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Contents

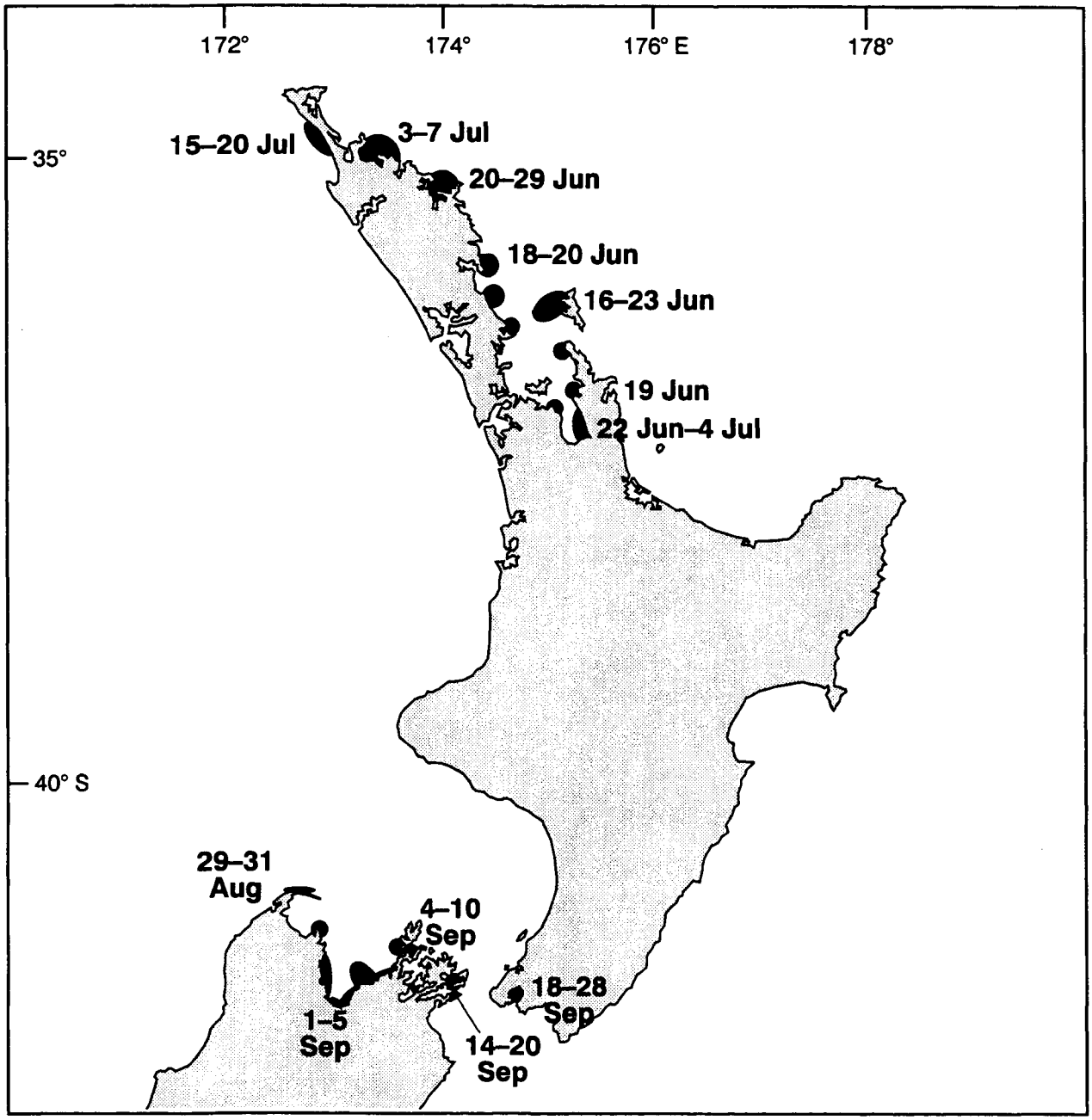

Figure 1: Locations and times of pilchard deaths in 1995.

Introduction

Large numbers of dead and dying pilchards were reported off the northeast and northwest coasts of the North Island during June and July 1995. In late August and September, pilchards were reported dead and dying in Tasman Bay, the Marlborough Sounds, and Wellington Harbour. No other species appear to have been directly affected by these events. Widespread pilchard mortalities were reported off Australia between March and June 1995, and preliminary conclusions were that stress, associated with environmental factors, may have been the primary cause of the kills coupled with a secondary herpeslike viral infection or amoebic infestations (Anon. 1995).

Tissue samples from dying pilchards and water samples from some kill sites in New Zealand were sent to specialist laboratories at the Animal Research Centre Wallaceville, the Cawthron Institute, Nelson, the Communicable Diseases Centre, Porirua and NIWA, Greta Point. Fixed tissue samples were sent to the Animal Health Laboratories, CSIRO, Victoria and to the Elizabeth MacArthur Institute, Menangle, New South Wales. Samples of dead pilchards from several localities were collected and frozen.

This report documents the location and timing of pilchard kills, the samples collected, and the results from preliminary laboratory tests; possible causes of the pilchard kills are discussed. Research on the herpesvirus is ongoing and additional funding has been sought through the Foundation for Research, Science and Technology in New Zealand

Pilchard kills

Pilchards are widespread around New Zealand from Spirits Bay to Stewart Island, but are more abundant around the northern South Island and North Island. Graham (1953) described abundant stocks off the east coast of the South Island during the first part of this century, but the species appears to be less common there now (D. A. Robertson, NIWA, pers. comm.).

The areas and timing of pilchard kills are summarised in Figure 1 and specific details are given in Appendix 1. The first reports of dead pilchards came from Great Barrier Island on 16 and 17 June and were quickly followed by reports from the Firth of Thames, Bream Bay, and the Bay of Islands. Dead pilchards were reported in the Firth of Thames over a 2 week period from 19 June to 4 July (*see* Figure 1). By early July there were reports of dead pilchards washing up in Whangaroa Harbour and in Doubtless Bay. The last large-scale kill in Northland was reported off Ninety Mile Beach (15–17 July) where dead fish were found along 35 km of beach. There was a small-scale kill in the Firth of Thames in early August. In most areas kills occurred over a few days with no further reports of deaths. Apart from the continued deaths in the Firth of Thames, there was evidence for a northwards shift in the onset of deaths from the Firth of Thames/Hauraki Gulf starting on 16 June to Tutukaka by 20 June, the Bay of Islands 20–23 June, and Whangaroa and Doubtless Bay by early July, with the last large-scale kill off Ninety Mile beach starting on 15 July. The pilchard deaths in northern New Zealand occurred over 500 km of coast over about a month from mid June to mid July. Kills were extensive in the Hauraki Gulf and Firth of Thames, but north of Taiharuru Head kills were patchy and reported only from the Bay of Islands, Whangaroa, and Doubtless Bay on the east coast. Within the Northland region pilchard kills were localised: in Doubtless Bay, when

dead pilchards were washing up on the beach there were schools of apparently healthy pilchards offshore. Off Whangarei, large schools of pilchards were caught within a few days of pilchard deaths occurring in the area (B. Florian, Whangarei, pers. comm.). Schools of pilchards were reported at Houhora in mid July, but there were no reports of large-scale deaths. It is possible that other kills occurred offshore but were not observed, although fishers were asked to report any unusual fish deaths in their region. There were no reports of pilchard mortalities off the east and west coasts of central and southern New Zealand during June and July.

In late August pilchard kills were reported from the northern South Island at Farewell Spit. Kills moved eastwards through Golden Bay, Tasman Bay, and into the Marlborough Sounds by mid September and to Wellington Harbour (*see* Figure 1).

Dying pilchards were found swimming slowly and erratically near the surface, with some trying to jump out of the water. Fish in the Bay of Islands were seen coming up head first, gulping at the surface, and then sinking tail first. In Whangaroa, pilchards were described as sinking from the surface and then swimming rapidly back to the surface, sometimes jumping out of the water before dying on the surface. Other descriptions mentioned fish appearing on their side about 1 m below the surface, rising to the surface head first and swimming in erratic circles before dying. Dying pilchards in Wellington Harbour were recorded on a short videotape (held in NIWA library, Wellington).

Large numbers of yelloweyed mullet were reported floating off Great Barrier Island on 16 June. However, dead specimens of yelloweyed mullet appear similar to pilchard when floating on the surface, and no specimens were collected for identification by Ministry of Fisheries or NIWA staff. Both jack mackerel and pilchard were found dead and dying at Port Fitzroy, Great Barrier Island on 23 June. All other kills appeared to be dominated by pilchards, with one or a few specimens of other species (kahawai, snapper, red cod, juvenile hoki, and ling). Predatory fish and sea birds were observed feeding on dead and dying pilchards, but there were no reports of other fish or sea birds dying in any numbers. In mid August large numbers of little blue penguins were reported dead on Ninety Mile Beach. However, there have been several large-scale mortalities of little blue penguins in Northland over the past 20 years and the two events, pilchard and penguin mortalities, may not be related (R. Pierce, DOC Northland, pers. comm.). Furthermore, there has been no evidence for a decline in sea bird numbers in Northland over the 1995–96 summer based on transect counts and a nest census (R. Pierce, pers.comm.).

Publicity about the pilchard kills resulted in further reports of fish kills over the previous year. Samples from a fish kill in the Bay of Islands in April 1995, which had been frozen for bait, were identified as pilchard (Allen, Ministry of Fisheries). Many baitfish, probably pilchard, were washed up on Waiuku Beach (Waikato) in March 1995; no specimens were available from this kill. Likewise, pilchards were reported washed up near Havelock in the Marlborough Sounds in May 1995. Another kill of small silver fish, possibly pilchard, occurred in the Catlins in February 1995: no specimens were collected. Specimens of small pelagic fish collected from a local kill on the Mahia Peninsula in February 1995 were identified as jack mackerel, *Trachurus novaezelandiae,* by Paul Taylor (NIWA). In addition, there were several reports of a few fish and birds washed up on beaches, but these are not unusual: the fish are probably discards from fishers and the sea birds immature birds that have died of starvation.

It is difficult to estimate the size of the kills, particularly where large numbers of dead pilchard were reported floating offshore. The kill on Ninety Mile Beach stretched for about 35 km and was estimated at 60–70 t; this is a minimum estimate as no allowance was made for fish which did not wash ashore. Estimates were made by weighing subsamples of all dead pilchards collected from a 10 m length of strandline. Samples were collected every few kilometres along the strandline and wherever the depth of the strandline appeared to change. Dead pilchards were also reported over a similar length of coast on the western Coromandel; counts were made of the number of dead pilchards per metre of strandline and multiplied by the length of coast to give an estimate of 180–200 t of pilchards (L. Curtin, Ministry of Fisheries, pers. comm.). Observations from the Whangarei pilchard fishery suggest that most (up to 80%) dead pilchards sink rather than float and so estimates of total dead pilchards are minimum estimates. Limited diver observations in the Chaffers Marina in Wellington Harbour were that there were far more dead pilchards on the sea floor than floating on the surface (S. Mercer, NIWA, pers comm.). The estimates of dead pilchards on Ninety Mile Beach and on beaches in the western Coromandel, coupled with the kills in east Northland and the large kills reported offshore, indicate that the total North Island kill could have been over 1000 t.

Estimates of the volume of pilchards killed in the South Island event are limited. An estimated 4 t were washed up on Nelson beaches, and considerably more dead fish were seen floating offshore. The weight of fish washed up on the beach was estimated by collecting all the fish from a 50 m length of the strandline and multiplying by the length of shoreline over which dead pilchards were observed. Dead pilchards were reported from several areas within the Marlborough Sounds, but there are no estimates of the volume of fish. In Wellington Harbour the City Council removed one truck load of dead pilchards from beaches, and large volumes were collected for bait by amateur and commercial fishers.

Reports from Whangarei indicate that large schools of pilchard remained in the area after the kills and there was no obvious short-term reduction in availability of local pilchards (B. Florian, Whangarei, pers. comm.). Large schools of pilchard were also observed in the Hauraki Gulf after the kills occurred (Allen, Ministry of Fisheries).

Historical pilchard kills

About 500 kg of pilchards were found dead in the Frank Kitts Park lagoon in Wellington Harbour in December 1993. The cause of death in this kill was reported to be anoxia brought about by the microalga *Tetraselmis* sp. sticking to and clogging the gills (Jones & Rhodes 1994). This species of alga was not implicated in the recent kills: it was not found on the gills of dead and dying pilchards, nor in water samples from kill sites in Northland and Tasman Bay. There was another unconfirmed report of a pilchard kill in the Frank Kitts Park lagoon in August–September 1994 from a member of the public. In August 1994 dead pilchards were collected for bait from Westshore Beach, Napier.

Pilchard kills are not new in New Zealand coastal waters. Graham (1953) reported several pilchard kills off the Otago coast in summer and autumn: "in 1900-1902 enormous numbers set in off Oamaru were washed ashore"; "at one time enormous quantities of pilchards were thrown up by the sea or left by the tide on the Portobello flats and shore".

The *New Zealand Graphic* (25 April 1903) reported large-scale pilchard kills in Picton: "the beach is strewn with dead herrings washed ashore by the tide sometimes to a

depth of several feet". (The Picton "herring" fishery was based on pilchards.) There are also reports of large numbers of pilchards or sprats being washed up in Milford Sound and Foveaux Strait last century (Thomson 1891), and of large numbers of pilchards dying near Picton in the 1920's (Phillips 1929). The publicity over the recent pilchard kills led to other reports of pilchard kills in the Marlborough Sounds; for instance in St Omer Bay, Keneperu, dead pilchards were taken off the beach by the wheelbarrow load in 1937.

Histology

Fixed tissue samples from the pilchard kills in the Bay of Islands (23 June 1995) and Whangaroa (6 July 1995), Tasman Bay (31 August and 1 September 1995), and Wellington Harbour (17 September 1995) were examined at the Animal Health Laboratory, Wallaceville, along with frozen material collected from a pilchard kill in the Bay of Islands in April. Results are presented in Appendices 2–7. Fixed tissue samples from the Bay of Islands were sent to the Fish Pathology Laboratory at the University of Guelph, Canada, and slides of gill tissues from pilchard kills in the Bay of Islands, Whangaroa, Tasman Bay, and Wellington to the Institute of Aquaculture at the University of Stirling for diagnosis.

The gills showed variable, often extensive, damage with sloughing of the epithelial cells covering the secondary lamellae. There was evidence of haemorrhage and exudate between the primary lamellae, along with bacteria. The gill lesions were all at the same stage of development, suggesting that the fish had been exposed to a single toxic event rather than an infectious agent that would produce different tissue responses over time. This gill pathology was interpreted as being consistent with acute exposure to a waterborne toxin (Appendices 3, 6) that caused a sudden extensive disruption to gill function with a loss of blood and fluids. Changes observed in the liver were also consistent with anoxia as the result of gill dysfunction. The gill changes were also interpreted as being consistent with a bacterial or toxic agent by H. W. Ferguson, University of Guelph (Appendix 8). From an analysis of a set of slides sent to the Institute of Aquaculture at the University of Stirling, H. Rodger agreed that the fish were killed by acute gill necrosis with widespread sloughing of the gill epithelium. However, he suggested that rapid changes in environmental conditions, exposure to pathogens, or a combination of these two were the likely cause of death and that the pathology in the samples was not typical of that associated with algal toxins (Appendix 9).

Initial results from samples of moribund pilchards from Australia showed loss of epithelial cells in the gills, and in some specimens excess mucus, suggesting the action of an irritant (Anon. 1995). More extensive examinations of specimens collected from the kills in New South Wales and Western Australia showed locally extensive to generalised inflammation and epithelial hyperplasia in the gills of all affected fish. The secondary lamellae were folded and distorted with cell debris and bacteria between adjacent lamellae. There were no significant lesions in the gills of pilchards tested at 5 and 25 days before kills occurred in Western Australia (R. J. Whittington, Elizabeth MacArthur Institute, New South Wales, unpubl. results).

Bacteriology and virology

Gill, spleen, and kidney tissue from dying pilchards sampled in the Bay of Islands (23 June 1995), Whangaroa (6 July 1995), and Tasman Bay (31 August and 2 September 1995) were cultured on blood agar and MacConkey agar. No bacterial pathogens were isolated.

Bacteria were observed on the gill sections taken from dying pilchards, but the bacterial populations were mixed and the numbers were low (*see* Appendices 6–8). The nutrient rich environment of necrotic tissue and blood tissue fluids would be rapidly colonised by bacteria, especially when normal gill function was compromised. Thus the mixed populations of bacteria observed in the samples taken from dying pilchards were to be expected, although Ferguson (*see* Appendix 8) cautioned against regarding bacteria as a secondary cause of mortality. There was no evidence for a dominant bacterium that was common to fish from the different kill sites.

Tissue samples from dying pilchards sampled in the Bay of Islands (23 June 1995), Whangaroa (6 July 1995), Tasman Bay (31 August and 1 September 1995), and Wellington Harbour (17 September 1995) were cultured on four cell lines, including brown bullhead BB recommended for the culture of herpesvirus. No virus was isolated during the 2 week tissue culture passages (*see* Appendices 2–7). Similar results were reported from tests carried out on Bay of Islands specimens by the Australian Animal Health Laboratory (Appendix 10). In addition, samples from the Bay of Islands (18 April and 23 June 1995) were subjected to a PCR (polymerase chain reaction) test to detect the presence of herpesvirus DNA. No herpesvirus sequences were detected (*see* Appendices 2, 3). However, initial PCR tests used primers designed for mammalian herpesvirus (*see* Appendix 2) and negative results may be due to genetic differences between the mammalian and pilchard herpesvirus rather than the absence of a herpesvirus in pilchard tissue samples. A PCR test with primers specific for channel catfish virus DNA also gave a negative result with pilchard tissues, but positive results with mammalian and avian herpesvirus DNA (*see* Appendix 2).

In Australia, a herpeslike virus was first identified in gill tissue fixed from dying pilchards in May. The herpesvirus has been found in samples of dying pilchard from the northern limits of its range off Western Australia, and off Victoria, New South Wales, and Queensland (B. Jones, Department of Fisheries, Western Australia, pers. comm.). The virus was detected only in samples of dying fish and not in samples collected before and after kills occurred within an area. Fixed gill tissue from three out of four dying pilchards from the Bay of Islands, tested at the Australian Animal Health Laboratory, were positive for the herpesvirus particles (*see* Appendix 10).

Subsequently, a herpeslike virus was found around the gills in 15 pilchards collected from a kill site at Doubtless Bay, but not in 9 pilchards collected in Wellington Harbour in July, when there had been no observed deaths (Hine 1995). The herpesvirus was found in 4 out of 4 pilchards sampled from the kill in Tasman Bay and in all 15 pilchards sampled from the kill in Wellington in September 1995. Infections were focal, but often extensive with detachment of infected epithelial cells soon after virus replication had begun (A. D. Hyatt, Australian Animal Health Laboratory, Victoria, unpubl. results). Blood gases of moribund pilchards sampled in Wellington in September showed extreme hypoxia. It was concluded that loss of the herpesvirus-infected respiratory epithelium may cause disturbance in osmoregulatory function and hypoxia, leading to death.

Phytoplankton and biotoxins

Most species of phytoplankton are harmless, but a few are capable of killing fish through depletion of oxygen in the water, physical damage to the gills, or the production of toxins. Generally, harmful phytoplankton tend to be non-specific and affect a range of fish and shellfish species, especially when kills are widespread (e.g., Tangen 1977, Boalch 1979, Williams & Williams 1988). Species-specific kills are usually associated with farmed fish (e.g., Jones *et al.* 1982, Chang *et al.* 1990) or localised and enclosed bodies of water (e.g., Jones & Rhodes 1994).

The gills of pilchards collected from the Bay of Islands showed no evidence of phytoplankton clogging the gills (L. Rhodes, Cawthron Institute, pers. comm.). A surface water sample from the Bay of Islands kill showed no evidence of a bloom and no evidence for high numbers of known ichthyotoxic species in the sample, and a surface sample from Port Fitzroy, Great Barrier Island (23 June 1995) showed a very low phytoplankton biomass (H. Chang, NIWA, pers. comm.).

Water samples collected throughout the water column from fish kill sites at Whangaroa (6 July 1995) and Doubtless Bay (7 July 1995) were tested at the Cawthron Institute: results are summarised in Appendices 11 and 12. Two water samples were collected from the surf zone on Ninety Mile Beach after the large pilchard kill; results are summarised in Appendix 13. The phytoplankton biomass was generally low with no evidence for large numbers of known fish-killing species, though at some sites there was much fine particulate matter in the water that may have originated from a decaying bloom of phytoplankton.

Phaeocystis spp. appeared in large numbers in samples from Doubtless Bay. *Phaeocystis* spp. produce an irritant (Sieburth 1960) and have been recognised as a potential hazard for fish cage culture. In the open sea some fish species appear to avoid high concentrations of *Phaeocystis* spp. (Rogers & Lockwood 1990). *Phaeocystis pouchetii* was responsible for the Tasman Bay slime outbreak in 1981 (Hurley 1982, Chang 1983) which killed several species of shellfish and finfish through clogging the gills with mucus. There were no reports of slime associated with the recent kills of pilchards. Specimens of *Phaeocystis* spp. from Whangaroa and Doubtless Bay were isolated and cultured for toxicity testing with the brine shrimp *Artemia*. Results from the *Artemia* tests were negative for *Phaeocystis* and for two other species of phytoplankton isolated from kill sites in Northland.

Water samples were collected in Tasman Bay (Delaware Bay) on 31 August 1995, 1 day after dead and dying pilchards were first reported from this area. The phytoplankton biomass was dominated by diatoms, in particular the large centric species *Coscinodiscus concinnus*. A few specimens of the dinoflagellate *Cochlodinium polykrikoides*, a species which has been implicated in fish kills in Japan, were detected, but numbers were too low (less than 500 cells per litre) for it to be the cause of the pilchard kills. Results are summarised in Appendix 14. The phytoplankton biomass was typical for this time of year (the start of the spring diatom bloom) but the biomass was low at two sites off the Abel Tasman National Park coast. There was no evidence from routine phytoplankton monitoring by the Cawthron Institute of unusual events in the weeks preceding the pilchard kills. Subsurface water samples collected in Wellington Harbour on 18 and 19 September 1995 showed a low phytoplankton biomass with no known fish-killing species (Chang, pers. comm.).

Samples of pilchards from Victoria, but not Western Australia, showed excess mucus around the gills. A phytoplankton species, *Thalassiosira*, which produces copious amounts of mucilage containing threads of chitin which could damage gills, was found in water samples from Victoria and New South Wales (Anon. 1995). However, in Western Australia phytoplankton were dismissed as a contributing factor to the pilchard deaths as there was no evidence for blooms or a consistent pattern of phytoplankton (Anon. 1995).

Samples of pilchards from the Whangaroa kill site and from Wellington Harbour in July (when there had been no reports of pilchard deaths) were screened for toxicity at the Communicable Diseases Centre in Porirua. Two pooled samples of gills and guts from about 70 fish from each area were screened for biotoxins. No toxin was detected in the aqueous extracts of either set of samples, but the lipid extract from both the Doubtless Bay and the Wellington Harbour samples was toxic to mice (*see* Appendix 15). This latter result indicates a general toxicity of pilchard gut to mice, but does not provide evidence of a toxin specific to pilchards from a kill site (D. Till, Communicable Diseases Centre, pers. comm.).

The Marine Biotoxin Monitoring Programme in Northland and Coromandel found no unusual biotoxin activity in shellfish from the Coromandel and Northland regions during June and July 1995. However, for the week ending 4 August 1995 a low level non-specific toxin detected in shellfish samples from Ninety Mile Beach to the Hauraki Gulf covered the same geographical range as the pilchard mortalities. This non-specific toxic event was restricted to the East Auckland current, suggesting that some unusual hydrographic conditions in this area triggered the biotoxin (W. Trusewich, Ministry of Agriculture and Fisheries Regulatory Authority, pers. comm.). Phytoplankton samples taken in early August from Parengarenga, Rangaunu, Mahurangi, Marsden Wharf, and Waimangu Point did not show any unusual numbers of known toxic phytoplankton species (Trusewich, MAF, pers. comm.). Likewise, results from the Marine Biotoxin Monitoring Programme did not show any unusual results for Tasman Bay and the Marlborough Sounds during August and September apart from a localised diarrhoeic shellfish poisoning (DSP) event in Queen Charlotte Sound (Trusewich, pers. comm.).

Water temperatures

In Australia the pilchard deaths coincided with a period of low water temperatures. An intrusion of cold subantarctic water was reported off South Australia in March and subsequently moved east and west along the shelf edge producing surface temperatures 3–4 °C colder than usual (Anon. 1995). More recent analyses of water temperatures have shown that there was no unusual cooling before the pilchard mortalities. There was an upwelling event 2–3 weeks before the mortalities in South Australia, but this was not unusual as larger upwellings have been detected in recent years (N. Bax, Division of Fisheries, Hobart, pers. comm.).

Sea surface water temperatures recorded at the Leigh Marine Laboratory were above average in April and May 1995, but fell rapidly in late May/early June to average levels. In November 1994, Dr Bill Ballantine (University of Auckland) issued a memorandum drawing attention to 4 consecutive years of colder than usual sea surface temperatures recorded at the Leigh Marine Laboratory. He predicted major ecological changes in the waters off northeast New Zealand as a result of recent climatic events which might occur over the next 3 years

because of lag effects in biological systems. The period of low sea surface temperatures ended in 1995; May 1995 was the warmest May on record and the April 1995 average has been exceeded only once in the previous 29 years.

Mean daily sea surface temperatures were 20.5 °C in April, 18.5 °C in May, and 15.5 °C in June 1995. There was a rapid drop in temperature over 2 weeks in late May early June (25 May, 18.5 °C; 8 June, 15.6 °C). The June temperature was close to the long term average, but the drop in temperature between May and June was two to three times more rapid than normal. Certainly these events were unusual, but many fish species face daily temperature fluctuations of 1–2 °C in the environment and it is unclear if these events alone were sufficient to trigger the pilchard deaths.

Water temperatures recorded in Tasman Bay in late August/early September 1995 were 11.2–11.8 °C, and are typical of this time of year as temperatures start to rise above the mean annual minimum of 9.5 °C.

Discussion

Marine fish and shellfish kills have been reported from around the world, but often the cause has remained elusive as samples were collected well after death; for example, the cause of Caribbean-wide kills of fish and invertebrates in 1980 is unknown (Williams & Williams 1988). Single species kills are generally associated with an infectious agent, particularly when kills are extensive (Lessios *et al.* 1984, Scheibling & Stephenson 1984, Lafferty & Kuris 1993), but have also occurred under unusual conditions when cage-reared fish have been exposed to local phytoplankton blooms (Jones *et al.* 1982, Chang *et al.* 1990). Multispecies kills are usually associated with local pollution events in enclosed bodies of water (Portnoy 1991, Dassenakis *et al.* 1994) and on a wider scale with toxic phytoplankton (Tangen 1977, O'Sullivan 1978, Boalch 1979, Guzman *et al.* 1990, Rabbani *et al.* 1990,) or major hydrographic events (Alsayed & Ghaddaf 1993).

There have been few documented fish kills in New Zealand waters in recent years. The Ministry of Fisheries has no database on fish and shellfish kills, though several multispecies kills were recorded during the algal bloom events of 1992–93 (Smith *et al.* 1993), and periodic multispecies kills of fish and shellfish have been reported (Chang & Ryan 1985, Taylor *et al.* 1985).

The initial gill histopathology of moribund pilchards sampled in the Bay of Islands, and at Whangaroa, Tasman Bay, and Wellington was consistent with a waterborne toxin that could have been derived through diet (Appendices 3, 6). Only adult pilchards (over 9 cm long) were found in the kills; adults feed on phytoplankton, whereas juveniles feed on zooplankton. However, gill lesions are nonspecific and can be produced under a number of different conditions (Mallat 1985). There was no evidence from phytoplankton data, pilchard toxicity tests, or the Marine Biotoxin Monitoring Programme to support a toxic event. Furthermore, any large-scale toxic events that occurred from Ninety Mile Beach to the Firth of Thames and from Tasman Bay to Wellington would most likely have affected a range of species. There is no evidence for large-scale mortalities of any other species over these areas, apart from jack mackerel dying at the Port Fitzroy wharf along with pilchards on 23 June. Diver surveys in the Leigh area at the time of the pilchard kills found no evidence for large-scale deaths of other

marine species (F. Anthoni, Seafriends Centre, Leigh, pers. comm.). Similar findings were made in Australia where only pilchards were reported to be dying.

A herpesvirus was found in all pilchards tested from kill sites in Australia, but not in pilchards from areas ahead of where kills subsequently occurred (Jones, pers. comm.). The herpesvirus was also associated with the pilchard mortalities in New Zealand, but the degree of infection was variable and light in some specimens (Hine 1995). The herpesvirus is the only consistent factor associated with the pilchard deaths around Australia and New Zealand. If the herpesvirus is responsible for the pilchard deaths, there are still some unanswered questions: what triggered the viral outbreak, how did the virus spread, and why did it affect some schools of pilchards and not others?

In Australia the pilchard kills occurred as single events in each area and appeared to spread over 6000 km in about 70 days from South Australia to Western Australia and Queensland (Anon. 1995). The kills in northern New Zealand appeared to shift northwards from the Firth of Thames and Hauraki Gulf to Doubtless Bay and to the northwest coast, a distance of about 500 km over 1 month. The South Island kills spread from Farewell Spit to Wellington Harbour, a distance of about 250 km, in 2 weeks. Large schools of pilchards were reported from the Bay of Plenty and Hawke Bay by commercial fishers, but there were no reports of kills in these areas. Most kills appeared to be brief, isolated events with dead fish reported over a few days to a week. However, in the Firth of Thames dead pilchards were reported from mid June to early July and again in early August, in the Bay of Islands a kill occurred in April before the main event in late June, and in the Marlborough Sounds there was an unconfirmed kill near Havelock in May, before the main event in September.

No oceanographic mechanism has been identified that could account for the rapid spread of pilchard deaths around Australia (Anon. 1995, O'Neill 1995). In New Zealand, the initial spread of kills was against the East Auckland current, which flows from north to south off the east coast of Northland. The second event moved eastwards with the D'Urville current across Tasman Bay and into the Marlborough Sounds and Wellington. In Australia it was suggested that the virus was spread by fish-eating sea birds or marine mammals moving out from a central focus, or even fast flowing deepsea waves (Anon 1995, O'Neill 1995). Sea birds and mammals could cross the Tasman Sea; immature gannets from New Zealand move to Bass Strait and return to New Zealand to breed (Wingham 1985).

Herpesviruses are usually host specific and are common in marine fish and shellfish, though there are no previous reports that include pilchards. The lack of a history of pilchard kills in Australia, coupled with the rapid shift of deaths from a central focus, led to the suggestion that the herpesvirus was recently introduced into Australian waters via pilchard imported as food for tuna farming operations (and subsequently introduced into New Zealand in imported pilchard bait), or in ballast water, or via migratory sea birds (R. J. Whittington, unpubl. results). At this stage there is little evidence to support the exotic virus hypothesis: preliminary virology tests in Australia have not detected the herpesvirus in samples of imported frozen pilchard, and screening of ballast water is not carried out, although some freshwater piscivorous birds are suspected of transmitting fish viruses. Proposed DNA testing of the Australian herpesvirus with other likely donor populations will help to resolve the exotic status of the herpesvirus. The historical kills in New Zealand indicate that large-scale pilchard deaths are not a new event, though in the absence of preserved samples the cause of the early kills will probably never be known.

Herpesviruses can remain latent for many years and engage in active replication only when the immune environment of the host changes. In some fish, herpeslike viruses are triggered by external factors, such as temperature stress, and internal factors, such as spawning when the immune system is suppressed. Such secondary pathogens do not harm hosts unless the defences are reduced by other stresses (Williams & Williams 1988). For example, in the European smelt, *Osmerus eperlanus*, a herpes disease is expressed only after fish reach sexual maturity and then only in the spawning season (Moller 1984). However, infections due to a latent herpesvirus would not be expected to produce rapid and extensive mortalities.

The onset of spawning in many marine fishes is determined by daylength and temperature; these triggers shift geographically, particularly in spring and autumn. In Australia, surface temperatures determine the distribution of spawning pilchards with the centres of spawning moving north in autumn and south in spring (Blackburn 1960). In New Zealand, pilchards spawn over much of the year in northern waters with peaks in spring and autumn, but only in spring and summer in central and southern regions (Baker 1972). Early work on pilchards suggested that the optimum temperature for spawning in central New Zealand was about 16 °C (Baker 1972). Pilchards sampled from kill sites showed a wide range in size classes (9–24 cm) but were all adult fish. The limited data on gonad samples showed spent and immature fish in Northland and immature fish in Tasman Bay. The rapid decline in water temperatures during late May and early June (18.5–15.6 °C) may have curtailed spawning activities in northern pilchards, and the northward shift of deaths mimicked the seasonal shift of completion of spawning activity. However, temperatures in Tasman Bay were about 11 °C at the time of the kills in September, typical for that time of year. The seasonal and water temperature differences between the Northland and South Island kills rule out a common physical factor in the two events.

In summary, the wide spread and specificity of the kills, coupled with evidence from phytoplankton and toxicity tests, and lack of unusual hydrographic events indicates that the pilchard mortalities in New Zealand were due to an infectious agent. Low numbers of bacteria and variations between kill sites suggest that bacteria had a minor role in the pilchard mortalities. The consistent finding of a herpesvirus in samples of dying pilchards from Northland, Wellington Harbour, and Tasman Bay and from kill sites in Australia, plus the absence of the herpesvirus in unaffected fish in Wellington Harbour, New South Wales, and Western Australia, implicates the herpesvirus in the pilchard mortalities. The large scale and rapid spread of the mortalities suggest that the virus may have recently arrived in Australian waters, though there are historical reports of large-scale pilchard kills in New Zealand. Further research is required to determine if the herpesvirus is an exotic species in New Zealand waters.

Acknowledgments

Initial phytoplankton analyses were undertaken by Dr Hoe Chang (NIWA) and Lesley Rhodes (Cawthron Institute). Barbara Bensoman undertook the biotoxin tests under contract to Ministry of Fisheries. Brett Wesney (NIWA) assisted with sample collection in Doubtless Bay. Bill Ballantine (Leigh Marine Laboratory) provided data on Northland water temperatures.

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Appendix 1. Pilchard kills reported around New Zealand, June September 1995. Reports of single or a few dead fish have not been documented.

A. Great Barrier Island

1. 16 June (Richard Beck)
Many yellow eyed mullet floating dead between Anchorite Rock and Port Fitzroy. No samples collected. Species identity not confirmed by Ministry of Fisheries or NIWA staff.

2. 17 June (Dave Allen, Ministry of Fisheries and Maurice Miles, Auckland Regional Health Board)
Hundreds of "bait" fish reported floating on surface north of the Needles. Species not identified, no samples collected.

3. Between Great Barrier Island and Takatu 19 June (M. Miles)
A 15 n. mile stretch of dead pilchards. Commercial fishers also reported sprats in the kill and estimated the length of fish at about 75 mm. Species identity not confirmed by Ministry of Fisheries or NIWA staff.

4. Port Fitzroy, 23 June (Lorraine Francis, Great Barrier Island, and Paul Taylor, NIWA).
Many pilchards and jack mackerel reported dead and dying at the Port Fitzroy wharf and in the outer harbour. Fish were swimming round in circles at the surface and dying. John dory, kahawai, shags, and gulls observed feeding on the dead fish. Two samples of jack mackerel (for tissue tests) and a water sample (for phytoplankton analysis) sent to NIWA, Greta Point.

B. Hauraki Gulf/ Firth of Thames

1. Omaha, 18 June (Floor Anthoni, Leigh)
Dead pilchards reported at Omaha and between Little Barrier Island and Leigh.

2. Hauraki Gulf, 19 June (Pete Saul, Ministry of Fisheries)
Reports of dead pilchards on the surface between Ponui Island and Coromandel Harbour.

3. Firth of Thames, 22–23 June (Arthur Hore, Ministry of Fisheries)
Many dead pilchards out from Utere Point.

4. Firth of Thames, 27 June (Dave Allen)
Many dead pilchards offshore on surface at Waiomu, south of Tapu. No samples collected. Twelve fish collected from Whakatete Bay, size range 16–18 cm, mean 17.1 cm (measured from frozen sample at Greta Point).

5. Port Jackson/Fantail Bay, 27 June (Dave Allen)
Large numbers of dead scallops in Goat Bay.

6. Firth of Thames, 28 June (Les Curtin, Ministry of Fisheries)
All beaches between Thames and Wilsons Bay (about 30 km) had a band of dead pilchards — about 150 fish per metre.

7. Firth of Thames, 2 July (Dave Allen)
Large number of dead pilchards reported on surface off Orere Point.

8. Kareta Bay, Coromandel, 4 July (Dave Allen)
Report of large number of dead pilchards washed up on beach, possibly dead for 2 days. Fish covered about 500 m of beach in a band about 1 m wide. Also two kahawai and one snapper. Pilchard size range, 16–21 cm, mean 17.6 cm, n = 20 (measured from frozen sample at Greta Point).

9. Kaiaua Bay, Firth of Thames, 4 August (Dave Allen)
Live and dead pilchards washed up on the high tide.

10. Leigh Harbour beach, no date (Dave Allen)
Size range 10–19 cm, mean 13 cm, n = 44 (measured from frozen sample at Greta Point).

C. Bream Bay

1. Hen and Chickens Islands, Mokohinau Island, Little Barrier Island, 17–19 June (Pete Saul)
Report of dead pilchards on the surface in an area between the Hen and Chickens Is., Mokohinau I. and Little Barrier I. Size range 10–18 cm, mean 13.3 cm, n = 31 (measured from frozen sample at Greta Point).

2. Te Arai Point and Mangawhai estuary, 19 June (Pete Saul)
Dead pilchards washed ashore. Fish longer than 10 cm.

3. Mangawhai Heads, 22 June (Dave Allen)
Dead pilchards washed ashore. Size range 15–20 cm, mean 18.3 cm, n = 22 (measured from frozen sample at Greta Point).

D. Bream Head/ Tutukaka

1. Bream Head/Tutukaka, 20 June (Pete Saul)
A large concentration of dead pilchards off Taiharuru Head. No reports of dead pilchards after 27 June.

E. Bay of Islands

1. Waikare Inlet, Opua, 18 April
Hundreds of dead pilchards floating near the Waikare Inlet. Frozen samples made available to Animal Health Laboratories, Wallaceville.

2. April-May (Craig Worthington, Bay of Islands)
Collected two bins of dead pilchards.

3. 20 June (Pete Saul)
Small numbers of dead pilchards reported throughout the inner Bay of Islands.

4. Russell, 21–22 June (Pete Saul)
Dead pilchards washed up on Russell beach, 21 June; many floating near Robertson Island, 22 June. Size range 17–21 cm, mean 19.3 cm, n = 27 (measured from frozen sample at Greta Point).

5. Veronica Channel, 23 June (Paul Taylor)
Tissues dissected from moribund specimens. Gill and brain tissues fixed for light and electron microscopy studies. Tissue samples sent to Animal Health Laboraories (Wallaceville), NIWA (Greta Point), and Animal Health Laboratories (CSIRO, Victoria). Freshly dead specimens frozen whole and sent to Animal Health Laboratories, Wallaceville, and Greta Point. Subsurface water sample collected from side of vessel and sent to NIWA Greta Point for phytoplankton analysis. Dead and dying pilchards occurred at 3–4 per m^2 over about 100 m^2 in channel. Size range 172–231 mm; sex ratio: 18 males, 15 females (Paul Taylor).

6. Russell, 25–26 June
Large volumes of dead pilchards washed up on beach. Northland Regional Council removed one truck load from the beach at Russell on 26 June.

7. Wairoa Bay, Waitangi, 27 June (Dave Allen)
Report of dead pilchards and yelloweyed mullet on beach with about 100 crabs (species not confirmed by Ministry of Fisheries or NIWA staff).

8. Tapeka Point, 28–29 June (Pete Saul)
Considerable numbers of pilchards washed ashore at Tapeka Point and Russell Beach. More than 4 t of dead pilchards were cleared from Russell and Tapeka beaches on 28 June by Northland Regional Council. Large volumes of dead pilchards reported in the water at Te Wahapu, Elephants Head, Matauwhi Bay, Watering Bay, Brampton, To Ti Ti beach, Pahia, and Okiato.

9. Inner Bay of Islands, 3 July (Pete Saul)
Large schools of pilchards observed with no reports of deaths.

F. Whangaroa

1. Stephenson Island, 22 June (Pete Saul)
No reports from commercial fishers of dead pilchards north of Stephenson Island.

2. Whangaroa Harbour, 6 July (John Holdsworth, Ministry of Fisheries)
Dying pilchards over about 3 hectares off Totara North. Fish seen swimming weakly near surface. Fixed tissue samples sent to NIWA (Greta Point) and Animal Health Laboratories (Wallaceville) and water samples for phytoplankton identification to the Cawthron Institute (Nelson).

G. Doubtless Bay

1. Manganoui and Hihi Beach, 3 July (Pete Saul)
Dead pilchards washed ashore.

2. Doubtless Bay 7 July (John Holdsworth).
Fish samples sent to NIWA (Greta Point) and to Animal Health Laboratories (Wallaceville). Water samples sent to the Cawthron Institute (phytoplankton) and NIWA, Hamilton (water chemistry).

H. Houhora

1. Houhora, 15 July (Pete Saul)
A few dead pilchards washed up, many live pilchards seen in the harbour. No samples collected. In the previous week large numbers of small jack mackerel had been caught at the Houhora wharf, but these disappeared and were replaced by pilchards which are not often caught there.

I. West coast

1. Waiuku, Waikato, March (Dave Allen)
Dead and dying fish (presumed pilchard) washed up over 3 km of beach.

2. Ninety Mile Beach, 15–20 July (Vic Hensley, Kaitaia and John Holdsworth)
Many dead pilchards washed up from 3 km south of Huketere stretching north for about 35 km. The kill started south of Huketere with 20–30 fish per 100 m of beach; about 8 km north of Huketere the dead fish increased to an endless line, two to three fish deep; about 20 km north the line increased to a 5 m wide band with fish overlaying each other and finally diminished about 35 km north of Huketere at Butlers creek. Fish were first found on the evening of 15 June after a very high tide: fish appeared to be all pilchards (many had disappeared by Saturday with gale force winds). Dead little blue penguins were also washed ashore. No tissue samples collected, but there was an estimated 60–70 t of pilchards on the

beach on 20 July. Size range at the Bluff 17–22 cm, mean 18.8 cm, n = 21; at Motupia Island size range 16–23 cm, mean 19.0 cm, n = 41 (measured from fresh sample).

J. Bay of Plenty

1. Tauranga, 7 July (A. Cameron, Ministry of Fisheries)
About 100 jack mackerel (about 30 cm long) washed up over 100 m of Papamoa beach. Purseseiners reported working in the area. No samples collected.

K. Mahia Peninsula

1. Mahia Peninsula (north side), 12 February (Gavin Fieldes, MAF Quality Management)
Large numbers of "pilchard" washed up on beach. Collected for bait; frozen samples made available to Animal Health Laboratories (Wallaceville). Identified as jack mackerel, *Trachurus novaezelandiae*, by Paul Taylor (NIWA).

L. Tasman Bay/Marlborough Sounds

1. Maori Bay, Havelock, late May (Mrs Firman, Havelock)
Unconfirmed report of 300–400 dead pilchards on beach; used for bait.

2. Farewell Spit, 29–31 August
Heavy concentration of dead pilchards on beach at southwest end of Farewell Spit with small numbers along western side of spit. Samples collected by DOC staff from Takaka.

3. Separation Point, 29–31 August
Large numbers of dead pilchards offshore. Species identity confirmed by Ministry of Fisheries staff, Nelson.

4. Delaware Bay, 30 August (Lincoln McKenzie, Cawthron Institute)
Dead and dying pilchards reported by scallop fishers in Delaware Bay associated with patches of discoloured water. This was the first day of the scallop season. Water samples collected on 31 August (*see* Table 9)

5. Awarua to Nelson, 1 September (Ron Blackwell, NIWA)
Thin line of dead pilchards in tidal front from Awarua to Nelson. Small numbers of juvenile hoki and ling reported by public, but no specimens found by Minsitry of Fisheries staff. Samples collected for Cawthron Institute, NIWA, Wallaceville, and Animal Health Laboratory, Victoria. Size range 15–19 cm, mean 16.5 cm, n = 56 (measured from frozen sample at Greta Point).

6. Croisilles Harbour-French Pass, 1 September (Kim Drummond, Ministry of Fisheries)
Many dead pilchards reported from Croisilles Harbour and Waikawa Bay. No samples collected. Species identity at Croisilles confirmed by Tracey Osborne (Southern Processors Ltd). Many dead pilchards washed up on D'Urville Island on 5 September.

7. Pelorus Sound, 4 September (Kim Drummond)
Many dead pilchard, 10–15 spiny dogfish, and 1 gurnard in Maori Bay, Inner Pelorus Sound.

8. Moutere Bluffs to Rabbit Island, 5 September (Lincoln McKenzie)
Many pilchards washed up on beach in a band about 2 m wide for several kilometres along strandline. Samples of chilled fish sent to NIWA, Greta Point. Size range 14–20 m, mean 16.4 m, n = 91. One red cod with pilchards. DOC reported four gannets, five shags, and two penguins washed up on local beaches. The estimated weight of fish on the beach was 4.5 t, based on quadrat samples from the strandline.

9. Pelorus Sound, 10–11 September (Ron Blackwell)
Dead pilchards east off Chetwode Is., and from Admirality Bay along western side of Pelorus Sound.

10. Port Ligar, 12 September (Ron Blackwell)
Dead pilchards on surface off Danger Point.

11. Punga Cove, Marlborough Sounds, 14 September (Scott Williamson, Ministry of Fisheries)
Report of dead and dying pilchards in Punga Cove. No samples collected.

12. Endeavour Inlet, Queen Charlotte Sound, 14–15 September (Roy Grose, DOC Picton).
About 20–30 pilchards washed up on beach, size 203– 215 mm.

13. Rabbit Island, Nelson, 14 September (Scott Williamson)
Report of dead hermit crabs and whelks on beach

14. Keneperu Sound, Double Bay, 18 September (Warren Wood, Keneperu Sound)
Small number (< 20) of dead pilchards washed ashore.

15. Keneperu Sound, St Omer Bay, 18–19 September
Many dead pilchards on beach, "knee-deep".

16. Tory Channel, Ruakaka Bay, 18–19 September Bay (Graham Coates, Regal Salmon)
Dead and dying pilchards washed up near salmon cages.

M. Wellington

1. 17–18 September
Many pilchards dead and dying in Evans Bay and in the inner harbour (Chaffers Marina and Oriental Bay). Water, fish and tissue samples collected by NIWA staff. Size range 10–22 cm, mean 16.71 cm, n = 109; males, 81; females, 26; unsexed, 2. Wellington City Council cleared one truck load of dead pilchards from Oriental Bay and from alongside Cobham Drive; amateur and commercial fishers collected large volumes from the inner harbour and Evans Bay.

19-28 September
Dying pilchards at Queen's Wharf and overseas terminal.

N. South Island

Catlins, 21 February (Richard Cook, Institute of Geological Science)
Many pilchard-like fish washed up between False Inlet and Cannibal beach, map ref H47 606098. Fish 3–5 per 10 m^2, about 15 cm long.

pendix 2. Report from Colin Anderson on pilchard samples from Bay of Islands 18 April.

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

20 July 1995

Accn No. 95-4173

Date Received: 9 June 1995

KOKICH - NORTHLAND REGIONAL COUNCIL
PILCHARD KILL - BAY OF ISLANDS

HISTORY

A pilchard kill was reported 18 April 1995 by a member of the public, who collected dead fish and held them frozen. Hundreds of dead pilchards were observed floating near Waikare Inlet, Opua, Bay of Islands. Sea gulls were feeding on them. The observer saw no obvious sign of pollution.

GROSS PATHOLOGY

There were no significant findings on examination of a wet smear of the gills.

The fish were very autolytic (decomposed) and unsuitable for either histological or bacteriological examination.

VIROLOGY

Pooled samples (6 fish examined) of gills and internal organs were prepared for culture on three cell types.

Results:

Cell death was observed in more than one cell type during the first, two week passage. However, this did not reoccur on the second passage and thus was most likely a toxic effect. Tissue during decomposition tends to produce products which are toxic for sensitive tissue culture cells.

No virus was isolated during the two, two-week tissue culture passages.

Central Animal Health Laboratory, MAF Quality Management
Wallaceville Animal Research Centre, Ward Street, PO Box 40063, Upper Hutt, New Zealand
Telephone (04) 528 1334, Facsimile (04) 527 7554

COMMENTS

This result indicates that viable virus was either not present in the tissues sampled or that the tissue culture systems used were not suitable for its culture.

Subsequent samples from a fish kill (? 22 June 1995) in the Bay of Islands, involving pilchards, have shown gill pathology consistant with exposure to a toxin/s, probably a marine biotoxin.

Colin Anderson.

Colin Anderson
Veterinary Fish Pathologist

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

Accn No. 95-4173

Date Received: 9 June 1995
Final report: 6 sept 1995

KOKICH - NORTHLAND REGIONAL COUNCIL
PILCHARD KILL - BAY OF ISLANDS

HISTORY

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VIROLOGY

TISSUE CULTURE

This is a further report to that of 9 June 1995.
Samples were cultured on a fourth cell line, brown bullhead [BB]. BB cells are recommended for the culture of the herpesvirus of channel catfish.
No virus was isolated after two, 14 day passages on this cell line.

POLYMERASE CHAIN REACTION [PCR] TESTING

1. A, PCR test designed to amplify segments of mammalian alpha herpesvirus was applied to gill and pooled tissues [liver, spleen and kidney] from 6 pilchards together with negative and positive controls and a fish herpesvirus, channel catfish virus.

RESULTS

There was no amplification from any of the pilchard samples or from the negative control. Viral sequences were not detected in the channel catfish virus sample.
Strong amplification was obtained from the bovine and wallaby herpesvirus positive controls.

COMMENT

These results indicate that if herpesvirus sequences were present they were not closely related to mammalian alpha herpesvirus.

2. A PCR assay developed for the rapid detection of channel catfish virus DNA, using 2 sets of specific primers, was used to test gill, liver, kidney and spleen from 6 pilchards together with positive and negative controls. Infectious bovine rhinotracheitis, Aujeszky's disease of pigs and infectious laryngotracheitis virus of chickens were included in the test and CCV was the specific positive control.

RESULTS

The CCV sample tested positive while all other samples were negative in the high stringency assay [annealing at 60 degree C].
At a low stringency of annealing [37 degree C], the CCV sample was again positive and the mammalian and avian herpesvirus DNA gave weak positive signals. The pilchard tissues were all negative.

COMMENTS

This PCR assay showed that the pilchard tissues were free of CCV and were unlikely to contain any known herpesviruses as evidenced by the negative results with amplification reactions performed at low stringency which can detect mammalian and avian herpesviral DNAs. The potential of the CCV PCR for the detection of the putative pilchard-specific herpesvirus requires further validation.

Colin Anderson.

Colin Anderson

for
Joseph O'Keefe
Kok Mun Tham
Virologists

pendix 3. Report from Colin Anderson on pilchard samples from Bay of Islands 23 June.

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

Accession number 95-4622
Date samples received 28 June-7 July
Report date 17 July
Final report: 6 September 1995.

MAFFisheries Greta Point
Pilchard Samples - Bay of Islands

HISTORY

Fish kill at the Bay of Islands, sampled 23 June 1995.

BACTERIOLOGY

Gill, spleen, and kidney tissue from 5 fish were cultured and no significant bacterial pathogens were isolated.

VIROLOGY

The gills and internal organs of five pilchards were sampled in pools of 2 fish and cultured separately, on 4 tissue culture cell lines [rainbow trout gonad, RGT; chinook salmon embryo,ChSE; epithelioma papillosum cyprini, EPC and brown bullhead, BB. The latter cell is recommended for culturing the herpesvirus, channel catfish virus.

A suspicious cytopathic effect, CPE, in the first passage of both gill pools, on ChSE cells was not seen on the subsequent passage. These gill samples were toxic to the BB cells and this toxicity may have been the cause of the 'CPE' with the ChSE cells.

No virus was isolated from any of the samples, over two, 2 week passages.

POLYMERASE CHAIN REACTION TESTING

Gills and pools of internal organs [liver spleen and kidney] sampled from 5 frozen pilchards were tested individually with the CCV PCR assay - [see Accession 95-4173 final report, for details].

All pilchard samples were negative.

Colin Anderson

Veterinary Fish Pathologist.

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Total Fees for Service $ 1,860-00

ıdix 4. Report from Colin Anderson on pilchard samples from the Whangaroa fish kill 6 July.

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

95-4844

20 July 1995

J. HOLDSWORTH - MINISTRY OF FISHERIES WHANGAREI
PILCHARD KILL

HISTORY

A pilchard kill had been reported in the Whangaroa Harbour and samples were collected near the Totara North Wharf on 6 July 1995. Pilchards were observed sinking to the bottom and then swimming rapidly to the surface sometimes jumping out of the water and falling back dead. Moribund fish were collected.

Another school of pilchards, a short distance from the wharf, were being herded by predators, and appeared to be swimming normally. Dead fish were not seen in this area.

Five formalin fixed and five chilled pilchards were submitted.

GROSS PATHOLOGY

There was a small amount of haemorrhage in the anterior chamber of the eye of 1 of 5 fish and one fish had some congestion of the base of the pectoral fins. The fins were not frayed.

There were breaks in the skin in the dorsal mid-body which were most likely inflicted by seagulls.

HISTOPATHOLOGY (5 fish examined)

Gills: The epithelium of the finest filaments was absent or degenerate and these filaments were fused and covered by a thin layer of epithelium. Necrotic cell debris was present

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between the fused lamellae, with early regeneration of the lamellar epithelium at this location. There was evidence of significant haemorrhage, and necrotic cellular debris between the larger filaments had been invaded by bacteria. The predominant inflammatory cell types were leukocytes and macrophages.

The pathology in all regions of the gills and in all fish, was of a similar age.

Spleen: There were nodules of congestion on the periphery of the spleen of one pilchard while the other spleens were mildly congested.

Heart, Liver, Pancreas, Adipose Tissue, Stomach and Skeletal Muscle: No significant pathology.

Intestines: Small foci of mucosal necrosis with excessive numbers of bacteria in the lumen of these sections of intestine. Bacteria were present in some of these lesions.

Kidney: Mild swelling and vacuolation of kidney tubules in 3 of 5 fish and cellular debris in the lumen of tubules in 1 of 5 fish.

BACTERIOLOGY (final)

No significant pathogens isolated from internal organs or gills.

VIROLOGY (interim)

No cytopathic effects observed on the first passage.

COMMENTS

Acute branchial necrosis has been the significant pathology in the pilchards sampled from kills, at both the Bay of Islands and the Whangaroa Harbour. The characteristics of the response in gill tissue was similar in both kills, with pathology in the pilchards from the Whangaroa Harbour being of longer standing.

The adverse effects of the necrosis on branchial function has been the immediate cause of death of pilchards at both locations. It is likely that marine biotoxins induced this lesion.

Colin Anderson

Colin Anderson
Veterinary Fish Pathologist

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

Accession number 95-4844

Date of collection 6 July 1995
First report 20 July 1995
Final report 7 Sept 1995

J. HOLDSWORTH - MINISTRY OF FISHERIES WHANGAREI
PILCHARD KILL

HISTORY

A pilchard kill had been reported in the Whangaroa Harbour and samples were collected near the Totara North Wharf on 6 July 1995. Pilchards were observed sinking to the bottom and then swimming rapidly to the surface sometimes jumping out of the water and falling back dead. Moribund fish were collected.

Another school of pilchards, a short distance from the wharf, were being herded by predators, and appeared to be swimming normally. Dead fish were not seen in this area.

Five formalin fixed and five chilled pilchards were submitted.

VIROLOGY

Five pilchards were cultured on RTG, ChSE, EPC and BB cell lines, in pools of 2 and 3 fish, over two, 14 day periods. The gills and internal organ pools were cultured separately.
There was some fungal contamination with the gill pools among the first three cell lines and extensive toxicity in the BB cultures.

No virus was isolated.

Colin Anderson

Veterinary Fish Pathologist

Central Animal Health Laboratory, MAF Quality Management
Wallaceville Animal Reseach Centre, Ward Street, PO Box 40063, Upper Hutt, New Zealand
Telephone (04) 528 6089. Facsimile (04) 527 7554

Total Fees for Service $1,450-00.

ppendix 5. Report from Colin Anderson on pilchard samples from Tasman Bay 31 August.

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

MAF

Accession number: 95 6233
Date received: 2/9/95
Report date: 25/9/95

DIXON - NIWA - NELSON
PILCHARD KILL
BARK BAY - ABEL TASMAN NATIONAL PARK

HISTORY

During collection of samples (Thursday pm. 31/8/95), live fish were seen approximately one meter below the water surface. Among these shoals the occasional fish would have difficulty maintaining it's balance and when it turned upside down, the white belly was visible. These fish were sampled for laboratory examination. Moribund fish were few in number relative to dead pilchards. All the dead fish observed were pilchards and it was estimated that the majority had been dead for 36 hours.

Moribund and dead fish had haemorrhages in the head region, the nostrils were red and there was a pink sheen to the flank. Shags were not seen to be eating the dead fish but many had their heads missing and bite marks on their bodies.
The launch master felt that the pilchards were moving southward with the current from the direction of Farewell Spit.

BACTERIOLOGY

The gills and internal organs of five pilchards were cultured.
No significant pathogens were isolated.

VIROLOGY

The second 2-week passage is in progress.

HISTOPATHOLOGY

The gills and internal organs of three pilchards and the gills of a further five pilchards were examined.

Central Animal Health Laboratory, MAF Quality Management
Wallaceville Animal Reseach Centre, Ward Street, PO Box 40063, Upper Hutt, New Zealand
Telephone (04) 528 6089, Facsimile (04) 527 7554

Total Fees for Service $ 390.00

There was extensive congestion, haemorrhage and necrosis of gill tissue. The finest filaments were fused together. The epithelial cell cover of these filaments was absent over much of the gill surface and in other areas this epithelium was degenerate. The remaining epithelial cells were in the early stages of multiplying to cover the denuded gill tissue.
The gonads of these fish, [two ovaries and a testes], were in an inactive state,[the ovaries were in maturation stage one].

COMMENT

This gill pathology is similar to that seen in the Northland pilchard kills.
The cellular response in these Bark Bay pilchards is some hours less advanced than that present in the pilchards sampled from the Boulder Bank.

Colin Anderson
Veterinary Fish Pathologist

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

Accession number: 95 6233

Interim report : 24/Oct/95

DIXON - NIWA - NELSON
PILCHARD KILL
BARK BAY - ABEL TASMAN NATIONAL PARK

VIROLOGY

No virus was isolated during three, 2-week passages, on three cell lines. Focal areas of cell death were seen in passage one and two, in the ChSE cells exposed to gill tissues, but this did not carry on to the third passage.

Colin Anderson
Veterinary Fish Pathologist

Central Animal Health Laboratory, MAF Quality Management
Wallaceville Animal Research Centre, Ward Street, PO Box 40063, Upper Hutt, New Zealand
Telephone (04) 528 1334, Facsimile (04) 527 7554

Appendix 6. Report from Colin Anderson on pilchard samples from Tasman Bay 1 September.

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

Accession number: 95-6232
Date received: 2 September 1995
Interim report: 21 September 1995

WILLIAMSON - MINISTRY OF FISHERIES NELSON

PILCHARD KILL - BOULDER BANK NELSON

HISTORY

Moribund pilchards were collected between The Glen and Snapper Point during a fish kill. At capture they were put in iced sea water on the afternoon of 1 September and transferred to ice at the laboratory. Gills were subsequently fixed in formalin approximately 7 hours after capture. Frozen pilchards were sent to NIWA, Greta Point.

BACTERIOLOGY

Gill and kidney samples from 6 fish were cultured for bacteria on 2 September. No significant bacterial pathogens were isolated and bacteria were not seen to be playing a significant role in the development of the lesions observed during histopathological examination.

VIROLOGY

There has been no evidence of viral growth in the tissue cultures during the first 2 week passage.

HISTOPATHOLOGY

There has been recent and extensive loss of the epithelial cell covering of the finest gill filaments which have stuck together. The remaining epithelial cells are growing over the fused groups of lamellae and in some areas remnant epithelial cells between the filaments are multiplying to recover the denuded filaments. Mucus cells are numerous among the regenerated epithelial cells. A considerable amount of necrotic cellular debris and in other cases haemorrhage, is trapped between fused filaments.

Central Animal Health Laboratory, MAF Quality Management
Wallaceville Animal Reseach Centre, Ward Street, PO Box 40063, Upper Hutt, New Zealand
Telephone (04) 528 6089, Facsimile (04) 527 7554

COMMENTS

The gill pathology seen in this kill is similar to that present in the pilchard kills at the Bay of Islands and the Whangaroa Harbour, in June/July 1995. It is likely that the cause of the gill pathology in this Nelson kill is also a marine biotoxin.

Colin Anderson
Veterinary Fish Pathologist

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

Accession number: 95-6232
Interim report: 24 October 1995

WILLIAMSON - MINISTRY OF FISHERIES NELSON

PILCHARD KILL - BOULDER BANK NELSON

VIROLOGY

Seven pilchards were tested. The gills and internal organs from three and four fish were pooled separately.

No virus was isolated after two passages in four cell lines.

Transient syncytia were seen, in some wells in passage one and two, in the ChSE cell line exposed to a spleen, kidney and liver pool.

Colin Anderson
Veterinary Fish Pathologist

Central Animal Health Laboratory, MAF Quality Management
Wallaceville Animal Research Centre, Ward Street, PO Box 40063, Upper Hutt, New Zealand
Telephone (04) 528 1334, Facsimile (04) 527 7554

ppendix 7. Report from Colin Anderson on pilchard samples from Wellington Harbour 17 September.

MINISTRY OF AGRICULTURE AND FISHERIES NEW ZEALAND
TE MANATU AHUWHENUA AHUMOANA AOTEAROA

Accession number: 95-6606
Interum report: 24 October 1995

P. SMITH - NIWA

PILCHARD KILL - WELLINGTON HARBOUR

HISTORY

Large numbers of pilchards were observed dying in the city wharf area and Evans Bay on 17 September 1995. Pilchards were collected that afternoon. Moribund fish were sampled and submitted fixed and on ice.

VIROLOGY

Nine pilchards were sampled. The gills and internal organs from four and five fish, were pooled separately.

No virus was isolated after two, 2 week passages on four cell lines.

Focal cell death was seen in passages one and two, in the RGT cell line exposed to one of the two gill pools.

HISTOPATHOLOGY

The gills and internal organs of six fish were examined.

Internal organs

The gonads were in a more advanced stage of seasonal development (stage 2), than the Tasman Bay pilchards.

There was an abnormal amount of blood break-down pigment in the spleen and kidney of some individuals.

No other consistent pathological change was observed.

Central Animal Health Laboratory, MAF Quality Management
Wallaceville Animal Research Centre, Ward Street, PO Box 40063, Upper Hutt, New Zealand
Telephone (04) 528 1334, Facsimile (04) 527 7554

Gills

There were similar changes to those present in previous New Zealand pilchard kills, with recent loss of the epithelial cover of the secondary lamellae which have then fused. There is also haemorrhage and oedema of the gill filaments. Necrotic epithelial cell debris fill many of the spaces between secondary lamellae and in some cases there is bacterial proliferation in this debris. Regeneration of epithelial cells is at a very early stage and mucous cells are not as evident in these Wellington pilchards as they were in the Tasman Bay fish.

COMMENTS

The gill pathology of this Wellington kill was similar to that of the Tasman Bay and Northland pilchard kills.

The tissue culture cell abnormalities which have been observed in the pilchard cases to date, have been inconsistent with regard to; their appearance, the tissue cell type effected, and the organ/s tested. These findings together with the failure of the tissue culture changes to passage beyond passage two, indicate that if viable virus is present the culture systems used do not support it's growth.

Colin Anderson
Veterinary Fish Pathologist

from the Bay of Islands.

UNIVERSITY of GUELPH

Fish Pathology Laboratory
ONTARIO VETERINARY COLLEGE
DEPARTMENT OF PATHOLOGY
GUELPH, ONTARIO, N1G 2W1

TELEPHONE (519) 824-4120 ext. 4631
FAX (519) 836-9819

TO: Stuart MacDiarmid, MAF

FROM: **Hugh W. Ferguson, BVM&S, PhD, DipACVP, MRCVS, FRCPath**

DATE: Jan. 30th, 1996

Dear Dr. MacDiarmid,

Many thanks for the fish samples, which arrived safely. I appreciated the opportunity of looking at the material, aand although I'm not sure that I'm going to be of much help, here is my opinion:

There seems to be little doubt that the fish died as the result of a severe gill disease (branchitis), and that the episode started roughly 4-7 days prior to these fish being killed. Thus the actual cause of mortality would have been a combination of gas exchange problems (mainly a hypoxemia) plus electrolyte\acid-base balance upset. All of the other changes in organs from fish from all sites sampled seemed to be relatively minor.

The gill changes themselves comprised severe lamellar fusion, lamellar epithelial hypertrophy, degeneration and necrosis (including chloride cells) with pronounced sloughing, and moderate acute-subacute branchitis with congestion and hemorrhage as major features. One common denominator with all sites was the presence of variable numbers of bacteria associated with the surface of the gills. Bacterial morphology varied from cocco-bacillary to almost filamentous. Cells were found between lamellae with the appearance of plankton (either dinoflagellates or diatoms), but these can be difficult to identify. A few viral inclusion-like bodies were seen in some lamellar epithelial cells (maybe the virus described by Dr. Hine?)..

The cause of these lesions was not readily apparent. One of the problems when looking at gill disease, especially when a problem hit the fish several days previously, is that the cause of the initial insult can be very hard to find, mainly because the gills are

clearing (flushing) themselves so efficiently. One of the other complicating factors (i.e. more excuses!) is that the initial insult leads to an outpouring of mucus, which in turn traps any debris in the immediate environment. Nevertheless, bacteria are almost always significant in our experience, and to regard them as secondary or as trapped in the mucus greatly underplays their true pathogenicity.

In summary, these fish are dying from gill disease, cause unknown, but I would tend to favour bacteria\plankton. Hemorrhagic gill lesions are usually the result of plankton in Canadian waters. A virus may be involved, but these samples would not this one way or the other. The inability to isolate mucosal pathogens (especially bacteria) doesn't surprise me as these are notoriously difficult to sort out. I just regret that I don't have access to fresh material and\or wet fixed gills, as I could then maybe help a little better with some SEM.

Hope this has helped - my thanks to Colin Anderson for providing the material.

Yours sincerely,

Hugh W. Ferguson

from the Bay of Islands, Whangaroa, Tasman Bay and Wellington.

INSTITUTE OF AQUACULTURE
UNIVERSITY OF STIRLING

Mr. Stuart MacDiarmid,
MAF Regulatory Authority,
ASB Bank House,
101-103 The Terrace,
PO Box 2526,
Wellington,
New Zealand

14th March 1996

Pathology report

Case no. R960024

Re. Pilchard histopathology in 1995

Dear Stuart,

We have finally all managed to screen the slides you forwarded in January and my apologies for the delay in replying.

Professor Roberts, Richards and myself have all examined the tissues and agree that the pathology that appears to have been killing these fish was acute gill necrosis with widespread sloughing of the gill epithelium and associated haemorrhage in some cases. The details we describe as follows:

Sample ref. 95/4622 - 13, 14, A - D (Bay of Islands)
The gills from these fish exhibited epithelial sloughing, haemorrhage, epithelial cell necrosis, occasional bacterial colonies and interlamellar algal detritus. Adhesion of the tips of the secondary lamellae were present and low numbers of epitheliocytis-like colonies were also present.

Within the livers there were numerous coccidia-like parasites and some degenerating hepatocytes. Helminths were obvious encysted in the visceral organs of some fish and in the heart of one but host reaction to these was minor.

Sample ref. 95/4844 - 1 & 2 (Whangaroa Harbour)
The gills from these fish had severe hyperplasia and fusion of the secondary lamellae, some epithelial sloughing and an irregular epithelial surface. Necrotic cells were present in the epithelium and amoeboid-like organisms were present in interlamellar vesicles and between lamellae.

Bacteria were obvious in the lumen of the intestine otherwise there was no significant pathology in the other organs.

INSTITUTE OF AQUACULTURE, UNIVERSITY OF STIRLING, STIRLING FK9 4LA, SCOTLAND
TEL: 01786 467878 FAX: 01786 472133

Sample ref. 95/6233 - 1 & 2 (Tasman Bay)
The gill pathology in the fish was similar to that seen in the Bay of Islands samples and in addition one of the fish had vesicle formation and amoeboid-like organisms present.

The internal organs had parasites present as in the Bay of Islands samples and some sloughing of the intestinal epithelium and bacteria present.

Sample ref. 95/6606 - 1 & 2 (Wellington Harbour)
The gills exhibited widespread epithelial sloughing and necrosis, some lamellar adhesion and occasional bacterial colonies (different bacteria to those in Whangaroa Harbour samples).

All other organs sampled showed no significant pathology.

Interpretation of findings

These fish have all suffered an acute insult to the gills. Rapid changes in environmental conditions, exposure to pathogens or a combination of these are the likely cause.

An algal bloom, toxic or otherwise, would affect other fish species as well as would sudden exposure to pollutants or other water borne toxins or influx of anoxic water and although low numbers of two other species were reported affected this was not a consistent finding. The pathology in these samples was not typical of that associated with algal toxins. Pathogens are more specific in terms of species affected and it is certainly possible that a primary pathogen such as a virus was responsible for such an acute pathology. The observations of bacteria and amoeboid-like organisms were not consistent in the samples and are more likely secondary invaders in the case of the bacteria or background problems in the case of the parasites. Some of the gill pathology observed could be due to the amoeba (e.g. Whangaroa Harbour), however the majority of changes observed were not typical of amoebic gill disease. The observation of the herpesvirus by workers in New Zealand and Australia is considered significant and highlights the most likely candidate for the gill pathology. The sporadic reports of previous pilchard kills around New Zealand indicate that this is not a novel problem however proving the involvement of the herpesvirus in the previous mortalities will be difficult without access to preserved material.

I hope these comments are of value,

Yours sincerely,

Hamish Rodger, BVMS, MSc, MRCVS
Veterinary Clinical Officer

Appendix 10. Virology and histopathology results for pilchard samples from the Bay of Islands, tested by the Australian Animal Health Laboratory, Geelong.

CSIRO AUSTRALIA

Australian Animal Health Laboratory
Division of Animal Health
Institute of Animal Production and Processing

Ryne Street, East Geelong, Victoria 3220
Postal Address: P.O. Bag 24, Geelong, VIC 3220
Telephone: (052) 26 5222. Fax: (052) 23 1424

DIAGNOSTIC SPECIMEN TESTING

Dr. Colin Anderson
Central Animal Health Laboratory,
Wallaceville Research Centre,
P.O. Box 40063, Upper Hutt,
NEW ZEALAND

SAN#: 950330
Date: 27 September 1995

ender	Dr. Colin Anderson Central Animal Health Laboratory, Wallaceville Research Centre, P.O. Box 40063, Upper Hutt, NEW ZEALAND	Sender's Ref. Received On 5 July 1995
wner	Wild fisheries New Zealand	
xamination	virology, EM and histology	

FINAL Report

Submitter: Central Animal Health Laboratories, Wallaceville, New Zealand.

Location: Bay of Islands, NZ.

Samples submitted: 10 whole fish on dry ice, formalin fixed organs and brains. No fixed gill material was submitted.

METHODS

I. Virology

Four fish were thawed and dissected. Gill, brain and pooled kidney, liver and spleen tissues were removed and processed for virus isolation. Brain from fish #3 and #4 was pooled. There were 11 pools in total. Samples were homogenised, suspended in maintenance medium, centrifuged and aliquots were filtered (0.45 um) prior to inoculation onto duplicate cultures of RTG-2 and CHSE-214 cell lines. Cultures were incubated at 15 C.

II. Histopathology

Gill, brain and internal organs were fixed in formalin after dissection. Fixed material received was also examined.

III. Electron Microscopy

continued on next page ...

SIRO
USTRALIA

Australian Animal Health Laboratory
Division of Animal Health
Institute of Animal Production and Processing

Ryrie Street, East Geelong, Victoria 3220
Postal Address: P.O. Bag 24, Geelong, VIC 3220
Telephone: (052) 26 5222. Fax: (052) 23 1424

DIAGNOSTIC SPECIMEN TESTING

Dr. Colin Anderson
Central Animal Health Laboratory,
Wallaceville Research Centre,
P.O. Box 40063, Upper Hutt,
NEW ZEALAND

SAN#: 950330
Date: 27 September 1995

Gill from 4 fish was fixed in gluteraldehyde for examination by electron microscopy.

RESULTS

I. Virology

Viral-like CPE was not observed in any of the CHSE-214 cell cultures. A blind passage was performed at 9 days post-inoculation and cell cultures were incubated for a further 11 days. Viral-like CPE was not observed. In contrast, most of the RTG-2 cell cultures showed focal plaques at 2-5 days post-inoculation (p.i.) and samples were submitted to EM. At 9 days p.i. all cultures were passed and incubated for a further 11 days. Again, plaques developed at 4-7 days p.i. but these had "healed" (with healthy cells growing into the plaques) by 11 days p.i. No viruses were isolated.

II. Histology

Tissues autolysed. However, it was possible to determine that there was inflamation characterised by cellular infiltration or proliferation.

III. Electron Microscopy

Three of the four fish submitted for examination were positive for herpesvirus particles in gill tissue. All cell culture material submitted for examination was negative for virus particles.
END OF REPORT

Laurence J Gleeson
Diagnosis & Epidemiology Project
for P.K. Murray
Head of Laboratory

Appendix 11. Phytoplankton results for the Whangaroa site.

CAWTHRON

DATE: 7 JULY, 1995
TO: Peter Smith
ORGANISATION: MAF Fish
FAX NUMBER: 04 386 0572
NO. OF PAGES: 1

SUBJECT: **Phytoplankton monitoring.**
CELLS / LITRE.

SITE: WHANGAROA HARBOUR	0M	3M	4.5M
DATE 6/7/95			
TOXIC IN SHELLFISH			
Alexandrium spp.	100		
Dinophysis acuminata			
Dinophysis acuta			
Gymnodinium cf breve.			
Gymnodinium mikimotoi			
Prorocentrum lima			
Pseudonitzschia-type	400	2200	1300
Rhizosolenia spp.			
ICTHYOTOXIC SPECIES			
Amphidinium carterae			
Chaetoceros convolutus			
Chrysochromulina spp.		100	200
Dictyocha speculum / fibula			
Fibrocapsa japonica			
Gambierdiscus toxicus			
Heterosigma akashiwo			
Phaeocysytis spp.			
Prymnesium spp.			
OTHER DOMINANT SPECIES			
Chaetocerous spp	9600	3800	
Skeletonema spp	2400	1300	1300

COMMENT: BIOMASS GENERALLY LOW, PREDOMINATELY DIATOMS. DINOFLAGELLATE SPP INCLUDED SEVERAL *GYMNODINIUM SPP*, *COCHLODINIUM SPP*, *PROROCENTRUM SPP*, *CACHONINA SPP* AND *OXYTOXUM SPP*, NONE IN HIGH NUMBERS. THERE WAS A LOT OF VERY FINE CLUMPED DEBRIS IN ALL THE SAMPLES. NONE OF THE SPECIES SEEN, IN THE NUMBERS PRESENT IN THE SAMPLES , WOULD BE AN OBVIOUS CAUSE OF FISH DEATHS.

WENDY GIBBS
PHYTOPLANKTON MONITORING

Appendix 12. Phytoplankton results for the Doubtless Bay site.

CAWTHRON

DATE: 23 MARCH, 1995
TO: Peter Smith
ORGANISATION: MAF Fish
FAX NUMBER: 04 386 0572
NO. OF PAGES: 1

SUBJECT: **Phytoplankton monitoring.**
CELLS / LITRE

SITE:	DOUBTLESS BAY
DATE	11/7/95
TOXIC IN SHELLFISH	
Alexandrium cf minutum.	160
Dinophysis acuminata	
Dinophysis acuta	
Gymnodinium cf breve.	
Gymnodinium mikimotoi	
Prorocentrum lima	
Pseudonitzschia-type	present
Rhizosolenia spp.	
ICTHYOTOXIC SPECIES	
Amphidinium carterae	
Chaetoceros convolutus	PRESENT
Chrysochromulina spp.	
Dictyocha speculum / fibula	
Fibrocapsa japonica	
Gambierdiscus toxicus	
Heterosigma akashiwo	PRESENT (LIVE SAMPLE)
Phaeocysytis spp.	PRESENT (HIGH NUMBERS)
Prymnesium spp.	
OTHER DOMINANT SPECIES	

COMMENT: BIOMASS MOD, PREDOMINATELY DIATOMS (MOSTLY *CHAETOCEROUS SPP*). SEVERAL TOXIC SPECIES PRESENT IN SAMPLE AS INDICATED ABOVE. WE HAVE ISOLATED SOME SPECIMENS OF *PHAEOCYSTIS SPP* FROM THIS SITE, AND FROM WHANGAROA , AND WILL TEST THESE ON *ARTEMIA* AS SOON AS POSSIBLE, FOR ICHTHYOTOXIC ACTIVITY.

WENDY GIBBS
PHYTOPLANKTON MONITORING

Appendix 13. Phytoplankton results for Ninety Mile Beach surf zone samples. n FAX 03 546 9464

FAX MESSAGE:

CAWTHRON

DATE: 21 JULY, 1995
TO: Peter Smith
ORGANISATION: MAF Fish
FAX NUMBER: 04 386 0572
NO. OF PAGES: 1

SUBJECT: **Phytoplankton monitoring.**

SITE:	MOTUPAIA ISLAND	TE WAKATEHAUA ISLAND
DATE	20/07/95	20/07/95
TOXIC IN SHELLFISH		
Alexandrium spp.		
Dinophysis acuminata		
Dinophysis acuta		
Gymnodinium cf breve.		
Gymnodinium mikimotoi		
Prorocentrum lima		
Pseudonitzschia-type		
ICTHYOTOXIC SPECIES		
Amphidinium carterae		
Chaetoceros convolutus		
Chrysochromulina spp.		
Dictyocha speculum / fibula		PRESENT
Fibrocapsa japonica		
Gambierdiscus toxicus		
Heterosigma akashiwo		
Phaeocysytis spp.		PRESENT
Prymnesium spp.		
OTHER DOMINANT SPECIES		
Chaetocerous spp.	PRESENT (HIGH NUMBERS)	PRESENT (HIGH NUMBERS)
Asterionellopsis spp.	PRESENT	PRESENT

COMMENT: BIOMASS FAIRLY HIGH AT MOTUPAIA, LOWER AT TE WAKATEHOUA, *CHAETOCEROUS SPP* DOMINANT SPP AT BOTH SITES. REMAINS OF SOME *PHAEOCYSTIS* CLUMPS SEEN IN TE WAKA., BUT IN LOW NUMBERS. WE HAVE SOME FROM THE LIVE SAMPLE WHICH WE WILL TRY TO GROW. RESULTS OF THE *ARTEMIA* TESTING ARE AT PRESENT INCONCLUSIVE, WITH NO APPARENT EFFECTS FROM A RANGE OF ORGANISMS AFTER EIGHT HOURS. WE WILL CHECK AGAIN AT 24 HOURS AND LET YOU KNOW IF ANYTHING HAS HAPPENED.

WENDY GIBBS
PHYTOPLANKTON MONITORING

Appendix 14. Phytoplankton results for Delaware Bay.

Water column analysis Delaware Bay 31 August 1995

Depth	0m	5m	10m	15m	
Temp (^{0}C)	11.8	11.2	11.2	11.2	11.2
Salinity (10^{-3})	34.8	34.9	34.9	34.9	34.9

	0m	5m	10m	15m
	Cells x10^3 l^{-1}			
Diatoms				
Coscinodiscus concinnus	2.8	2.8	4.4	7.4
Chaetoceros spp.	4.2	3.1	2.1	1.1
C. convolutus	< 0.1	1.0 (1 chain)	< 0.1	< 0.1
Eucampia zoodiacus	< 0.1	< 0.1	1.2	< 0.1
Lauderia annulata	1.0	< 0.1	< 0.1	< 0.1
Stauroneis sp.	< 0.1	0.6	< 0.1	< 0.1
Dinoflagellates				
Ceratium furca	< 0.1	0.2	0.1	< 0.1
Ceratium fusus	< 0.1	0.2	< 0.1	< 0.1
Cochlodinium polykrikoides	0.1	0.4	0.4	0.5
D. acuminata	< 0.1	< 0.1	0.1	< 0.1
Polykrikos schwartzii	< 0.1	0.1	< 0.1	< 0.1
M. rubrum	8.2	0.2	< 0.1	< 0.1
Cryptomonads	ND	80.0	ND	62.0
Small naked dino's	ND	43.0	ND	49.0
Other small flagellates	ND	105.0	ND	118.0

Appendix 15. Results from biotoxin screen tests carried out on pilchard samples from a kill site in Northland and from Wellington Harbour.

ESR
HEALTH

24 July 1995 File: 42/1/2/1

Peter Smith
NIWA
PO Box 14901
Kilbirnie
WELLINGTON

Dear Peter

RESULT OF PILCHARD BIOTOXIN SCREEN TEST

Pilchards were analysed for toxicity by the intraperitoneal inoculation of mice. The test sample (MB498) was from Northland and a control sample was taken from Wellington (MB497).

Methodology Used

Liquid extraction was as in Hannah et al. Extraction of Lipid-soluble Marine Biotoxin. Journal of AOAC International Vol 78, 1995.

Aqueous extraction was as in the AOAC Standard Methods 15th ed 1990, Section 959.08.

The mice were observed for 24 hours after inoculation for symptoms of toxicity.

Results

Pilchard Sample No.	Lipid Extraction		Aqueous Extract
	Lipid	Aqueous Residue	
MB497	Detected	Not Detected	Not Detected
MB498	Detected	Not Detected	Not Detected

In this analysis the lipid bound agent present in both the test and the control sample of pilchards was lethal to mice.

The results apply to the samples as received.

Yours sincerely

Barbara Benseman
Manager, Marine Biotoxin Laboratory

Institute of Environmental Science & Research Limited
Communicable Disease Centre
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