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Gross pathology, histopathology, virology, serology and parasitology

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1. GROSS PATHOLOGY

All post-mortem examinations of New Zealand sea lions were conducted in the field under less than ideal conditions, apart from three pups that were shipped frozen to Massey University. In total, 10 pups, one sub-adult female, and 5 adult females were examined from the Auckland Islands. Four of these females were euthanased on humane grounds. One adult female sea lion was autopsied on Campbell Island and limited samples were collected for histopathology.

1.1 Pups

Of the 10 pups examined, 5 were in poor body condition with marked to severe depletion of the blubber layer and marked dehydration. The remaining pups were in fair or moderate body condition. One pup had ulceration of the anal muco-cutaneous junction and one appeared to have suppurative arthritis of a stifle joint. The remaining pups had no significant gross lesions.

1.2 Adults

The sub-adult female was in excellent body condition. There was focal haemorrhage in the blubber and sub-cutaneous tissues. The most significant change in this animal was multifocal hepatic necrosis characterised by large (up to 3 cm) white foci of necrotic tissue in the subcapsular parenchyma. Approximately 10% of the liver mass was affected.

Among the adults examined, body condition was described as adequate for two animals and very poor for a third, while no comments were recorded on the remaining three animals. Of the four adults euthanased on Enderby Island, one had cellulitis of the right side of the neck and multifocal raised nodular skin lesions on the ventral surface. The cellulitis was described as a diffuse exuberant inflammatory response in the region of the parotid salivary glands and retropharyngeal lymph nodes. The skin lesions were approximately 1 cm diameter, raised, red and firm that oozed blood-stained fluid on cut surface. There were no other lesions. A second female that was extremely weak and had been observed convulsing prior to death was found to have multifocal skin lesions as described for the previous animal but no other significant gross lesions. A third female was also weak and had

numerous skin lesions but no internal abnormalities. The fourth female was in very poor body condition and appeared reluctant to move, but there were no significant gross findings in this case. One female that was found dead had a subcutaneous abscess on one pelvic limb but no other significant lesions. No gross lesions were noted for the female found dead on Campbell Island.

2. HISTOPATHOLOGY

2.1 Pups

Pneumonia was the most significant histological lesion in three pups. In two pups this was classified as mild acute interstitial pneumonia of unknown aetiology; the third pup had mild suppurative bronchopneumonia associated with nematode parasites. One of the pups with interstitial pneumonia also had moderate lymphoid depletion in peripheral lymph nodes and it had acute suppurative cellulitis affecting the stifle region. One pup had suppurative encephalitis and had mild neuronal degeneration with formation of axonal spheroids. One pup had acute suppurative lymphadenitis. The remaining four pups had no significant lesions.

2.2 Adults

The sub-adult female had acute random multifocal hepatic necrosis that was largely confined to the sub-capsular parenchyma. The necrotic foci consisted of degenerate hepatocytes and large numbers of neutrophils associated with bacterial colonies. This female also had mild suppurative bronchopneumonia consistent with bacterial infection and mild lymphoid depletion indicative of reduced immune system capacity.

Mild focal parasitic bronchopneumonia was present in two adult females. The lesion in these animals consisted of a mild neutrophilic and eosinophilic inflammatory response associated with nematodes in the bronchioles. The skin lesions were characterised as focal acute suppurative dermatitis and vasculitis with oedema and haemorrhage. There was fibrinoid necrosis of the affected arterioles associated with large colonies of pleomorphic gram-negative bacteria and marked infiltration of the vessel wall by neutrophils. The perivascular tissue was often heavily infiltrated by neutrophils and also had oedema and haemorrhage. These lesions were located both in superficial and deep dermis and often extended into the superficial blubber. There was frequently severe fibrinopurulent lymphadenitis of superficial lymph nodes and tonsils. Severe suppurative lymphadenitis with extension to surrounding tissues accounted for the neck swelling in one female. In one case there was focal acute fibrinoid necrosis of a pulmonary artery in addition to arteritis in the dermis. The pulmonary and dermal lesions were associated with the proliferation of gram-negative pleomorphic bacteria similar to those seen in the previous case. In one of the euthanased females, in addition to dermal and lymphoid changes, there was mild hepatic atrophy, acute adrenal cortical haemorrhage and mild focal thalamic haemorrhage. These changes were consistent with severe septicaemia and a period of inanition.

3. VIROLOGY

Tissue samples from the sea lions were tested for the presence of viruses by Auckland University (AU); Massey University (MU) and the Central Animal Health Laboratory (CAHL) in Wallaceville; the Institute of Virology, Erasmus University Hospital (EU) Rotterdam, The Netherlands; and by the Foreign Animal Diseases Diagnostic Laboratory (FADDL), U.S. Department of Agriculture, Plum Island, New York.

Tests on tissues included:

- Electron microscopy (AU, MU, EU, FADDL);
- Inoculation and passaging on the following cell cultures: VERO, SekC, SeFB, seal PBMC, MDCK, CRFK, Pk-15, and primary porcine kidney (CAHL, MU, EU, FADDL);
- Guinea pig red blood cell agglutinating test for haemagglutinating viruses, e.g. Influenza A (CAHL);
- IgG and IgM enzyme-linked immunosorbent assay (ELISA) for morbillivirus (EU);
- Polymerase chain reaction (PCR) assay for herpesvirus and morbillivirus (EU).

Using electron microscopy, no viral particles were observed in skin lesions or in cell cultures. Cell cultures were negative for haemagglutinating viruses and also for cytopathic viruses (morbilliviruses and herpesviruses) based on at least five passages in cell culture. Tests for viral antigen in tissues including morbillivirus and herpesvirus were negative using an ELISA test and viral genome (phocine distemper virus and phocid herpesvirus 1 and 2) was not detected using a PCR assay.

4. SEROLOGY

Serum samples from 28 convalescent adult female sea lions were collected in February and tested at FADDL for antibodies against canine distemper virus (CDV) and phocine distemper virus (PDV). All samples were negative against CDV but 4 of 28 (14%) were positive with moderate titres against PDV. A further 3 (11%) had low/borderline titres against this virus. These results indicate likely exposure of the females to PDV or a PDV-like virus at some time in the past. However the prevalence of antibodies and also the magnitude of the serological responses were too low to implicate PDV or a similar virus in the aetiology of the event.

5. PARASITOLOGY

One of the pups examined at Massey University had a heavy burden of hookworms, *Uncinaria* spp., with 1428 adult worms in the intestine. This pup was in poor body condition, markedly dehydrated, and it also had suppurative encephalitis associated with bacterial infection.

6. INTERPRETATION OF RESULTS

Of the ten pups examined, few had either gross or histopathological lesions which makes interpretation of the high pup mortality difficult. Among the few with evidence of disease, bacterial infections appeared to be the cause of death as in the case of the pup with suppurative meningoencephalitis. However, it is likely that other factors such as malnutrition, and hookworm infection played a role in exacerbating the effects of bacterial infection. The role of hookworm infection in natural mortality of New Zealand sea lions is unknown but this pathogen is considered significant for other otariids such as the northern fur seal, *Callorhinus ursinus* (Olsen and Lyons, 1965). Further investigation of this parasite in New Zealand sea lions is warranted.

The gross and histopathological findings in the sub-adult and adult females are consistent with fulminating septicaemia and vasculitis associated with bacterial infection. In most cases, there were numerous pleomorphic gram-negative bacteria present that were fastidious and difficult to maintain in culture. Unfortunately, this precluded specific identification of these organisms which had the morphological characteristics of *Haemophilus* spp. A species of that genus, *Haemophilus somnus*, causes similar pathology in cattle (Andrews et al. 1985; Jubb and Huxtable 1993).

A variety of *Salmonella* serotypes were isolated from several pups and adults; however, based on the available tissue samples it was not always possible to associate isolates with histopathological lesions. The role that salmonellosis played in the epidemic was probably that of an opportunistic invader in which it was responsible for the death of some animals. *Salmonella enteritidis* was isolated from the brain of a pup with encephalitis and has previously been reported as the cause of meningomyeloencephalitis in a northern fur seal pup found dead on St George Island. Alaska (Stroud and Roelke 1980). Salmonellae were also isolated from several adult sea lions and may have been the cause of lymphadenitis and vasculitis. *Salmonella newport* was isolated previously from a captive New Zealand fur seal (*Arctocephalus forsteri*) that had enteritis (Cordes and O'Hara 1979). Intestinal samples were available from only three pups examined here but they did not have enteritis.

Surveys for *Salmonellae* in marine mammals have shown that these bacteria are prevalent in many regions including California (Gilmartin et al. 1979; Thornton et al. 1998), the Pribilof Islands, Alaska (Jellison and Milner 1958), and the British Isles (Baker et al. 1995). They are associated with disease but may also be isolated from apparently healthy animals. Thus, the isolation of these bacteria from New Zealand sea lions may be an indication of the normal bacterial flora of this species that under certain circumstance, can invade the host and cause death. The reservoir host for *Salmonellae* in the Auckland Islands is unknown. Based on preliminary data it is possible that adult sea lions are carriers for some serotypes: faecal samples collected post-epidemic on Enderby Island contained *S. derby.* Further work on archived samples and from samples collected in the future will be required to determine their origin and role in sea lion mortality.

The absence of histopathological lesions that could be attributed to viruses, and the negative virology results from a battery of tests by several different laboratories, are strong evidence that pathogenic viruses were not implicated. The serological findings also support the contention that morbilliviruses, the most pathogenic marine mammal viruses, were not involved in the mass mortality (Duignan et al. 1995, 1997).

The cause of death for many of the sea lions examined was overwhelming bacterial infection resulting in vasculitis and septicaemia. The laboratory diagnostic tests described above allow the following hypotheses to be proposed:

(a) The event may have been caused by a previously unknown—or difficult to identify—gram-negative pleomorphic bacterium. This organism may be a highly pathogenic organism in its own right that has been recently introduced into a naive population, or it may be a normal commensal that became pathogenic because of some change in the normal host/pathogen relationship, swinging the balance in favour of the pathogen.

(b) Some event predisposed the sea lions to a suite of bacterial infections. This could have been infection by a previously unknown virus, a marine biotoxin, or a drastic environmental change associated with the El Niño/ Southern Oscillation phenomenon. There are cogent arguments against the role of viruses or toxins some of which are given above. The role of environmental change is difficult to quantify. However, whether or not it played a primary role in the event, it is likely that it at least played some facilitating role.

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Investigation of the involvement of algal biotoxins

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1. INTRODUCTION

The sudden die-off of New Zealand sea lions at the Auckland Islands during 1998 was first brought to AgResearch's attention by DOC's Southland Conservancy on 28 January. Sampling protocols were rapidly written and radioed to the field workers on Enderby Island, who were able to despatch blood, tissue, milk, and stomach content samples to the mainland by helicopter on 29 January. Following biosecurity clearance, the samples were analysed at AgResearch for the detection of marine biotoxins.

Marine biotoxins have been implicated in a number of mortality events involving marine mammals: for example, Hawaiian monk seals in 1980, Florida manatee in 1982 and 1996, U.S. east coast dolphins in 1987/88, and Mediteranean Monk seals in 1997 (e.g. Anderson & White 1989; Wilkinson 1996; Baden et al. 1998; Bossart et al. 1998; Weaver 1998). Most recently, sea lion deaths have been conclusively linked with the toxin domoic acid, produced by the microalga *Pseudonitzschia australis,* following a large algal bloom in California. In this incident, over 62 adult sea lion deaths were recorded. Domoic acid was detected in serum, urine, and faeces; and brain lesions characteristic of domoic acid poisoning were recorded. Anchovies which died in great numbers were thought to be the vector of toxin transfer: these bait fish contained high levels of toxin, and their guts were packed with *Pseudonitzschia* cells. Algal cell counts peaked above 100,000 cells/L (Scholin 1998).

The study of algal blooms, and their impact upon the food chain, is of growing interest world-wide. New Zealand has a good understanding of the toxins produced by algae, gained during a number of years of study of shellfish toxins following a series of algal bloom events in 1993 (Jasperse 1993).

There is little information regarding the algal ecosystem surrounding the subantarctic Auckland Islands, although satellite imaging has identified the presence of significant amounts of chlorophyll in the waters during January, and there were reports of mucilaginous slime in the sea lion feeding area (see papers by Murdoch and Mackenzie, these proceedings).

Algal biotoxins are classified into groups based on the observed symptoms (primarily following shellfish ingestion) and on the solubility of the toxins.

Water-soluble toxins include:

- Amnaesic shellfish poison (ASP)—Symptoms include loss of memory and balance, nausea, vomiting, fatality;
- Paralytic shellfish poison (PSP)—Symptoms of numbness, tingling of lips and skin, paralysis, fatalities.

Lipid soluble toxins include:

- Neurotoxic shellfish poison (NSP)—Symptoms include incoordination, paralysis, convulsions, fatalities;
- Diarrhoetic shellfish poison (DSP)—Symptoms of diarrhoea.

ASP and NSP toxins have been implicated in previous sea mammal deaths. Other toxicities also exist, including fish kill due to gill clogging/anoxia and respiratory disorders in mammals.

The symptomology of the mass mortality event was not immediately suggestive of known algal toxins. There were also no reports of large-scale deaths in other species; however the potential involvement of marine biotoxin warranted attention.

2. ANALYSIS FOR KNOWN BIOTOXINS

Enzyme linked immunosorbent assays (ELISA) have been developed for ASP toxins (Garthwaite et al. in press), DSP (Matsuura et al. 1994), NSP (Garthwaite et al. 1996), PSP (Usleber et al. 1991). The neuroblastoma cell bioassay capable of detecting PSP, NSP, gymnodinime and other sodium channel active toxins was used as per Garthwaite et al. (1996). Mouse bioassays were conducted using minor modifications of standard procedures (Hannah et al. 1995).

Blood samples and a number of tissue samples were obtained from badly affected animals immediately following euthanasia. Other tissues were obtained at post-mortem examination.

Extracts of blood, stomach contents, liver, and kidney from a number of affected animals were analysed by ELISA and cellular assay for the detection of the following known toxins:

- Brevetoxin, including the neurotoxic shellfish poisoning toxins;
- Saxitoxin, and other members of the paralytic shellfish poisoning group of toxins;
- Domoic acid, the causative agent of amnesic shellfish poisoning;
- Okadaic acid, diarrhetic shellfish toxins including *Dinophysis* toxin DTX1.

Fluid samples were analysed by direct addition to the ELISA, and following extraction in aqueous media, acetone or methanol as appropriate. Tissue samples were extracted for analysis.

A single milk sample was available, and was analysed for brevetoxin and domoic acid.

There was a general masking of the assays due to the unusual sample matrix, however there was no indication of the presence of NSP, PSP, DSP or ASP group toxins in these samples.

Extracts were prepared and analysed by the neuroblastoma cell culture assay which detects cytotoxicity, and the presence of sodium channel active toxins (NSP, PSP and other toxins). A low level of cytotoxicity was observed in a single sample of 0.2-mm filtered blood at 1/10 dilution, however this effect disappeared when the sample was diluted 1/30. No other indication of toxin or toxicity was observed.

No toxins were detected in samples of limpet collected at the Sandy Bay haul-out site.

3. ANALYSIS FOR UNKNOWN BIOTOXINS

Stomach contents of animals euthanased on Dundas and Enderby Islands (specimens D4, E5) were extracted with acetone and partitioned against dichloromethane. Residual water was removed, and the sample dried *in vacuo*. The sample was resuspended in PBS-Tween 60 and injected into mice, and the animals observed for symptoms of toxicity. This is a modification of the standard bioassay for shellfish toxins; 1 mL injection volume represented 10 g (10 mL) of stomach contents.

An aliquot of the dichloromethane fraction was also analysed by neuroblastoma assay, with and without control toxin spike.

Mice showed no adverse reaction to the injected material. The animals were observed for 24 hours with no signs of toxicity, indicating the absence of detectable levels of NSP toxin, gymnodinime and DSP toxins. The neuroblastoma assay gave no indication of toxicity or sodium channel activity. Cytotoxicity was not observed in any sample, and indeed, the blood sample analysed directly supported improved cell growth.

4. ANALYSIS OF MUCILAGINOUS 'SEA-SLIME' MATERIAL COLLECTED FROM THE FEEDING GROUNDS

The logistics of sampling from the remote feeding grounds, and indeed from around the Auckland Islands during the event, were such that no suitable water samples were recovered for phytoplankton identification.

A mucilagenous slime was reported to form large sheets throughout the water column during January. A sample of this material was obtained from the cod end of a scampi trawl from a depth of 400–480 m on 6 February 1998 close to the sea lion feeding grounds in the vicinity of Auckland Islands, 50°51.9'S, 167°22.4'E (surface water temp = 11.1°C).

This material was analysed under the microscope by Lincoln Mackenzie of the Cawthron Institute, Nelson (see following paper) and for toxicity by oral dosing of mice. The semi-plastic material was homogenised with a small volume of water, and introduced to the subject by gavage (force-feeding). No signs of toxicity were observed following repeated dosing with this material.

5. GYMNODIMINE? LINKS TO ALGAL BLOOMS ON THE MAINLAND?

During the January/February period over which this mortality event took place, blooms of *Gymnodinium* sp. cf. *mikimoitoi* were observed along the east coasts of both the North and South Islands of New Zealand, and a large bloom occurred in Wellington Harbour, killing much of the marine life in that harbour (see paper by Murdoch, these proceedings).

A further round of assays was conducted, employing procedures designed to detect toxins and bioactive compounds known to be produced by this organism.

The mouse bio-assay was negative. The standard neuroblastoma assay, and the extended neuroblastoma assay, for detection of gymnodimine, were negative. Cytotoxicity was not observed in any sample.

6. ANALYSIS OF SHELLFISH FOR DETECTION OF TOXIN SIGNATURES

Many shellfish are known to depurate slowly, and can be used as sentinel species for toxin detection. Shellfish samples were collected from around the haul-out site on Enderby Island shortly after the event (24 February 1998), and analysed for toxin. These were negative by ELISA, neuroblastoma and mouse bio-assay.

7. CONCLUSIONS

Samples were analysed for known marine biotoxins, using specific assays, and for general toxicity using the non-specific mouse bio-assay. We were unable to detect the involvement of a marine biotoxin in the mass sea lion mortality event. No toxins were detected in the blood or tissue samples analysed, or in shellfish collected. However, the limited availability of samples suitable for analysis is a concern.

The preparation and adoption of contingency plans will better address this aspect of the investigation during any future events, although it must be noted that the remote nature of the breeding colonies of the New Zealand sea lion poses considerable obstacles to such an investigation.

Suggestions for shellfish and animal tissue sampling follow the References.

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9. APPENDIX: SHELLFISH AND ANIMAL TISSUE SAMPLING PROTOCOL

The aim of this sampling is to preserve any toxin intact in animal tissue, or in shellfish which may be used an a sentinel species (due to their capacity to concentrate toxins while filter feeding).

Collect samples from all species affected in the toxicity incident.

The sampling kit should consist of:

- 10 mL Vacutainers for blood sampling;
- 70 mL wide-mouth pottles for samples of urine, stomach contents, feaces and body tissues;
- labelled bottles containing alcohol as preservative;
- plastic bags for collecting shellfish and dead fish;
- gloves.

9.1 Sampling procedures

Blood: Samples may be taken and stored as plasma or serum. These should be stored as cold as possible.

Urine and stomach contents: Prepare two pottles per sample. Fill one as full as possible and seal; fill a second container 1/3 full, then top up with alcohol before sealing.

Feaces: Collect into a pottle, label and store as cold as possible.

Tissue: Tissue samples are useful for a number of analyses. Two samples must be taken for each tissue, such as liver, kidney, stomach, lungs, brain.

One set of samples should be preserved for histology. A second set will be extracted for analysis of toxin. These should be stored as cold as possible, preferably frozen.

9.2 Despatch of samples

Send samples to Dr Ian Garthwaite, AgResearch Ruakura, East Street, Hamilton as soon as practicable (e.g. when the ship returns to port).

Contacts

If the persons doing the sampling have any queries they should directly contact Ian Garthwaite, Neale Towers, or Katherine Ross at Ruakura for advice.

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Examination of 'slime' collected near the Auckland Islands, February 1998

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1. SAMPLE DESCRIPTION

A sample of slime was collected from the cod end of a scampi trawl from a depth of 400–480 metres on 6 February 1998, in the vicinity of the Auckland Islands, 50°51.9'S, 167°22.4'E (surface water temp = 11.1°C).

- The "slime" was a cohesive, grey, quite solid material that was difficult to tease apart for microscopic examination. Its cohesiveness was lessened when suspended in distilled water or HCL, when a pale stringy mucilaginous material was released.
- The "slime" had a high calcareous content mainly due to the large numbers of foraminifera tests embedded in it.
- The "slime" was coloured with algal pigments. Intense red chlorophyll auto fluorescence was apparent when viewed with UV epifluorescent microscopy; most of the pigment did not appear to be associated with intact cells.
- There were few identifiable micro-algal remains apart from the frustules of several diatom species (including two *Rhizosolenia* spp.), these were not especially numerous. There was quite a lot of siliceous detritus (e.g. radiolarian skeletons) within the "slime".
- Embedded within the pale mucilage were large numbers of small (5–10 μm) cells. The identity of these cells is unknown though they are suggestive of the colonial form of the prymnesiophyte alga *Phaeocystis*.

2. INTERPRETATION

- The sticky mucilage (polysaccharide?) forming the matrix of the "slime" was almost certainly produced by phytoplankton but most of the identifiable organisms and their remains were probably adventives and had nothing to do with its production.
- Mucilage-producing diatoms such as *Rhizosolenia* were present, though their occurrence in oceanic waters is not unusual and they were probably not sufficiently numerous to be the main causative agent.

This paper was presented at the workshop by Dr Ian Garthwaite on behalf of the author.

- The prime candidate for the origin of this material are the small cells embedded in the mucilage (though diatom exudates cannot be entirely ruled out). More detailed examination of these cells using electron microscopy and possibly genetic probes would be required to try and conclusively establish their identity. These cells have a resemblance (possibly superficial) to the colonial stage of the prymnesiophyte alga *Phaeocystis*.
- Various diatom species and *Phaeocystis* spp. are well known to form blooms which can result in the accumulation of very large quantities of mucilage in the water column. *Phaeocystis* blooms are common in temperate and high-latitude (e.g. Antarctic) waters. These slimes can persist for long periods and cause problems for fishers due to net clogging and fish avoidance of affected areas.
- Phytoplankton species which produce slime blooms are not generally associated with the production of known biotoxins as such, though they may produce some bio-active compounds (e.g. acrylic acid production by *Phaeocystis*).
- Based on these observations, an association between the Auckland Island "slime" and the sea lion mortalities is purely speculative.

3. PHYTOPLANKTON AND MARINE SLIME SAMPLING PROTOCOL

The aim of this sampling is to try and identify the organisms present in the water, to detect any known toxins present, and establish the origin of the marine slime which was causing net-clogging in the vicinity of the Auckland Islands during the event.

We may be able to identify the organisms causing this occurrence by examining preserved samples of seawater and slime under the microscope, and could potentially detect toxin in the seawater samples by ELISA.

The sampling kit should consist of:

- 250 mL plastic bottles for sea water samples;
- 400 mL wide-mouth bottles for samples of slime which may be adhering to the trawl nets;
- labelled bottles containing Lugol's iodine, 5% glutaraldehyde and formaldehyde preservatives. Acidified formalin solution (c. 40% formaldehyde) is made by mixing equal parts of formalin and concentrated acetic acid. For preservation, add 2 mL of this solution to 100 mL sample. This gives 0.4% formaldehyde solution. The Lugol's iodine is relatively harmless but will stain fingers and clothes; the glutaraldehyde and formaldehyde solutions are poisonous and should be handled with care (gloves).

3.1 Sampling procedures

Surface seawater

Surface seawater samples should be collected every day from a boat using a clean bucket or from a deck hose for two weeks from the commencement of any sign of toxicity, and at any other time, when, or if, the sea has an unusual turbidity or colour.

Samples must be taken off shore, away from the surf zone where these fragile cells are often damaged and broken.

The procedure is to label one 250 mL bottle (before it gets wet) with the date, time and location (note latitude and longitude), fill about ¾ full (i.e. about 200 mL in each one) and top up (i.e. about 50–70 mL) with Lugol's iodine. Screw the top on tightly and shake, then store in a box. These samples need not be refrigerated.

Slime

If unusual quantities of gelatinous slime are seen on trawl nets, fill one of the wide-mouth pottles with this material and freeze immediately. In addition, fill another three wide-mouth pottles about ¾ with the same slime material (i.e. about 300 mL in each one) and top up (i.e. about 100 mL) each with one type of preservative (i.e. glutaraldehyde, formaldehyde or Lugol's iodine). Screw the top on tightly and shake vigorously before storing. The pottles should be labelled with the date, location, the type of preservative used ("glut", "form", "Lugs") and any other comments which might be relevant (e.g. "grey/green slime off net" etc. The preserved samples do not need to be refrigerated but it would be preferable if they could be (**do not freeze the samples which have preservative!).** The slime samples will not be of much use if they are contaminated with mud from the sea floor. Only "clean" slime, that is from mucilage on the net which is collected from material suspended in the water, is useful.

3.2 Despatch of samples

Send seawater samples to the Cawthron Institute when the ship returns to port. Sub-samples will be passed on to Ian Garthwaite, AgResearch for toxin analysis.

Contacts

If the persons doing the sampling have any queries they should directly contact either Lincoln Mackenzie or Kirsten Todd at the Cawthron Institute for advice.

Phone: 03 548 23 19 or 0800 80 98 98
Fax: 03 546 94 64
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kirsten@environment.cawthron.org.nz

Oceanographic conditions at the time of the 1998 event

Rob Murdoch

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1. INTRODUCTION

Changes in oceanic phytoplankton abundance and community structure are suggested to be associated with variability in the weather, and climatic and physical oceanographic conditions (Chang et al. 1998a,b). These environmental fluctuations and perturbations influence phytoplankton population dynamics through changes to the stability of the surface layers of the ocean. This stability is determined by rainfall, and associated river run-off in coastal zones, wind speed and direction, and solar radiation. (Chang et al. 1992; Rahmstorf 1992). Such factors influence the frequency of upwelling of deep nutrient-rich waters in coastal regions, and the depth of the mixed layer, which will in turn determine levels of primary production, and consequently the distribution and abundance of higher trophic levels. Seasonal and annual changes in local oceanographic conditions can also be linked to the presence in some years of toxic phytoplankton in New Zealand waters, although the causative factors involved in these links are poorly understood.

The present paper examines information available on the oceanographic conditions around New Zealand at the time of high mortality of New Zealand sea lions at the Auckland Islands over the 1997/98 summer. The aim of this analysis is to provide data to test the hypothesis that the sea lion mortality was linked to the presence of toxic phytoplankton within the vicinity of the Auckland Islands.

2. RESULTS AND DISCUSSION

2.1 Climate and SST

The climatic conditions around New Zealand that prevailed over the 1997/98 summer were linked to an El Niño/Southern Oscillation (ENSO) event (Fig. 1). As with previous ENSO years, the surface waters around New Zealand, including the subantarctic region, were colder than average. In December 1997 surface waters were 1 to 2°C colder than average (Fig. 2), a feature typical of ENSO events in previous years. This pattern continued through January, but not February (Fig. 3). Waters in northern New Zealand became warmer than average, a situation unusual for an ENSO event. Temperatures of surface waters in the subantarctic region of New Zealand, however, remained below average over the entire summer period.

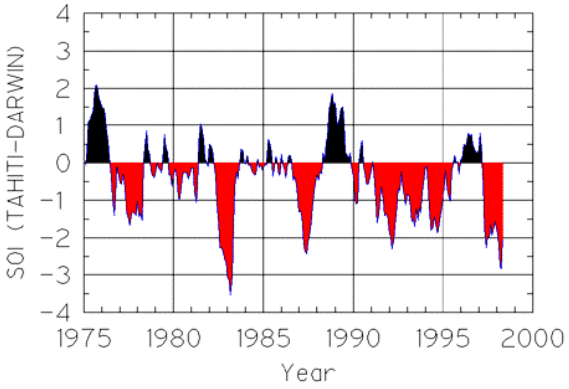

FIGURE 1: PLOT OF THE SOUTHERN OSCILLATION INDEX (SOI) DERIVED FROM PRESSURE MEASUREMENTS AT TAHITI AND DARWIN. THE NEGATIVE EXCURSIONS REPRESENT EL NIÑO EVENTS.

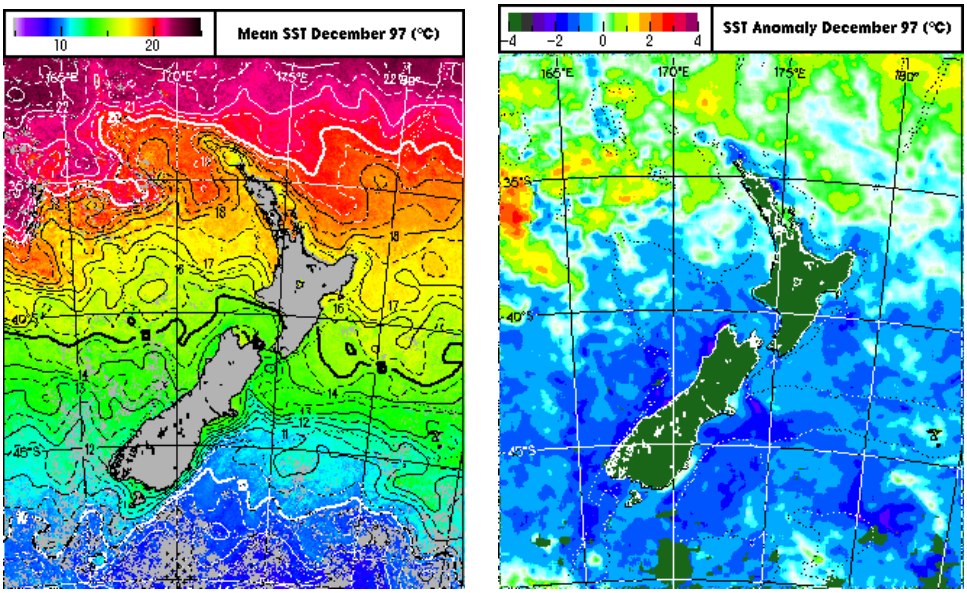

FIGURE 2: MEAN SEA-SURFACE TEMPERATURE IN °C (COLD AREAS BLUE AND WARM AREAS RED, AS INDICATED BY THE COLOUR SCALE) ESTIMATED FROM DAILY IMAGES FOR THE ENTIRE MONTH OF DECEMBER 1997 (LEFT), AND THE SEA-SURFACE TEMPERATURE ANOMALY FOR DECEMBER 1997 AROUND NEW ZEALAND (RIGHT). THE ANOMALY IS THE TEMPERATURE DIFFERENCE IN °C BETWEEN THE DECEMBER 1997 MEAN AND THE AVERAGE OF THE DECEMBER MEANS FROM 1993 TO 1997; BLUE AREAS ARE COLDER AND RED AREAS WARMER THAN AVERAGE AS INDICATED BY THE COLOUR SCALE.

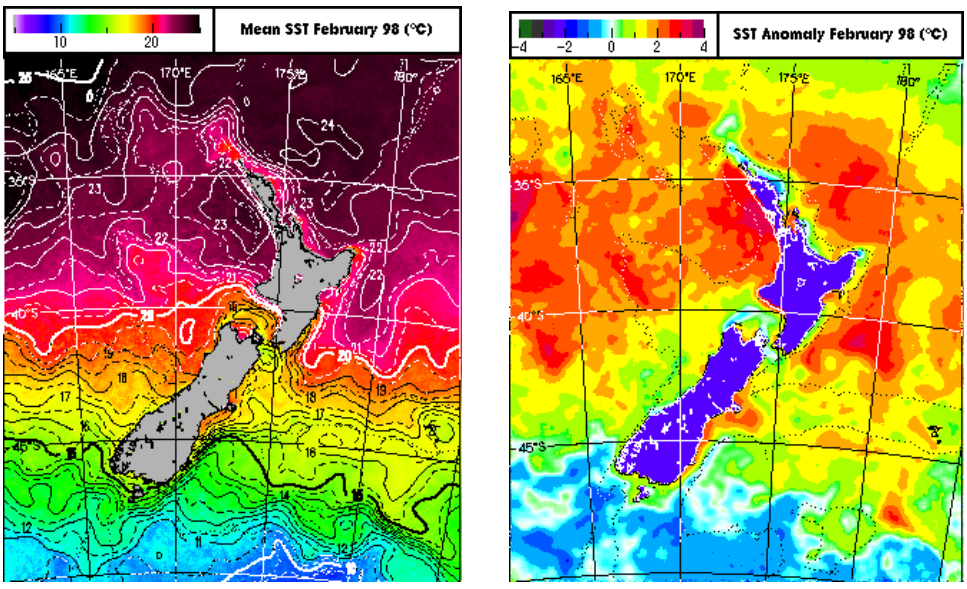

FIGURE 3: MEAN SEA-SURFACE TEMPERATURE (LEFT) AND THE SEA-SURFACE TEMPERATURE ANOMALY FOR FEBRUARY 1998 AROUND NEW ZEALAND (ESTIMATION METHODS AND COLOUR REPRESENTATION AND SCALES SAME AS FOR FIGURE 2).

2.2 Toxic phytoplankton

Throughout January and March of 1998, toxic phytoplankton were recorded off the east coast of North Island and also as far south as Kaikoura. This was associated with fish kills, and reports of respiratory complaints from bathers and surfers (Chang 1998a; Chang et al. 1998b). Off the Wairarapa coast striped marlin, tuna, broad bill swordfish, sea urchins, starfish and paua were reported killed (Chang et al. 1998b,c). Off Kaikoura kills of paua, kina and starfish were also reported. The most dramatic effects of the toxic algae were observed in Wellington Harbour where significant kills of marine life occurred including a range of finfish, shellfish, crustaceans and echinoderms (Chang 1998b,c). These kills coincided with the presence of a bloom of a *Gymnodinium* species. Cell concentrations in excess of 33 million cells per litre were recorded in parts of Wellington Harbour (Chang 1998c; Chang et al. 1998c). Phytoplankton samples collected off the Wairarapa coast during a NIWA oceanographic research voyage on RV *Tangaroa* revealed that the same species was very wide-spread and possibly responsible for the kills both off the Wairarapa coast and within Wellington Harbour; this species appeared to be associated with the unusually warm offshore waters around northern New Zealand at this time (Chang et al. 1998a). Recent studies indicate that this toxic alga is a new species of *Gymnodinium* with especially potent toxin(s) compared with previously known species within New Zealand waters (Chang et al. 1998c, Chang in press). Current analysis of cultures of this species suggests that it may produce novel toxins (P. Northcote pers. comm.).

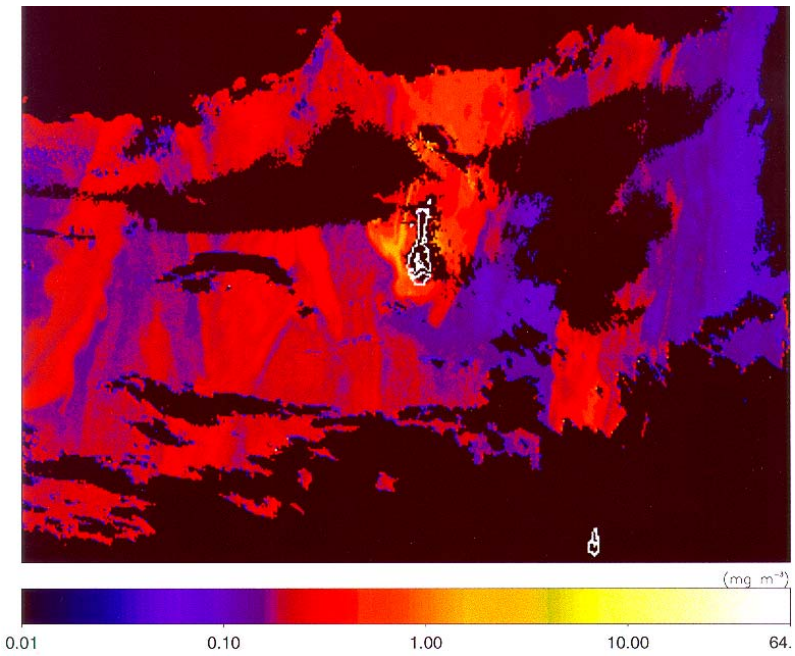

FIGURE 4: SEAWIFS OCEAN COLOUR IMAGE OF THE AUCKLAND ISLANDS REGION (CENTRE OF IMAGE) SHOWING CONCENTRATIONS OF CHLOROPHYLL (MG M^{-3}), 26 JANUARY 1998. BLACK REPRESENTS CLOUD; HIGH AND LOW CHLOROPHYLL LEVELS ARE SHOWN IN LIGHT RED TO YELLOW AND PURPLE, RESPECTIVELY (SEE SCALE). DATA FROM NASA, GODDARD SPACEFLIGHT CENTRE; PROCESSED AT NIWA.

2.3 Oceanographic data

Oceanographic observations and measurements, physical or biological, within the subantarctic region, either in the past or over the summer of 1997/98, are extremely few. An analysis of physical oceanographic data and measurements of chlorophyll concentrations south of 45° latitude was reported by Heath & Bradford (1980). Their study indicated that higher phytoplankton abundance occurred over the shallow regions of the Campbell Plateau and around the subantarctic islands relative to deeper regions. It was concluded that this was most likely associated with shallowing of the mixed layer as a consequence of shallowing water depths. A single SeaWIFS ocean colour image (Fig. 4), from the Auckland Island region on 6 February 1998, similarly indicates that phytoplankton abundance around the islands was higher than the surrounding ocean, consistent with the observation of Heath & Bradford (1980). The species composition of the phytoplankton assemblages at this time, however, is unknown.

3. CONCLUSION

Sea surface temperature data indicate that water temperatures were below average around the Auckland Islands at the time of the sea lion deaths. This situation prevailed over southern New Zealand for the entire summer, and is consistent with the effects of ENSO events in previous years. Toxic phytoplankton were recorded around central New Zealand, and were found to be responsible for substantial kills of marine life and respiratory complaints in humans exposed to blooms. Studies indicate that this is a new species of *Gymnodinium*, possibly with new toxin(s).

At this point, links between sea lion death and marine biotoxins have not been established. However, given:

(1) that new species of toxic algae continue to be discovered in well sampled regions around the New Zealand mainland;
(2) that high chlorophyll concentrations have been observed around the Auckland Islands; and
(3) the almost non-existent sampling of phytoplankton in the past from subantarctic waters south of New Zealand,

the presence of a toxic algal species at the time of the sea lion deaths cannot be ruled out as the causative agent of these deaths.

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Organochlorine contamination of collected sea lions

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1. INTRODUCTION

The presence of organochlorine contaminants in the bodies of marine mammals has been proposed to be a contributing factor to the recent global increase in marine mammal mass mortalities (Simmonds 1991; Simmonds and Symoens 1992). In particular, it has been suggested that suppression of the immune system leads to exposed animals being more susceptible to pathogenic organisms (Dietz et al. 1989; Reijnders 1994; Swart et al. 1994; Ross et al. 1995).

Organochlorine concentrations in New Zealand marine mammals are considerably lower than those in northern hemisphere species (Jones et al. in press). However to investigate any possible contribution of chemical contaminants to the mass mortality event, three samples from affected animals were analysed for the presence of organochlorine pesticides and a range of polychlorinated biphenyl (PCB) congeners.

2. SAMPLE DESCRIPTION

Samples were provided by Prof. Per Madié of Massey University. They had been collected at the time of post-mortem and were stored frozen until analysis. Samples of blubber from the following individuals were submitted:

2.1 Sample E11

Adult female caught and euthanased at Sandy Bay, 16 February 1998 (post-epidemic). She had numerous skin lesions on her ventral and lateral surfaces. Apart from the skin lesions there were no other significant gross findings.

Histopathology

1. Dermatitis, deep dermal, focal, suppurative, acute with haemorrhage and oedema, centred on blood vessels associated with gram-negative bacteria.
2. Cellulitis (blubber), focal, suppurative, acute.
3. Tonsillitis, acute, suppurative with haemorrhage.
4. Hepatic atrophy, mild, diffuse.
5. Thyroid degeneration, cystic, marked, bilateral, diffuse.

6. Adrenal necrosis, focal, acute, with haemorrhage.
7. Thalamus, focal haemorrhage, acute, mild.

Bacteriology

No isolates on salmonella plates. The inflammatory (vasculitis, heamorrhage, necrosis) changes are bacterial septicaemia possibly salmonella (although not isolated) or an unknown gram-negative bug.

2.2 Sample E12

Adult female captured and euthanased at Sandy Bay, 16 February 1998. She was in poor condition and stiff and lethargic. She was observed lying at the highwater mark for 3 days before capture. No gross lesions noted.

Histopathology

1. Thyroid degeneration, cystic, moderate, bilateral, diffuse.
2. Broncho-pneumonia, suppurative, with haemorrhage and haemosiderin laden macrophages, intra-lesional nematodes (*Parafilaroides* sp.?).
3. Blubber, plerocercoid cysts.

Bacteriology

Salmonella isolated from faeces. The findings here are probably not significant.

2.3 Sample 28330

Also coded Dundas-6. This was a pup shipped intact to Massey, 29 January 1998. It was in poor body condition. It had haemorrhagic intestinal contents and congested meninges, but no other remarkable gross lesions.

Histopathology

Encephalitis, thalamus and mid-brain, suppurative, acute, with neutrophilic perivascular cuffing.

Parasitology

It had a heavy burden of hookworms.

Bacteriology

Salmonella enteritidis isolated. The bacterial encephalitis and the hookworm enteritis are both significant findings.

3. ANALYTICAL PROCEDURES

For analysis in the laboratory, blubber samples were removed from the freezer and allowed to partially thaw. The semi-frozen blubber was dissected in a containment hood and a portion was removed and chopped into roughly mm sized cubes. The sample (approx. 10 g) was weighed accurately and placed into an Accelerated Solvent Extraction cell. Before extraction a range of isotopically labelled internal standards was added to each sample. Samples were extracted by accelerated solvent extraction with a mixture of acetone

and hexane at 100°C under pressure. The samples were then subjected to a range of chemical and chromatography clean-up procedures to remove interfering substances and to isolate the most toxic co-planar PCBs from other PCB congeners. Samples were analysed by standard isotope dilution procedures (Jones et al. 1996). Analytes of interest were polychlorinated biphenyls (PCBs) and a range of organochlorine pesticides

Full analytical details are available on request as are details of the extensive quality assurance procedures used in the laboratory. All analyses were performed under the laboratory's IANZ (formerly Telarc) accreditation.

4. RESULTS

As part of the analytical procedure, a portion of the extract is used to determine the amount of "hexane extractable lipid" (% HEL). While this is not a strict analytical measure, it provides a very good estimation of the lipid content of the blubber. The blubber from specimen E11 had a much higher HEL content than samples E12 and 28330. This is in general accord with the observations on the condition of the animals (see above). The concentrations detected in E12 and 28330 are particularly low for marine mammal blubbers which are generally in the range of 50 to 90 % HEL.

The results of organochlorine pesticide and PCB congener analyses are in a separate report, available from DOC upon request. A summary of the data is provided in Table 1. PCB congeners were detected in all samples. The sum of the congeners analysed ranged from 11 to 23.3 ng/g wet weight. The biological potency of the PCB mixture was calculated as dioxin equivalents (TE) using the TEF values of Ahlborg et al. (1994). Total TE values ranged from 1.63 to 2.19 pg/g wet weight.

The most abundant organochlorine residue detected was p,p'-DDE (a metabolite product of DDT): concentrations ranged from 21.7 to 30.6 ng/g wet weight. Heptachlor epoxide was also detected in all samples. This organochlorine was not detected in a range of albatross egg samples recently analysed, but was detected in New Zealand fur seals (Day 1996) at similar concentrations (mean 4.2 ng/g, range 0.25 to 22.9 ng/g wet weight, n=18).

TABLE 1. SUMMARY OF ANALYTICAL FINDINGS, SEE TEXT FOR DISCUSSION.

	E11	E12	28330
% HEL	64.0	12.0	11.9
S-PCBs	23.3	16.0	11.0
TE	2.19	1.63	1.90
p,p'-DDE	30.6	27.7	21.7
Heptachlor epoxide	1.77	9.31	6.53

5. DISCUSSION

The HEL content of the blubber samples indicates relatively poor condition of two of the three animals, in accord with observations on the whole animals.

Concentrations of organochlorines in the submitted blubber samples were low compared to similar species from the Northern Hemisphere. Ross et al. (1995) detected a total TE concentration of 61.8 pg/g in harbour seals fed relatively "uncontaminated" Atlantic herring for a period of two years. They also measured 208.7 pg/g total TE in seals fed herring from the Baltic Sea over the same time period. While these studies also included TE derived from polychlorinated dibenzo-*p*-dioxins and dibenzofurans, these contaminants are in general only minor contributors (< 15%) to TE measured in New Zealand marine species (Jones 1998, Jones et al. in press). In the above feeding experiments, significant differences in the immune function of the seals receiving contaminated fish were detected (Ross et al. 1995). It is however not clear whether the "control" animals in this study were showing immuno-suppression.

It seems unlikely that the contaminants measured in the New Zealand sea lions could have been a major contributing factor to the observed mortality. This conclusion is, however, drawn with no knowledge of the threshold dose for immuno-suppression in this species. Given that pinniped populations in the Northern Hemisphere survive with higher burdens of these contaminants, it seems likely that the threshold for effects is above levels currently observed in northern hemisphere animals. However, if the threshold dose is less than the observed concentration in the New Zealand sea lions (i.e. < 2 pg/g TE) then these contaminants may have had some bearing on the mass mortality.

It should be noted that the complexity of the immune system is not yet fully understood let alone the subtle effects of low-level contaminants.

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