provided.

In the field it was not possible to produce infections on demand. Uninfected oysters placed on trays with infected oysters at any stage of the infection did not themselves become infected unless a new wave of infection swept through the estuary.

Did local oysters have any resistance? I placed on racks samples of oysters from two areas where QX appears to have been endemic for many years (Tin Can Bay and Moreton Bay, both in Queensland) and alongside them put oysters from Port Stephens, where QX has never occurred. After a QX outbreak, equal numbers had become infected so there was no obvious inherited protection from infection (Ref. 31). It is possible fewer endemic oysters would have died but preliminary observations and general observations on wild oysters suggested that when an outbreak occurs in an estuary and many imported oysters die, wild oysters also suffer heavy mortality.

Several drugs were tested to determine if they would kill the parasite. None were effective at doses that did not harm the oyster (Ref. 31). In fact it appeared that once the parasite had sporulated in the oyster it had come to the end of its development and no further spread occurred through the oyster tissues.

Oysters shed spores while they are alive. The spores are shed in groups of two, referred to as sporonts. Some oysters appeared to be able to shed all their sporonts and recover. Even

oysters that died of the disease shed most of their sporonts before death (Ref. 17). The sporonts, and the spores they enclose, are fragile structures that lack a sturdy wall. Both are surrounded only by a sheath of membranes. We found that sporonts usually disappeared within days in the sediment. Spores died within 4 weeks in filtered seawater in the laboratory.

When infections occur in the field half the oysters in an estuary may become infected within 1 or 2 days. Prior to this there is frequently little or no infection in any of the oysters. Because of this, because of the difficulty of transferring infection directly from oyster to oyster, and because of the fragility of the spore, it appears there must be a reservoir of infection somewhere in affected estuaries. We therefore sought an alternate host.

The spore consists of three cells, the first inside a cytoplasmic vacuole in the second, and the second inside a vacuole in the third. The outer cell has dark objects, the haplosporosomes, in its cytoplasm. Exactly the same features are seen in the PKX organism that causes proliferative kidney disease in rainbow trout in Europe and North America. The PKX cells are believed to be the early stages of a myxosporan, Sphaerospora sp. The only life cycle of a myxosporan known is that of Myxosoma cerebralis, the cause of whirling disease in rainbow trout; it requires a Tubifex worm to complete its development. Thus it is possible that an estuarine fish such as a mullet could be the alternate host for the QX organism, particularly as local mullet and bream are infected with several species of myxosporans.

QX spores which we fed to mullet and bream developed thickened walls (Ref. 37) and appeared to form some sort of discharge tube. To trace the sporoplasm as it emerged from the spore and entered the fish we developed a fluorescent antibody test (Refs. 12 and 17). However, we found no evidence that the parasite penetrated into the fish. The fluorescent antibody did not attach to any putative sporoplasms in the tissues or to any myxosporan infections already present, and fish fed large numbers of spores did not develop any protozoan infections. We tested several species of fish (mullet, bream, blennies, trumpeter). As the results were negative, we then turned to invertebrates likely to feed on shed sporonts and fed infected oyster tissue to yabbies and polychaete worms. Again our fluorescent antibody did not highlight anything in their tissues. They did not develop any new infections nor could we find anything unusual in yabbies and polychaetes collected from infected oyster leases.

Thus, the life cycle remains unknown. The life cycle of the closely related Marteilia refringens, a parasite that decimated the French oyster industry in the 1970s is also unknown. French growers have switched from growing Ostrea edulis to the less highly valued species Crassostrea gigas to avoid the catastrophic losses caused by M. refringens and a second parasite Bonamia ostreae.

In Australia, the Sydney rock oyster has an excellent market value. Nevertheless, an alternate species may be valuable to

growers in the northern part of its range where QX is a problem. The Pacific oyster, Crassostrea gigas, does not seem to do well in southeast Queensland, particularly when exposed to the air for extended periods. Possibly changes in culture and processing operations would overcome this. Native species such as the blacklip and milky oysters may be viable if spat could be obtained cheaply. For Sydney rock oysters, until we can find the reservoir for QX the main way to minimise losses continues to be to reduce the numbers of oysters on leases during the high risk months of January, February and March.

OTHER PROJECTS

Milky kingfish

I found that the milky texture of many yellowtail kingfish was due to the kingfish protozoan Unicapsula seriolae. The texture remains normal if fillets are cooked rapidly; tissue only breaks down if the fish is baked slowly (Ref. 5). This was brought to the attention of fish wholesalers (Ref. 1).

Crumbling abalone

The muscle of some Australian abalone crumbled when canned in Japan. We showed that this was the result of processing methods in Japan, and not because it was an inferior product; in fact it was because the product was a little too fresh. The condition arose when freshly caught abalone were so quickly processed and frozen that the muscle did not have time to undergo rigor. If these animals were then rapidly thawed and canned, the muscle fell apart. The same problem occurred at Australian canning plants when abalone that had been frozen within an hour or so of being caught were quickly thawed and canned (Ref. 10).

Holes in trevally muscle

Holes in the flesh of trevally Caranx dentatus and occasionally snapper Chrysophrys auratus were shown to be an abnormal extension of the swim bladder and not the result of any infectious agent (Ref. 21).

Jack mackerel

A survey of the parasites of jack mackerel at different times in the seasonal fishery off Tasmania showed that the majority caught belonged to a single subpopulation. A sample taken at the end of the season had a different history and probably belonged to a different stock. Thus heavy fishing at the start of the season is likely to affect catches later as only at the end of the season do fish apparently move in from another area (Sewell and Lester, in preparation).

Rock lobster

Black inclusions up to 30 mm long in the muscle of rock lobsters in South Autralia were found to be old haematomas and

were not associated with an infective agent. They occurred near the base of the tail on the ventral side and were sometimes associated with irregularities in the adjacent exoskeleton. They were apparently the result of wounds from octopus attacks (Lester, unpublished results).

Other problems

Not all problems were solved. We sought the definitive host of Gymnorhynchus thyrsitae, the trypanorhynch worm that causes 'wormy couta' in couta Thyrsites atun. Adult trypanorhynchs occur in the gut of sharks. Over 77 sharks were examined without success. Since then a large project in South Australia on trypanorhynchs in Australian sharks has been carried out, also without finding the definitive host (Dr I. Beveridge, personal communication). I suspect the host is one of the pelagic sharks such as the blue whaler which is rarely caught because commercial shark fishing uses bottom gear. The project was discontinued when the couta fishery disappeared in 1982/3.

Preliminary work on a bucephalid that causes the gonad of southern scallops to swell and become bright red was discontinued when the scallop fishery in Port Phillip Bay went into decline (Ref. 3).

Some enquiries resulted in further studies here that were funded by other agencies or by a separate grant from FIRTA. Our observation that an amoeba was associated with gill disease on sea-caged Tasmanian salmon and trout led to a project funded by the Australian Research Council that is developing techniques to minimise losses (Refs. 20,33 and 40).

Our detection of the protozoan Perkinsus olseni in dying abalone Haliotis laevigata in South Australia (Ref. 11) led to a FIRDC-funded project to replenish the coast now the parasite has apparently lost some of its virulence.

An examination of parasites in the viscera of the deep water fish, orange roughy (Hoplostethus atlanticus), led to a FIRTA-funded project in which we showed that roughy are essentially sedentary and not widely migratory as had been believed (Ref. 16). This was later borne out by the fishing out of a small area of the New Zealand fishing grounds by the commercial fishery. We now work to determine how far fish move to join aggregations.

A preliminary examination of prawns for parasites led to the discovery of the first marine virus reported from Australia (Ref. 22) and the first virus from Australian cultured prawns (Ref. 15). Our prawn virus work is now a separately funded project (FIRDC and ARC).

TRAINING

An important role of the Centre as established originally by FIRTA was to train graduate students and others in marine

parasitology and marine disease. Two Ph.D. students, one Masters Qualifying student, and 18 honours students have graduate from the unit. There are currently 4 Ph.D., 1 M.Sc. and 1 B.Sc. Hons students enrolled. The popular B.Sc. undergraduate subject, Parasites of Marine Animals (a 3rd year subject started under the auspices of FIRTA), was upgraded from 7 to 10 credit points in 1981. It attracted 30 students in 1989.

A facility for aquaculture has been established at The University of Queensland. The marine parasitology unit will use two of their brackish water ponds in addition to our extensive aquarium and laboratory facilities to teach about diseases in aquaculture in a new B.Sc. undergraduate subject 'Aquaculture' to be introduced at The University of Queensland in 1991.

I organised two workshops, 'Parasites of the southern Great Barrier Reef', the proceedings of which form Volume 37(1), Australian Journal of Zoology (1989), and 'Methods for Stock Identification' (proceedings submitted for publication). I was an invited speaker at six other workshops (Refs. 23,25,27, 28,29 and 30). Members of my group are regular contributors at industry meetings such as those of the Australian Mariculture Association.

Teaching material on infections in seafood products has been supplied to the Queensland University of Technology, Tasmanian Institue of Technology and the Australian Maritime College.

THE FUTURE

Marine parasitology will continue to be important to the fishing industry in three distinct areas: in marketing to overcome problems associated with loss of quality as a result of infections; in aquaculture to overcome disease constraints; and in fisheries management where parasites are used to elucidate the movement of species which otherwise are difficult to tag. Our current research addresses all three topics.

Appendix 1. Enquiries directed to the consultancy service Sept 88 to June 89 (i.e. from last progress report to end of FIRTA 81/20)

1988
Oct. Michael Pierce, NT Temnocephalids from Cherax.
Oct. Peter Haaker, Dept. California Fish & Game. Abalone disease.
Nov. Sydney Morning Herald. Anisakids in local fish.
Dec. Joan Alexander FITC,NSW. Swimbladder ectasia in trevally.

1989
Jan. Judith Ham, Qld. Watery muscle in bream from Caloundra.
Mar. Chris Johnson, SA. Poisonings and nematodes from scallops.
Mar. Shane Hall, Qld. Fungus in yellowbelly (freshwater).
May Rosemary Burgess, Qld. Sores on goldfish (A. hydrophila).
May Chris, Qld. Oscar with dropsy.
June Chris Estreich, Qld. Cichlid with probable mycobacterium.
June Sharon McGladdery, Canada. Perkinsus in scallop broodstock.
June Paul Presidente, Vic. Anisakids in fillet of orange roughy.

Appendix 2. Publications arising from work done under FIRC 81/20. Those with an asterisk were funded solely by FIRC 81/20; those without an asterisk were started under FIRC 81/20 and completed with funds from other sources.

* 1. Lester, R.J.G. and B. Goodrick 1981. How to get the best from yellowtail kingfish. Circular to Buyers, Queensland Fish Board.

* 2. Lester, R.J.G. and G.H.G. Davis 1981. A new Perkinsus species (Apicomplexa, Perkinsea) from the abalone Haliotis ruber. Journal of Invertebrate Pathology 37: 181-187.

* 3. Sanders, M.J. and R.J.G. Lester 1981. Further observations on a bucephalid trematode infection in scallops (Pecten alba) in Port Phillip Bay, Victoria. Australian Journal of Marine and Freshwater Research 32: 475-478.

* 4. Shaharom, F.M. and R.J.G. Lester 1982. Description of and observations on Grillotia branchi n. sp., a larval trypanorhynch from the branchial arches of the Spanish Mackerel Scomberomorus commersoni. Systematic Parasitology 4: 1-6.

* 5. Lester, R.J.G. 1982. Unicapsula seriolae n. sp. (Myxosporea, Multivalvulida) from Australian yellowtail

kingfish Seriola lalandi). Journal of Protozoology 29: 584-587.

* 6. Lester, R.J.G. and W.R. Kelly 1983. Tumour-like growths from southern Australian marine fish. Tasmanian Fisheries Research 25: 27-32.

* 7. Lester, R.J.G. and W.R. Kelly 1984. Ankylosing spondylosis in the giant perch Lates calcarifer (Bloch). Journal of Fish Diseases 7: 193-197.

* 8. Nearhos, S.P. and R.J.G. Lester 1984. New records of Bopyridae (Crustacea, Isopoda) from Queensland waters. Memoirs of the Queensland Museum 21: 257-259.

9. Lester, R.J.G. 1984. A review of methods for estimating mortality due to parasites in wild fish populations. Helgolander Meeresuntersuchungen 37: 53-64.

* 10. Lester, R.J.G. and D.E. Bottrill 1984. Fast-thawed abalone muscle breaks down on cooking - why? Australian Fisheries 43(3): 48-49.

* 11. Lester, R.J.G. 1986. Abalone die-back caused by protozoan infection? Australian Fisheries 45(6): 26-27.

* 12. Roubal, F.R. and R.J.G. Lester 1987. New test developed for QX parasite in oysters. Australian Fisheries 46 (10): 38.

13. Goggin, C.L. and R.J.G. Lester 1987. The occurrence of Perkinsus species (Protozoa, Apicomplexa) in bivalves from the Great Barrier Reef. Diseases of Aquatic Organisms 3: 113-117.

14. Lester, R.J.G., P.J. Ketterer and J.L. Paynter 1987. Intranuclear inclusion bodies in the hepatopancreas of the brown tiger prawn Penaeus esculentus. Aquaculture 67: 238-239.

15. Lester, R.J.G., A. Doubrovsky, J.L. Paynter, S.K. Sambhi and J.G. Atherton 1987. Light and electron microscope evidence of baculovirus infection in the prawn Penaeus plebejus. Diseases of Aquatic Organisms 3: 217-219.

16. Lester, R.J.G., K.B. Sewell, A. Barnes and K. Evans 1988. Stock discrimination of orange roughy Hoplostethus atlanticus by parasite analysis. Marine Biology 99: 57-63.

* 17. Roubal, F.R., J. Masel and R.J.G. Lester 1989. Studies on Marteilia sydneyi, agent of QX disease in the Sydney rock oyster, Saccostrea commercialis, with implications for its life cycle. Australian Journal of Marine and Freshwater Research 40: 155-167.

18. Goggin, C.L., K.B. Sewell and R.J.G. Lester 1989. Cross infection experiments with Australian Perkinsus species.

Diseases of Aquatic Organisms 7: 55-59.

19. Lester, R.J.G. and K.B. Sewell 1989. Checklist of parasites from Heron Island, Great Barrier Reef. Australian Journal of Zoology 37: 108-128.

20. Roubal, F.R., R.J.G. Lester and C.K. Foster. Studies on cultured and gill-attached Paramoeba sp. (Gymnamoebae: Paramoebidae) and the cytopathology of amoebic gill disease in Atlantic salmon, Salmo salar L., from Tasmania. Journal of Fish Diseases (in press).

Papers in Proceedings of Meetings

* 21. Lester, R.J.G., M.A. Wilson and P. Machin 1982. Swim-bladder ectasia in the trevally Caranx georgianus. In Fowler, M.E. ed. Wildlife diseases of the Pacific Basin, Proceedings of the 4th International Conference of the Wildlife Disease Association. University of California, Davis, pp. 170-174.

22. Paynter, J.L., D.V. Lightner and R.J.G. Lester 1985. Prawn virus from juvenile Penaeus esculentus. In Rothlisberg, P.C., B.J. Hill, and D.J. Staples, eds. Second Australian National Prawn Seminar. NPS2, Cleveland, Australia, pp. 61-64.

23. Lester, R.J.G. 1986. Parasites and parasitic diseases of aquatic animals. In Humphrey, J.D. and J.S. Langdon eds. Proceedings of the Workshop on Diseases of Australian Fish and Shellfish. Australian Fish Health Reference Laboratory, Victoria, pp. 87-101. (Invited speaker).

24. Paynter, J.L., R.J.G. Lester and P.J. Ketterer 1987. A review of the diseases of penaeid prawns in Australian aquaculture. In Jones, T. ed. Proceedings of the Australian Mariculture Association Annual Meeting. Pp. 46-59.

25. Lester, R.J.G. 1988. Metazoan diseases of fish. In Bryden, D.I., ed. Fish Diseases. University of Sydney, pp. 115-124.

26. Paynter, J.L. and R.J.G. Lester. 1988. Diseases of cultured prawns in Australia. In Evans, L.H. and D. O'Sullivan, eds. Proceedings of First Australian Shellfish Aquaculture Conference. Curtin University, pp. 161-179.

27. Lester, R.J.G. 1989. Diseases of molluscs. In D.I. Bryden, ed. Invertebrates in Aquaculture. University of Sydney. Pp. 129-135.

28. Lester, R.J.G. 1989. Diseases of molluscs - practical. In D.I. Bryden, ed. Invertebrates in Aquaculture. University of Sydney. Pp. 137-142.

29. Lester, R.J.G. and J.L. Paynter. Diseases of cultured prawns in Australia (part 1). In Barret, J. ed. Advances in Tropical Aquaculture. IFREMER. (In press).

30. Lester, R.J.G. Parasites and infectious diseases of cultured molluscs in Australia. In Barret, J. ed. Advances in Tropical Aquaculture. IFREMER. (In press).

Chapters in books

* 31. Lester, R.J.G. 1986. Field and laboratory observations on the oyster parasite Marteilia sydneyi. In Dobson, C., Cremin, M. and Moorhouse, D.E.M. eds. 'Parasite Lives', University of Queensland Press, pp. 33-40.

32. Lester, R.J.G., C.L. Goggin and K.B. Sewell. Perkinsus in Australia. In T.C. Cheng & F.O. Perkins eds. Pathology in Marine Aquaculture. Academic Press (in press).

33. Munday, B.L., C.K. Foster, F.R. Roubal and R.J.G. Lester. Paramoebic gill infection and associated pathology of Atlantic salmon (Salmo salar) and rainbow trout (Salmo gairdneri) in Tasmania. In T.C. Cheng & F.O. Perkins eds. Pathology in Marine Aquaculture. Academic Press (in press).

Published abstracts

* 34. Lester, R.J.G. and W.R. Kelly 1983. Ankylosing spondylosis in the giant perch Lates calcarifer. 1st International Conference of the European Association of Fish Pathologists, Plymouth Polytechnic, England.

* 35. Lester, R.J.G. 1984. The economic importance of marine parasites. Joint meeting, New Zealand and Australian Societies for Parasitology, University of Canterbury, New Zealand. (Invited speaker).

36. Paynter, J.L., D.V. Lightner and R.J.G. Lester 1984. Viral diseases of juvenile prawns. 1984 Annual Meeting, Australian Marine Science Association, Geelong.

* 37. Lester, R.J.G. and J.M. Healy 1986. Possible ultrastructural evidence for a fish alternate host in the life cycle of the oyster pathogen Marteilia sydneyi (Protozoa, Ascetospora). 6th International Congress of Parasitology, Brisbane. Australian Academy of Science, Canberra.

38. Roubal, F.R., T.C. Jones and R.J.G. Lester 1988. Studies on the ultrastructure of cultured and gill-attached Paramoeba sp. and the cytopathology of amoebic gill disease in Atlantic salmon, Salmo salar. 1988 Annual Meeting, Australian Society for Parasitology, Sydney.

39. Lester, R.J.G., C.L. Goggin and K.B. Sewell 1988. Perkinsus olseni and other Perkinsus infections from Australian molluscs. 3rd International Colloquium on Pathology in Marine Aquaculture. Virginia Institute of Marine Science, pp. 45-46.

40. Munday, B.L., C.K. Foster, F.R. Roubal and R.J.G. Lester 1988. Paramoebic gill infection of Atlantic salmon (Salmo salar) and rainbow trout (Salmo gairdneri). 3rd International Colloquium on Pathology in Marine Aquaculture. Virginia Institute of Marine Science, pp. 53-54.

Appendix 3. Industry meetings at which we presented results of our oyster disease research.

Annual meeting of the Australian Oyster Growers and Producers Association, Northern Rivers Branch, 4 May, 1982 (Dr R.J.G. Lester)

Annual meeting of the Australian Oyster Growers and Producers Association, Northern Rivers Branch, 5 April, 1983 (Dr R.J.G. Lester)

Annual meeting of the Australian Oyster Growers and Producers Association, Northern Rivers Branch, 3 April, 1984 (Dr R.J.G. Lester)

Workshop on oyster parasites, Port Stephens, 18-20 October, 1982 (Dr R.J.G. Lester)

Excecutive meeting of the Queensland Oysters Farmers Association Brisbane. November, 1988. (Dr F.R. Roubal)

Annual conference of the Australian Mariculture Association, Lismore, NSW. June, 1988. (Dr F.R. Roubal).
