

Epizootic mortality in the pilchard *Sardinops sagax neopilchardus* in Australia and New Zealand in 1995. I. Pathology and epizootiology

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ABSTRACT: A large-scale epizootic occurred in the Australasian pilchard *Sardinops sagax neopilchardus* between March and September 1995 over more than 5000 km of the Australian coastline and 500 km of the New Zealand coastline. Affected fish died within a few minutes of clinical signs of respiratory distress and death was associated with hypoxaemia and hypercapnea. Significant lesions were confined to the gills and comprised acute to subacute inflammation followed by bizarre epithelial hypertrophy and hyperplasia. The lesions were initially focal but progressed to become generalised over about 4 d. Pathological changes in affected fish from western Australia, eastern Australia and New Zealand were similar, suggesting a common aetiology. The lesions were unlike those associated with ichthyotoxic algae, siliceous algae, physicochemical factors, fungi, bacteria, dinoflagellates, amoebae, other protozoa and metazoa. A herpesvirus was consistently present in gills of affected fish and absent from unaffected pilchards and is proposed as the aetiological agent. The rate of spread of the mortality front (approximately 30 km d⁻¹) in relation to the migration rate of pilchards and prevailing currents suggests that a vector was involved. The disease may have been newly introduced into Australian waters.

KEY WORDS: Clupeoidei · Pilchard · *Sardinops sagax neopilchardus* · Gill diseases · Pathology · Epizootiology · Mortality · Herpesvirus

INTRODUCTION

The Australasian pilchard *Sardinops sagax neopilchardus* is a prolific, small, pelagic, planktivorous fish of the suborder Clupeoidei which is found in waters of the continental shelves of Australia and New Zealand south of latitude 25°S (Fletcher 1990). Closely related species are found in waters off South Africa, South America, California and Japan (Fletcher 1990).

In 1995 an extraordinary epizootic affected schools of this species in Australasia. No other species of fish

were affected. Mortalities on a massive scale commenced in March 1995 in central southern Australian coastal waters and spread in both easterly and westerly directions. Floating rafts of dead pilchards as large as 3 km by 1 km were observed and many tonnes of dead pilchards were washed ashore on nearby beaches. The epizootic was observed progressively over 4 mo along more than 5000 km of the Australian coastline, representing the total range of the species in Australia. A similar phenomenon commenced in north-eastern New Zealand waters in June 1995, eventually affecting pilchards along 500 km of the coast. There have been no other progressive large-scale epizootics affecting pilchards in Australasia and to our knowl-

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edge this is the largest mortality event ever recorded in any fish species in terms of both numbers affected and geographic range.

Although oceanographic disturbances were suspected to be the cause of the epizootic in the first few weeks, it was soon clear that infectious disease would also need to be considered. This report details the results of the investigation undertaken by a network of institutions in Australia and New Zealand and provides strong evidence for an infectious aetiology. A companion paper (Hyatt et al. 1997) provides more details of a herpesvirus, which is the most likely aetiological agent.

MATERIALS AND METHODS

Occurrence and spread of disease. The occurrence and spread of the disease was monitored by observers at sea, along the coastline in inhabited areas and from aircraft. Observers included commercial fishermen, Government officers and members of the public in both countries. Data were collated for this report from information supplied with specimens submitted for laboratory examination and from the records of New South Wales Agriculture, New South Wales Fisheries, the Fisheries Department of Western Australia and the National Institute of Water and Atmospheric Research, New Zealand. Data for South Australia and Victoria were obtained from the Department of Primary Industries and Energy, Canberra. In Australia, the distance and elapsed time of the disease front from its origin were calculated as the shortest distance in km by sea from the site at which dead pilchards were thought to have been seen first (Anxious Bay, South Australia) and the time in days from the date of origin to the first date at each site at which dead pilchards were seen, respectively. Data analysis was carried out using SYSTAT.

Clinical signs. Affected and healthy pilchards were observed from the surface of the water, underwater by SCUBA divers, and also by viewing underwater video camera recordings.

Blood gas analysis. During the outbreak in Wellington Harbour, New Zealand, in September 1995, pilchards ($n = 16$) that were >11 cm total length and that were swimming listlessly at the surface were removed using a hand net. The tail was severed and blood was taken into heparinized 75×1 mm capillary tubes (Drummond Scientific Company, Broomall, Pennsylvania, USA), chilled over ice, and pools of 2 samples were read on an AVL 995 automatic blood gas system analyser. Normal pilchards were sampled from Wellington Harbour in December 1995, 3 mo after the outbreak. Eight were caught by rod and line and placed immediately into seawater, taken to a laboratory aqua-

rium and maintained for 1 to 5 d in a dimly lit environment. They were then removed one by one with minimal excitement and blood was sampled and processed as above. Results for the 2 groups were compared using a *t*-test assuming normality of the data.

Collection of specimens. The epizootic commenced in a relatively remote area and initially only a few opportunistic collections were made. Later, field staff were deployed at sea specifically to collect healthy, moribund and freshly dead fish. Healthy live pilchards were collected in purse-seine nets (Australia) or with rod and line (New Zealand). Affected fish were removed from the sea by dip netting. Whole fish were placed immediately on wet ice or wet ice/sea water slurry in insulated boxes, or into 10% v/v buffered neutral formalin or 10% v/v formalin in seawater. Some specimens were fixed directly in 2.5% v/v glutaraldehyde in filtered seawater. Fixation of specimens from some collections in Western Australia was delayed by up to an hour. In some instances one operculum was removed and/or the abdominal cavity was incised to allow more rapid penetration of fixative. Fish were transported to laboratories for examination within 24 h of collection.

Prospective investigations were undertaken in New South Wales (Iluka) and Western Australia (Albany, Rottnest Island). Specimens were collected from schools of pilchards at these locations before the arrival of the mortality front, during the mortality event, and after the mortality front had passed.

Gross pathology and histopathology. Fish collected from New South Wales and from Bremer Bay, Western Australia, were subjected to a full necropsy. Gills, brain, skeletal muscle and viscera were examined histologically from up to 10 fish from each collection. Tissues were embedded routinely in paraffin wax, sectioned at $5 \mu\text{m}$ and stained with haematoxylin and eosin. Selected sections of gill were stained also by the periodic acid Schiff reaction, the method of Gram and by Grocotts, Warthin-Starry, Feulgen, Giemsa and Ziehl Neelsen methods. Longitudinal sections of the second holobranch of each fish were prepared and histological descriptions were based on sections midway along the length of the primary lamellae. Lesions in gills are referred to as generalised when all secondary lamellae on all primary lamellae were affected, as locally extensive when large regions of secondary lamellae were affected but other regions on the same primary lamellus were normal, as multifocal when multiple small regions of secondary lamellae were affected and as focal when a single area of secondary lamellae was affected.

Direct microscopic examination for bacteria, fungi, protozoa and metazoa. Gills from affected fish from selected collections in New South Wales and Western Australia were placed in a drop of saline and examined

for the presence of filamentous bacteria, fungi, protozoa and metazoa at 10 and 100 times magnification using transmitted light. In addition, parasitological examination was conducted on whole-body scrapings and gills.

Electron microscopy. Gills were freeze dried without cleaning off the adhering debris, mounted on stubs, coated with gold-palladium and examined using a Philips XL20 scanning electron microscope. Gills from moribund New Zealand pilchards were excised, cut into 1 mm³ pieces, fixed for 1 to 2 h in 2.5% glutaraldehyde in 0.22 µm filtered seawater (FSW), washed twice in FSW, post-fixed in 1% osmium tetroxide for 1 h, dehydrated through graded ethanol solutions, embedded in Araldite, sectioned, stained with 5% uranyl acetate for 10 min and 5% lead citrate for 5 to 6 min, and examined on a Philips 420ST electron microscope. Gills and internal organs from Australian pilchards were prepared as described by Hyatt et al. (1997).

Bacterial culture. Gills were cultured on blood agar, MacConkey agar, marine agar, cytophaga agar and thiosulphate citrate bile salt sucrose medium. Kidney and spleen were cultured on marine agar and blood agar using standard techniques.

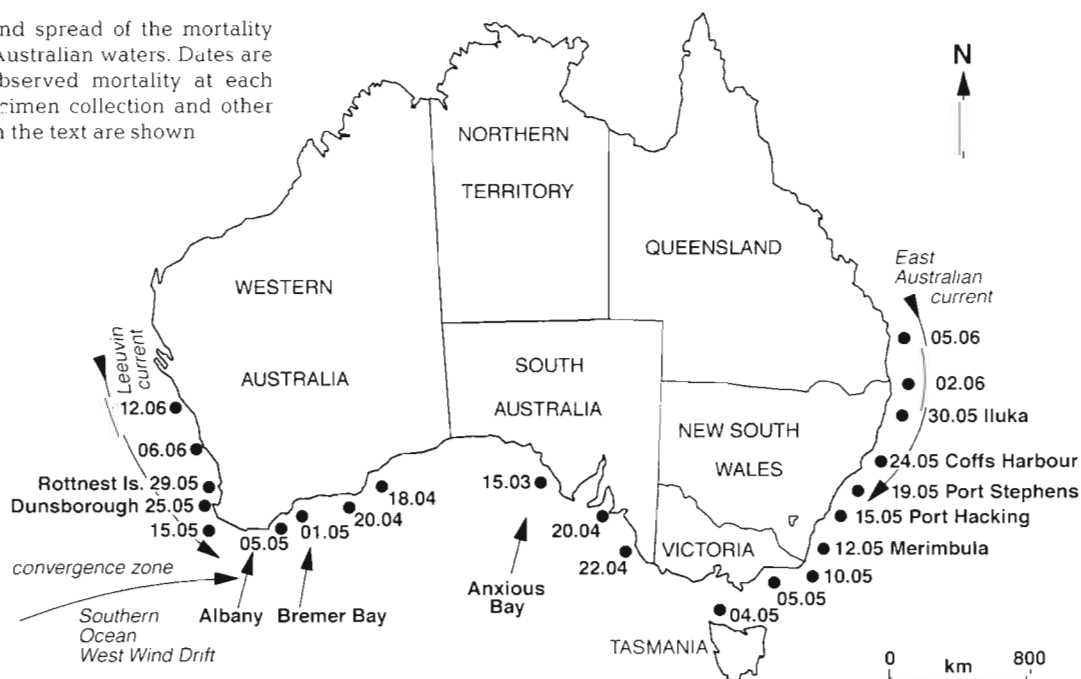
RESULTS

Occurrence and spread of disease

The mortality commenced in the vicinity of Anxious Bay, South Australia, in March 1995 and spread both

west and east covering more than 2500 km in each direction over 3 mo (Fig. 1). A plot of the elapsed time of the mortality front by its distance showed that the rate of spread to the west was slower than that to the east (Fig. 2) so the directional data were analysed separately. In both directions the Spearman rank correlation between elapsed time and distance was significant (to the east, $r = 0.922$, $n = 41$; to the west, $r = 0.940$, $n = 22$). Linear regression was attempted, but the patterns of the residuals were unacceptable. To the east, variance decreased with distance from the origin, while to the west the pattern in the residuals was cyclic. Therefore, to estimate the rate of spread of the disease, distance was divided by elapsed time for each site. Examination of plots of these rates with distance as the independent variable showed that to the east the pattern changed after 26 April (at the western approaches to Bass Strait) from a median 12.1 km d⁻¹ (range 1.3 to 21.2 km d⁻¹, skew -0.04) to a median 32.4 km d⁻¹ (range 8.0 to 36.2 km d⁻¹, skew -0.97) through Bass Strait and northwards up the coast to Queensland. To the west the rate, after removal of 3 data points in South Australian waters prior to 24 March which had rates of 68 to 275 km d⁻¹, showed a median rate of 30.4 km d⁻¹ (range 3.3 to 43.2 km d⁻¹, skew -1.36). The rate of spread to the west was against the eastward current of the Southern Ocean across the south of Australia and against the southward flowing warm Leeuwin Current which converges with the Southern Ocean waters (Fig. 1). Spread to the east was unaffected by the generally eastward movement of water to Tasmania and was not apparently affected by

Fig. 1. Occurrence and spread of the mortality front in pilchards in Australian waters. Dates are those for the first observed mortality at each location. Sites of specimen collection and other sites named in the text are shown



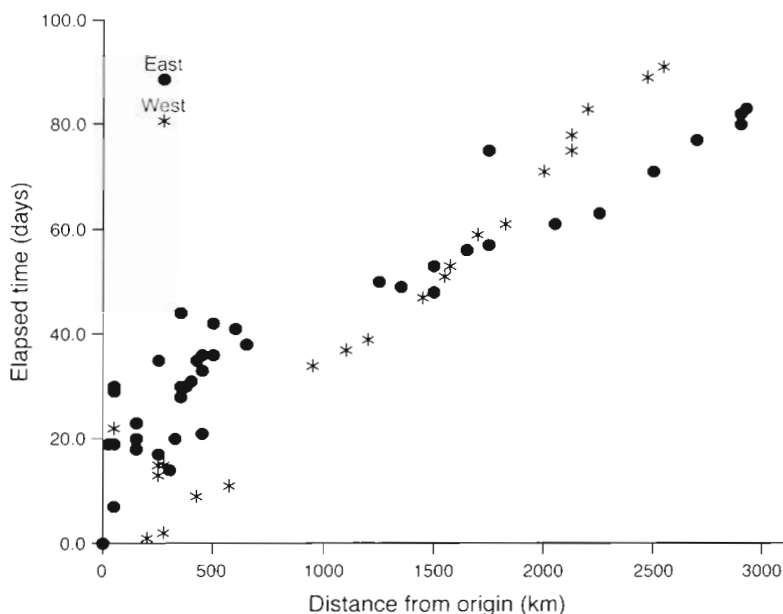


Fig. 2. Elapsed time of the mortality front in relation to distance from its origin in South Australian waters

the convergence zone between the warm East Australian Current flowing down the Queensland and New South Wales coast and the southern cold water (Fig. 1). The spread of the disease was not impeded by storm events; there were 3 during its progress through Western Australian waters, and one prolonged storm event during the spread in New South Wales waters.

In New Zealand, large-scale mortalities were noted first in Hauraki Gulf and the Firth of Thames on the east coast of the North Island in June 1995 and spread north through the Bay of Islands, rounding the tip of the North Island to reach Ninety Mile Beach by mid-July (Fig. 3). There were no reports of mortalities in central and southern waters of the North Island over this period or later. In August another mortality event commenced off the northwest tip of the South Island and spread east, reaching Wellington Harbour in mid-September (Fig. 3).

The eventual distribution of the disease was discontinuous but observations were also discontinuous. Mortality was noted for only a few days at each location, consistent with an advancing disease front. Apparently healthy schools of pilchards were noted offshore from affected schools in western Australian, New South Wales and New Zealand waters and normal catches were recorded in each area within days of the mortality front passing. The exceptions were embayments such as Wellington Harbour where pilchards were rarely sighted in the weeks after the mortality event. Pilchards were the only species affected in Australia and in New Zealand.

Clinical signs

Adult pilchards of both sexes were affected (Table 1) and based on weight-for-age data (Fletcher 1990) these fish were at least 2 or 3 yr old. Affected fish along the south coast of Western Australia (Bremer Bay, Albany) were ≥ 4 yr old, 2 and 3 yr old fish being absent from the fishery, while the affected fish off the west coast of Western Australia were ≥ 2 yr old (R. Fletcher pers. comm.). The mortalities occurred at different times in relation to spawning (from several months before to several months after, depending on location) suggesting a lack of relation to the reproductive cycle. In Western Australia, juvenile fish were seen schooling under and in the vicinity of schools of affected adults but were not themselves clinically affected.

Observers on boats noted that affected pilchards comprised a small proportion of the pilchards in schools that appeared otherwise to behave normally. However, many more dead fish appeared when the schools were chased. Dead fish floated to the surface to form large rafts while apparently unaffected fish schooled 0.5 to 3 m below. One observer in New Zealand reported that affected fish left the school, ceased swimming, turned onto their sides, resumed swimming sluggishly and then died in extremis within a few minutes. When disturbed, moribund fish jumped erratically from the water. Underwater observations in New Zealand

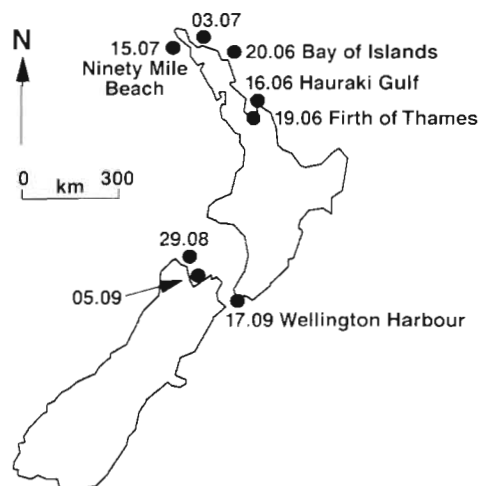


Fig. 3. Occurrence and spread of the mortality front in pilchards in New Zealand waters. Dates are those for the first observed mortality at each location. Sites of specimen collection and other sites named in the text are shown

Table 1. *Sardinops sagax neopilchardus*. Sampling locations, clinical status, morphometrics and results of histological and electron microscopical examination of gills. Fish collected before and after the mortality event were taken live from clinically unaffected schools; those examined during the mortality event were dead or moribund and taken from schools affected by mass mortality. nr: not recorded; NSW: New South Wales; WA: Western Australia; NZ: New Zealand. Note: herpesvirus was detected in fish from Wellington, NZ, during the outbreak, but histology was not undertaken

Location	Stage of mortality event	Most common form of lesion in gill	Total length (cm) ^a	Weight (g) ^a	No. of fish examined	Percentage of fish with finding					Herpes-virus ^b	
						Epithelio-cystis	Gram-positive bacterial rods in colonies	Other bacterial colonies	Amoebae	Other proto-zoa		Meta-zoa
Iluka, NSW	4 d before	Focal	18 ± 1.2	38 ± 8.6	10	30	90	10	20	0	0	Negative
Iluka, NSW	2 d before	Locally extensive	19 ± 1.4	44 ± 10.1	10	0	60	10	10	0	0	Positive
Iluka, NSW	During	Generalised	17 ± 2.2	30 ± 12.3	10	0	10	40	80 ^c	0	10	Positive
Iluka, NSW	8 d after	Focal	18 ± 1.0	38 ± 8.6	10	30	50	10	0	0	0	Negative
Coffs Harbour, NSW	During	Generalised	20 ± 1.2	52 ± 8.6	5	0	0	60	80 ^d	0	0	Positive
Port Stephens, NSW	During	Generalised	17 ± 1.2	37 ± 10.4	10	0	0	20	40	0	0	Positive
Port Hacking, NSW	During	Generalised	20 ± 0.9	51 ± 5.8	4	25	25	50	100	25	0	Positive
Merimbula, NSW	During	Generalised	nr	nr	4	0	0	25	50	0	0	Not tested
Albany, WA	5 d before	Nil	17 ± 5.7	42 ± 4.8	10	10	50	0	10	0	0	Negative
Albany, WA	During	Multifocal	17 ± 6.2	35 ± 4.2	4	0	50	25	50	0	0	Positive
Albany, WA	8 d after	Multifocal	18 ± 7.0	41 ± 5.1	10	0	0	10	10	0	0	Negative
Southwest, WA	During	Generalised	18 ± 8.4	38 ± 5.6	4	0	25	0	50 ^d	0	0	Positive
Rottnest Island, WA	25 d before	Nil	18 ± 3.8	43 ± 6.4	10	0	60	0	0	0	0	Negative
Rottnest Island, WA	During	Generalised	17 ± 5.6	38 ± 4.8	10	0	10	30	60	0	0	Positive
Rottnest Island, WA	18 d after	Multifocal	18 ± 12.2	43 ± 10.7	10	0	30	0	0	0	10	Negative
Bay of Islands, NZ	During	Locally extensive	17 ^e	nr	5	20	0	40	40	0	0	Positive
Bay of Islands, NZ	During	Locally extensive	nr	nr	3	0	0	33	33 ^g	33	0	Not tested
Bay of Islands, NZ	During	Locally extensive	17 ^e	nr	10	20	0	0	0	0	0	Positive
Wellington, NZ	18 d before	Focal	17 ^e	nr	10	10	0	0	10	20	10	Negative

^aSample size 25, mean ± SD; ^bfor details see Hyatt et al. (1997); ^cmoderate to large numbers in 3 fish; ^dmoderate to large numbers in 1 fish; ^erange 10 to 22 cm

revealed that affected schools contained individuals of varying size and that only the larger fish were dying. Death occurred within minutes of the first signs of distress while the remaining fish were schooling normally. The rate of mortality increased suddenly when the school was startled; flashing behaviour commenced within 15 s and death within 3 to 5 min. Many dead pilchards had sunk to the bottom. Accurate estimates of the mortality rate were not made at any location, although 10% was suggested in Western Australia (R. Fletcher pers. comm.).

Gross pathology and direct microscopic examinations

Apart from traumatic lesions associated with netting, there were few gross abnormalities and most fish were in good bodily condition. Some had died with the mouth open and the operculae abducted. Vascular congestion around the eyes was observed in some moribund and dead pilchards in New Zealand. The gills of freshly collected affected fish were dark red to dark brown while those of unaffected pilchards were bright red. It was uncertain whether this difference was related to the time since death, since in general the affected fish were removed dead from the water while the normal fish were removed alive. However, blood collected from live affected pilchards that were caught after being startled by SCUBA divers was noticeably darker than blood of healthy pilchards caught by rod and line. Mucus was not apparent on the gills. One affected fish had pale streaks in the myocardium later found to be due to microsporidian cysts. The degree of fill of the gastrointestinal tract varied between schools without apparent relationship to the presence of disease. The spleen varied from a pale brown structure 10 mm (length) × 2 mm (maximum width) to a dark red to black structure 20 mm × 5 mm but size did not correlate with disease status.

No filamentous bacteria, fungi, protozoa or metazoa were observed in direct mounts of gills of affected fish from New South Wales or Western Australia. No parasites were observed in whole-body

scrapings of 10 fish caught at Iluka 4 d before the mortality event, while small numbers of gliding bacteria were seen in gill smears of 2 of 15 fish from this site. Gill scrapings and smears from 5 freshly dead fish collected off Rottneest Island, Western Australia were specifically examined for amoebae but none were found.

Histopathology of tissues other than gills

Liver, kidney, spleen, brain, heart, pancreas, gastrointestinal tract, gonad, skin and skeletal muscle were examined from pilchards that were collected from Iluka, New South Wales, 4 d before the mortality event ($n = 5$), 2 d before the event ($n = 5$), during the event ($n = 5$) and 8 d after the event ($n = 5$) and from affected fish from Port Hacking ($n = 4$) and Port Stephens ($n = 10$). There were occasional insignificant abnormalities in a range of tissues from occasional fish from each group, including microsporidian cysts in myocardium and skeletal muscle unassociated with inflammation, metazoan parasites in the gastrointestinal tract and peritoneal cavity, intestinal coccidia and scattered inflammatory foci in various organs. The exocrine pancreas was autolytic or scant in many fish. The large spleens were engorged with erythrocytes, while small spleens contained few erythrocytes. There was mild to marked vacuolar change of the liver in most fish and mild to severe autolysis of the gastrointestinal tract. There were no significant findings in most affected fish from Western Australian waters although a coccidian, *Goussia* sp., was found in the liver of some. The oocyte diameter was approximately 20 μm while the spore size was 9–10 \times 6 μm with residuum, which is consistent with *G. clupearum* (Levine 1983). *Kudoa thyrsites* was occasionally seen in the musculature of pilchards from Western Australia as previously recorded by Langdon (1991).

Histopathology of gills

Histology of unaffected fish. Secondary lamellae were of uniform length, straight and evenly distributed along the primary lamellae. Secondary lamellae were covered by a single thin layer of epithelium that was closely apposed to the pillar cells. The epithelial cell nuclei were flattened, while the cytoplasmic processes were indistinct (Fig. 4). Chloride cells were abundant on some primary lamellae but were absent from others; this was determined by the plane of section. Where present, chloride cells were restricted to the bases of

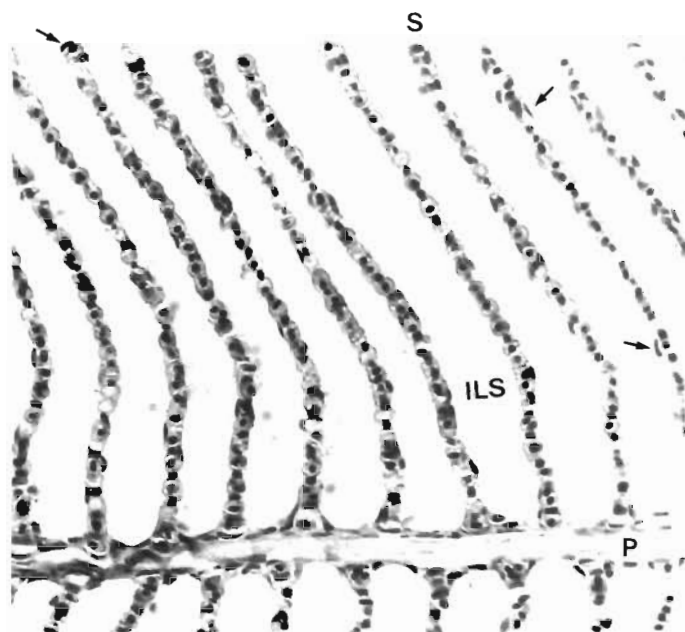


Fig. 4. *Sardinops sagax neopilchardus*. Normal pilchard gill, Iluka, New South Wales. In this section the primary lamellus is devoid of chloride cells. P: Primary lamellus; S: secondary lamellae; ILS: interlamellar space. Note flattened epithelial cell nuclei (arrows). $\times 450$

the interlamellar spaces and were found singly, in pairs or groups along the entire length of the primary lamellus, either within or below the epithelium. Mucous cells were rarely present in the epithelium of the secondary lamellae but they did occur on the tips of the primary lamellae in some fish.

Iluka, New South Wales—4 d before the mortality event. There were single or multiple, small foci of subepithelial proteinaceous oedema along the secondary lamellae in 4 of 10 fish. A separate focus of branchitis in one of these was characterised also by proliferation of chloride cells and mild lymphocytic infiltration (Fig. 5). There was a single small focus of mild epithelial hyperplasia in each of 2 fish.

Iluka, New South Wales—2 d before the mortality event. There were multifocal to locally extensive inflammatory lesions affecting some primary lamellae in 10 of 10 fish. In 8 fish there was also focal (4 fish) or multifocal (4 fish) epithelial hyperplasia; in the remaining 2 fish there was multifocal to locally extensive epithelial hypertrophy. The secondary lamellae were normally aligned or were folded, mildly distorted and apparently shortened. Inflammation was characterised by subepithelial and luminal exudates of small to moderate numbers of eosinophilic granulocytes, chloride cells, lymphocytes and occasional neutrophils (Figs. 6 & 7) while macrophages were also present in 2 fish. In affected areas the epithelial cell

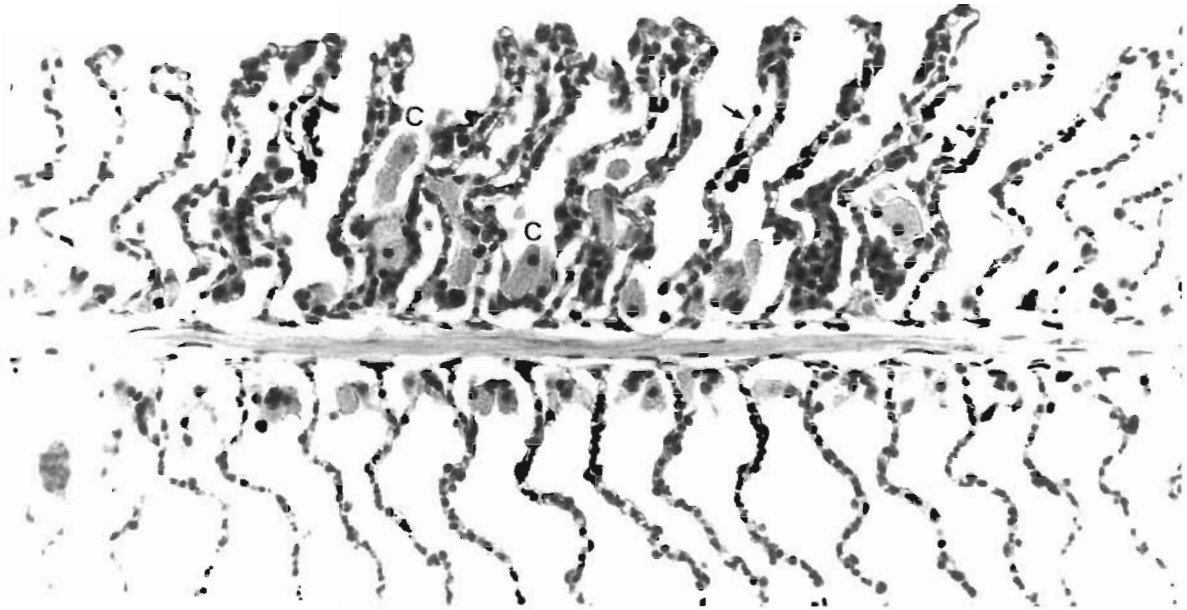


Fig. 5. *Sardinops sagax neopilchardus*. Fish collected (live) 4 d before the mortality event at Iluka, New South Wales. There is focal branchitis with subepithelial proteinaceous oedema (arrow), chloride cell proliferation (C), a mild lymphocytic infiltrate and early epithelial hypertrophy. $\times 450$

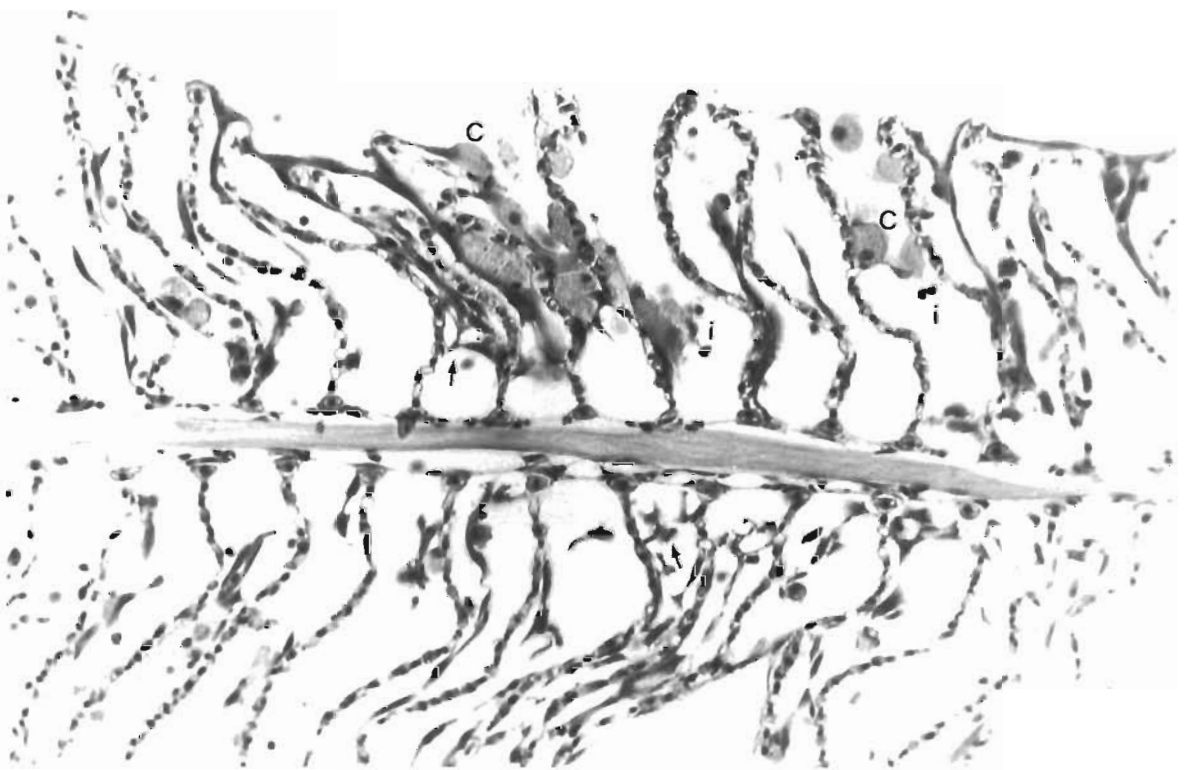


Fig. 6. *Sardinops sagax neopilchardus*. Fish collected (live) 2 d before the mortality event at Iluka, New South Wales. Part of primary lamellus with locally extensive branchitis. There is proliferation of chloride cells (C), mild infiltration of mixed inflammatory cells (i) and epithelial cell elevation and hypertrophy forming cytoplasmic bridges (arrows) between adjacent secondary lamellae. $\times 450$

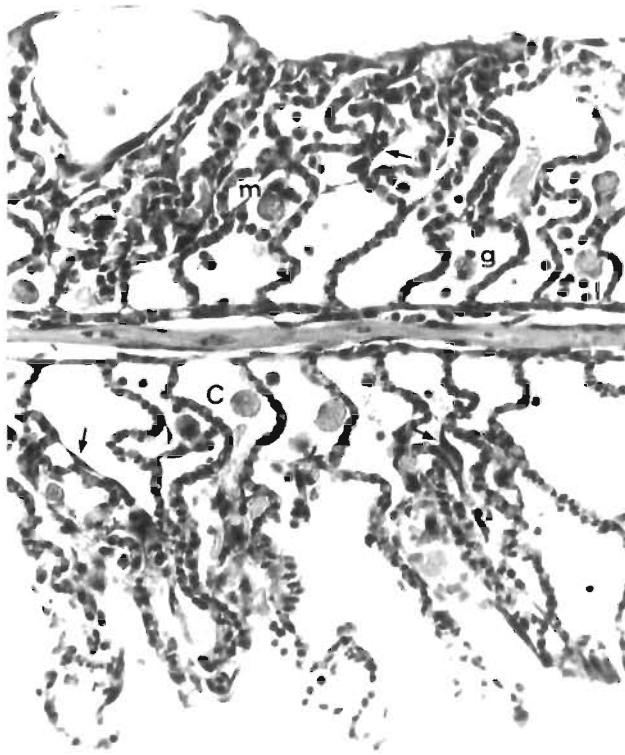


Fig. 7. *Sardinops sagax neopilchardus*. Fish collected (live) 2 d before the mortality event at Iluka, New South Wales. Part of primary lamellus with multifocal branchitis. There is a mixed inflammatory cell exudate in distended subepithelial and interlamellar spaces with epithelial hypertrophy (arrows). l: lymphocytes; m: macrophage; g: eosinophilic granulocyte, C: chloride cell. $\times 450$

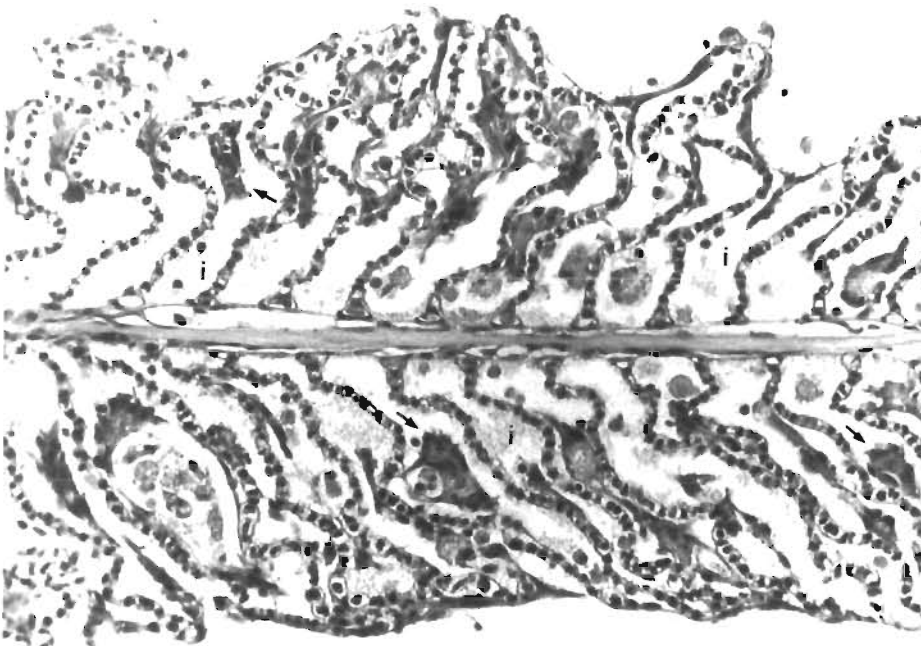


Fig. 8. *Sardinops sagax neopilchardus*. Fish collected (dead) during the mortality event at Iluka, New South Wales. Part of primary lamellus with generalised branchitis and mild epithelial hyperplasia forming islands (arrow) and cystic spaces. Infiltrate with proteinaceous fluid and inflammatory cells (i). $\times 450$

nuclei were elevated and enlarged and their cytoplasmic processes were abundant, intensely basophilic and extended to bridge adjacent secondary lamellae to form intraepithelial spaces (Fig 7, see also Fig. 14). In 2 fish the hyperplastic response resulted in the formation of epithelial islands in the interlamellar spaces. An inflammatory exudate was often present in the subepithelial space adjacent to focal proliferative lesions.

Iluka, New South Wales—during the mortality event.

There was moderate to severe, locally extensive to generalised inflammation, epithelial hypertrophy and epithelial hyperplasia in 10 of 10 fish (Fig. 8). Secondary lamellae were folded, distorted and apparently shortened. Islands of hyperplastic epithelium and cystic spaces containing inflammatory cells and cell debris were present in interlamellar spaces (Figs. 8 & 9). In 7 fish the interlamellar spaces were filled by hyperplastic epithelium (Fig. 9). The epithelial cells were hypertrophic, deeply basophilic and detached segmentally from the basement membrane. The subepithelial space was empty or contained eosinophilic fibrillar and granular material and/or a mixed inflammatory cell exudate with small numbers of eosinophilic granulocytes, chloride cells, lymphocytes, occasional neutrophils, macrophages and cell debris. The exudate appeared to be less well-developed than in the samples collected before the mortality event. Autolysed, free erythrocyte nuclei within the vessels of the secondary lamellae resembled viral inclusions in one fish (Fig 10); true viral inclusion bodies were not observed in any fish.

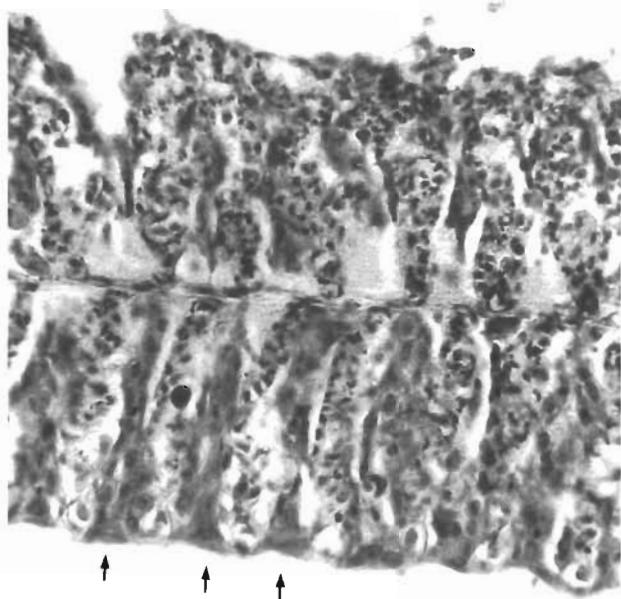


Fig. 9. *Sardinops sagax neopilchardus*. Fish collected (dead) during the mortality event at Iluka, New South Wales. Autolysed gill. Consolidation of interlamellar spaces with hyperplastic epithelium (arrows). $\times 450$

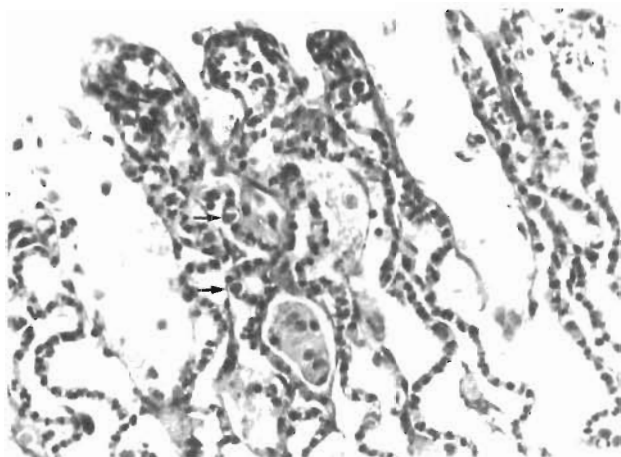


Fig. 10. *Sardinops sagax neopilchardus*. Fish collected (dead) during the mortality event at Iluka, New South Wales. Autolysed gill with inflammation, epithelial hypertrophy and erythrocyte nuclei resembling viral inclusion bodies (arrows). $\times 500$

Iluka, New South Wales—8 d after the mortality event. There were no lesions in the gills of 6 of 10 fish. There was locally extensive acute branchitis in one fish; the subepithelial exudate consisted of eosinophilic granulocytes, chloride cells, lymphocytes and occasional neutrophils and there was early epithelial hypertrophy. In a second fish there was a single focus of epithelial hypertrophy and hyperplasia with a subepithelial exudate of eosinophilic granulocytes, lym-

phocytes, chloride cells and occasional neutrophils and macrophages. This lesion spanned apposing secondary lamellae on adjacent primary lamellae. Adjacent secondary lamellae were united discontinuously along their length and at their tips by attenuated cytoplasmic projections of hypertrophic epithelial cells and 2 intraepithelial cystic spaces were present at the margin of the lesion. There were scattered, small, focal, lamellar lesions in 2 other fish. These consisted of fusion of 2 to 4 adjacent secondary lamellae with epithelial hypertrophy and hyperplasia but did not resemble the process described in fish before or during the mortality event.

Albany, Western Australia. There were no significant lesions in the gills of 10 of 10 fish collected 5 d before the mortality event. There were multiple foci of mild inflammation and epithelial hypertrophy in the gills of 2 of 4 apparently healthy fish which were netted from a school at the onset of the mortality event. The lesions resembled those in Fig. 5. Eight days after the mortality event there were focal to multifocal inflammatory lesions with epithelial hypertrophy in the gills of 3 of 10 apparently healthy fish.

Rottnest Island, Western Australia. There were no significant lesions in the gills of 10 of 10 fish collected 25 d before the mortality event. One day after the onset of the mortality event there was locally extensive to generalised inflammation and epithelial hyperplasia in the gills of 10 of 10 affected fish. The gill was consolidated by epithelium in 2 fish and there were widespread islands of hyperplastic epithelium in 4 fish. Chloride cells, lymphocytes and neutrophils were prominent in the inflammatory exudates while eosinophilic granulocytes were uncommon. Eighteen days after the mortality event there was multifocal to locally extensive inflammation in the gills of 3 of 10 apparently healthy fish. The cellular exudates consisted predominantly of chloride cells and lymphocytes.

Other locations. Findings in the gills of affected fish from other locations were similar to those described above and varied only in the degree of inflammation and hyperplasia. The epithelial hyperplasia in affected fish from New Zealand was less advanced than in most of the affected fish from Australia (Figs. 11 & 12); generalised lesions were present in 4 of 18 affected fish from the Bay of Islands. Degranulating eosinophilic granulocytes were more abundant in the inflammatory exudates in some affected pilchards from New Zealand (Fig. 11) than in most of those from Australia.

Bacteria, protozoa and metazoa in gill sections. A range of microorganisms was observed in the gills of normal and affected pilchards and their prevalence is given in Table 1. With the exception of a few affected fish that had large numbers of amoebae (Table 1), none

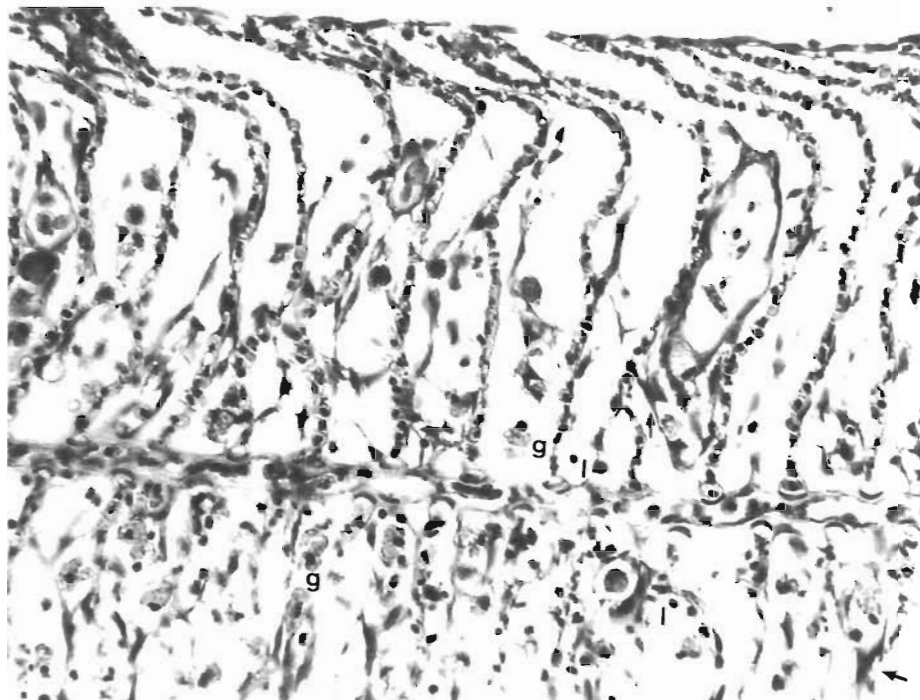


Fig. 11. *Sardinops sagax neopilchardus*. Fish collected (dead) during the mortality event at Bay of Islands, New Zealand. There is generalised branchitis, epithelial hypertrophy and mild epithelial hyperplasia (arrows). g: eosinophilic granulocytes; l: lymphocytes. $\times 500$

of these organisms were numerous. The amoebae appeared as round, crescentic to irregularly shaped, pale, amphophilic organisms with a few cytoplasmic granules (Kent et al. 1988, Roubal et al. 1989) and were present on the superficial epithelium, on the epithelium of the interlamellar spaces and in intraepithelial cystic spaces. Epitheliocystis (Inglis et al. 1993) were

present in the epithelium in very small numbers. Bacteria were not seen on gills of the majority of affected fish but a few small colonies of robust Gram-positive coccobacilli were found in the interlamellar spaces of fish regardless of disease status. Small colonies of Gram-negative, fine, short to filamentous rods were sometimes found associated with cell debris in intra-

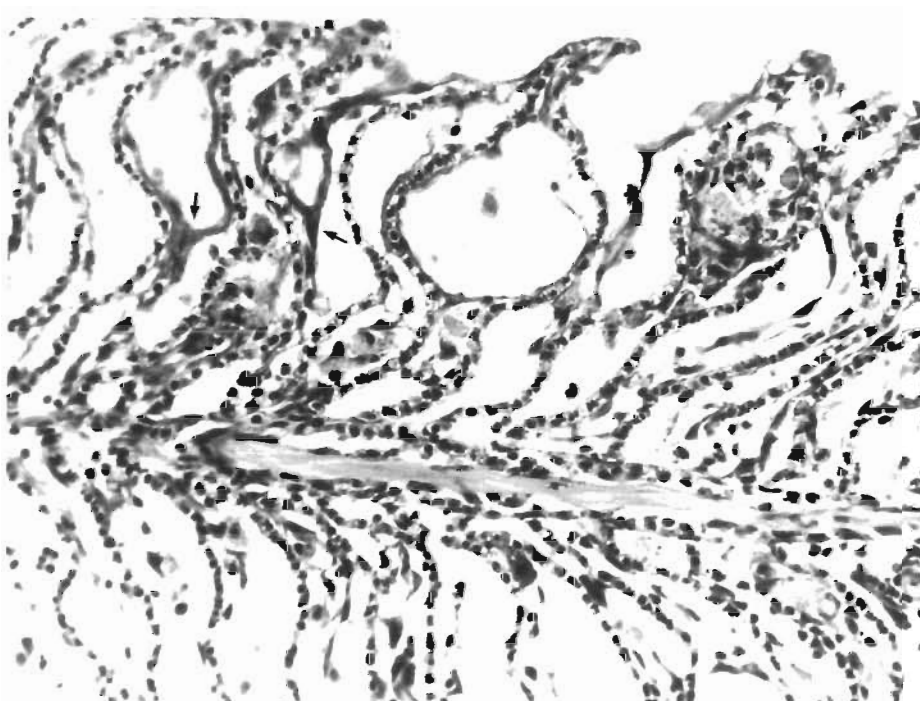


Fig. 12. *Sardinops sagax neopilchardus*. Fish collected (dead) during the mortality event at Bay of Islands, New Zealand. There is generalised branchitis, epithelial hypertrophy and mild hyperplasia (arrows). $\times 450$

epithelial cystic spaces and on the epithelial surface of affected fish. These bacteria were never numerous and were obvious only in the most severely affected gills, for example those from Port Hacking and Coffs Harbour. Single metazoa were found on the gills of 3 fish.

Electron microscopy

In scanning electron micrographs there were no adhering algae or algal spines (Yang & Albright 1992) or evidence of mechanical damage in the gills of 2 moribund and 2 dead fish from Bremer Bay, Western Australia. A small copepod was found embedded in the base of the secondary lamellae of one fish; this was confirmed histologically but whole specimens for taxonomic identification were not obtained. There are no records of copepods on Australasian pilchards.

In transmission electron micrographs granulocytes containing large granules with a condensed or crystalline plate or rod were observed in the subepithelial space and moving through the epithelium to the interlamellar space (Fig. 13). Cytoplasmic processes from

hypertrophic epithelial cells contacted those from adjacent secondary lamellae and were joined by desmosomes (Fig. 14). The cytoplasm of these cells was rich in ribosomes. Herpesvirus particles were observed in the epithelial cells of affected fish from the locations shown in Table 1 and from Wellington Harbour, New Zealand, during the outbreak; additional ultrastructural findings and viral prevalence data are given in Hyatt et al. (1997). Viral particles were not observed in kidney or spleen from 4 affected fish from Iluka, New South Wales nor from pancreas from affected fish from Port Hacking, New South Wales.

Bacterial culture

No significant bacterial growth was obtained from gills of 5 fish collected at Iluka 4 d before the mortality event nor from kidney and/or spleen of 6 fish collected 4 d prior to the mortality event in Western Australia. There were no significant bacterial isolates from kidney and/or spleen of 13 freshly dead fish collected during the mortality event at Albany and Rottnest Island, Western Australia.

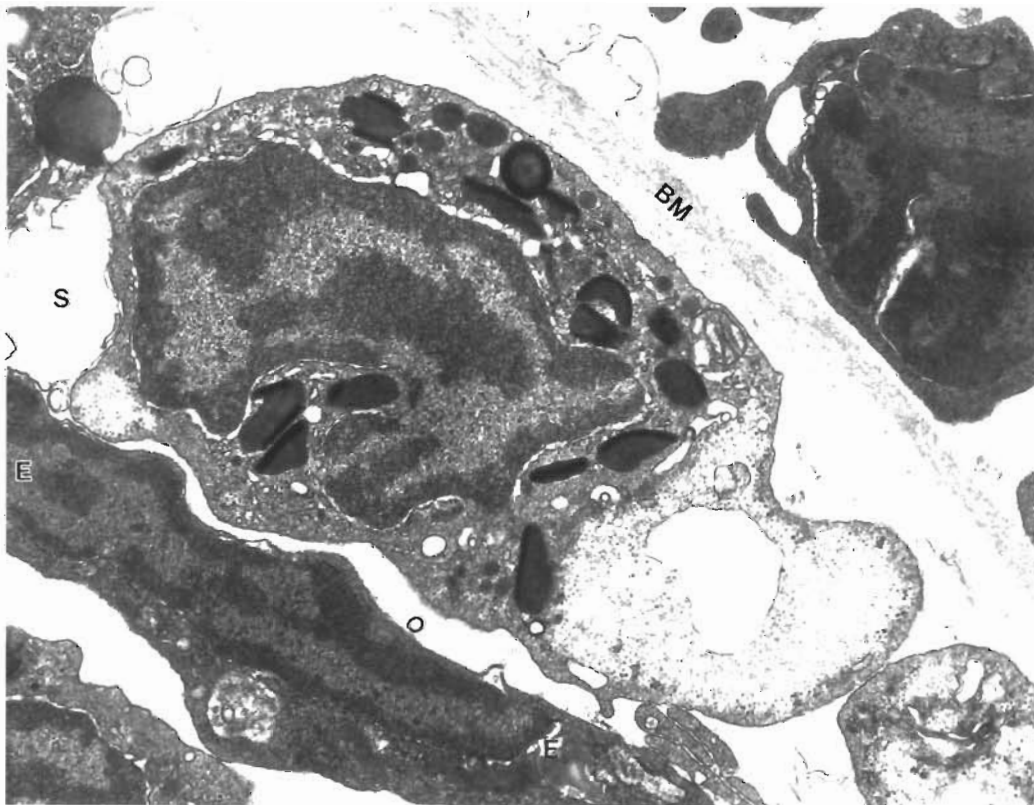


Fig. 13. *Sardinops sagax neopilchardus*. Transmission electron micrograph, fish collected at Bay of Islands, New Zealand. Eosinophilic granulocyte in the subepithelial space. It contains dense granules, each with a peripheral condensation. BM: basement membrane; S: subepithelial space; E: epithelial cell. $\times 19\,500$

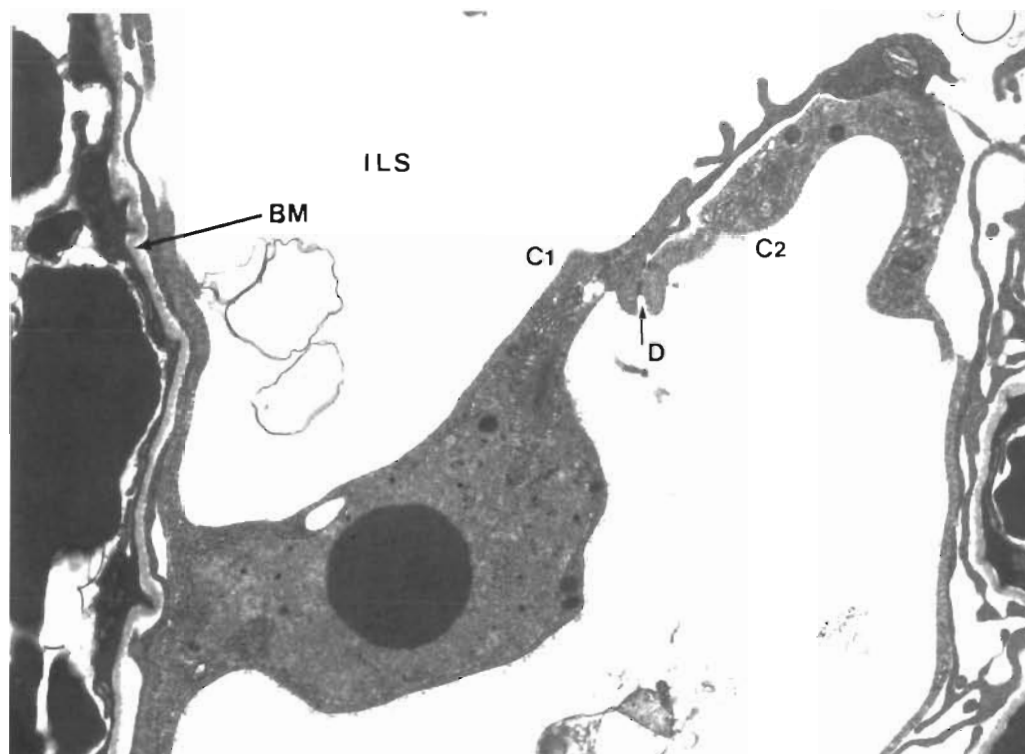


Fig. 14. *Sardinops sagax neopilchardus*. Transmission electron micrograph, fish collected at Bay of Islands, New Zealand. Hyperthrophic epithelial cell infected with herpesvirus (see Hyatt et al. 1997). A cytoplasmic process (C1) crosses the interlamellar space (ILS) to join by desmosomes (D) to the cytoplasmic process (C2) from another epithelial cell. BM: basement membrane. $\times 12\,000$

Blood gas analysis

Affected pilchards had a significant reduction in the partial pressure of oxygen and percent oxygen saturation in blood compared to normal pilchards (Table 2). In addition affected pilchards had significant reductions in blood pH and bicarbonate concentration and a significant increase in the partial pressure of carbon dioxide, suggesting both respiratory and metabolic acidosis.

Table 2. *Sardinops sagax neopilchardus*. Blood gas analysis of normal and affected pilchards from Wellington Harbour, New Zealand. Blood was collected from 8 normal pilchards and 12 affected pilchards and was analysed after pooling into 4 and 6 samples, respectively. Data are means \pm SD

Parameter	Normal	Affected	p
pH	7.3 \pm 0.18	6.7 \pm 0.09	0.0048
pCO ₂ mmHg	25.3 \pm 7.28	59.1 \pm 8.79	0.0003
pO ₂ mmHg	23.8 \pm 4.73	16.05 \pm 6.66	0.0700
HCO ₃ ⁻ mmol l ⁻¹	10.9 \pm 1.48	6.90 \pm 0.76	0.0077
O ₂ saturation %	31 \pm 12.2	6 \pm 4.5	0.0290

DISCUSSION

Clupeoids are among the most numerous and readily observable of the marine fishes but little is known of their diseases. Stranding induced by predators and weather conditions, physicochemical factors and *Ichthyophonus* infection have been responsible for mass mortalities of clupeoids in North America (Sindermann 1990). However, the cause of many mortalities was unknown, for example when 1000 t of Pacific herring *Clupea harengus pallasii* died in British Columbia in 1949. More recently in Alaska, 15 to 43% of spawning Pacific herring had external ulcers or haemorrhages associated with viral haemorrhagic septicemia virus (Meyers et al. 1994). This virus might also have been responsible for mass mortalities associated with subcutaneous haemorrhage in Pacific sardines *Sardinops caerulea* and Pacific herring in 1941-1942 in British Columbia (Sindermann 1990).

Diseases of pilchards are poorly documented, although several parasites have been recorded. We are aware of only one other pilchard kill in Australia. Copas (1982) reported that thousands of 15 to 25 cm pilchards were washed ashore in northern Tasmania and had signs 'consistent with crushing'. The localised

nature of this event and the dramatic gross pathology clearly distinguish it from the present epizootic. In New Zealand, isolated pilchard kills are relatively common. Jones & Rhodes (1994) reported an algal bloom that killed about 500 kg of pilchards in a small lagoon in Wellington Harbour. There were other fish kills in New Zealand in 1994 and 1995 that were thought to involve pilchards but these occurred inshore or in embayments, did not spread and the species involved were not confirmed. There are earlier, mostly anecdotal accounts of pilchard deaths in New Zealand (Graham 1954). These occurred in 1900–1902, 1921 and 1934 when quantities of fish were washed ashore and processed for human consumption. On some occasions the pilchards had been 'thrown up by the sea' or chased ashore by predatory fish.

Thus to our knowledge the event described in this report is the only extensive and progressive epizootic to have affected the Australasian pilchard. A longitudinal study was undertaken to define the condition and its aetiopathogenesis.

The disease observed in fish from New South Wales and Western Australia had a logical progression from mild acute focal to severe subacute generalised branchitis. A few residual cases were present after the main disease front had passed but there was no evidence of resolution of the severe lesions observed during the mortality event, suggesting a high case fatality rate. The primary lesion was inflammatory; there was leakage of protein from pillar capillaries and exudation of inflammatory cells into the subepithelial space. Chloride cells detached from their basal location, moved into subepithelial spaces along the secondary lamellae and may have proliferated. Eosinophilic granulocytes were common in the exudates and their degranulation might have played a role in the pathogenesis of the lesions by inducing vascular leakage. Neutrophils, lymphocytes and a few active macrophages were also present. Further studies to classify the granulocytes are in progress. Inflammation subsided as epithelial hypertrophy and hyperplasia supervened; secondary lamellae became distorted. The epithelial cells enlarged and extended cytoplasmic processes to join to those from adjacent secondary lamellae (Fig. 14). The nuclear to cytoplasmic ratio increased as the hyperplastic response matured. Islands of epithelium appeared in the interlamellar spaces, eventually obliterating them. The longitudinal data from Iluka, New South Wales, suggested that mild focal lesions became generalised and severe over about 4 d.

The clinical signs of the disease correlated well with the pathology. The gill lesions were of sufficient severity to compromise gas exchange, as evidenced by the relative hypoxaemia and hypercapnea in affected fish. We also demonstrated respiratory and metabolic aci-

dosis, but other disturbances of ionic regulation and failure of excretion of wastes may also have contributed to the pathogenesis of the disease. A range of lesion severities was seen in affected fish as clinical signs and death were precipitated by external stressors which would be expected to vary from place to place. Subclinically affected fish were thus in compensated respiratory and/or osmoregulatory failure prior to the stress.

As the pathology was relatively consistent across the geographic range of the mortality event, a common aetiology is suggested. The gills of teleosts are susceptible to a wide range of insults and the lesions that result are often not specific or even indicative of their aetiology. Common lesions such as lamellar oedema, epithelial degeneration and necrosis, epithelial hypertrophy and hyperplasia and lamellar fusion may be due to environmental, infectious, nutritional and other causes (Eller 1975, Mallatt 1985). Furthermore there is little information on the responses of different species of fish to similar insults. However, the morphogenesis of the lesions in pilchard gills from focal to generalised and the development of lamellar fusion through contact of attenuated cell extensions followed by hyperplasia were remarkable and do not appear to have been described before (Eller 1975, Gardner 1975, Daye & Garside 1976, Daoust et al. 1984, Chevalier et al. 1985, Mallatt 1985, Karlsson-Norrgren et al. 1986a, b, Ferguson 1989, Roberts 1989). The lack of mucous cell activity, epithelial degeneration and necrosis and microbial pathogens visible at the light microscope level were also notable.

As the epizootic was thought by many observers to be environmentally mediated, some discussion of this is warranted. Environmentally mediated chemical and toxicological insults cause generalised rather than focal changes in gills and these become more severe with time; lamellar fusion occurs by contact of cell processes from plump epithelial cells which may already be hyperplastic (Daoust et al. 1984), changes clearly dissimilar to those in affected pilchards. Chloride cell hyperplasia may occur in response to aluminium toxicity and exposure to acid water (Karlsson-Norrgren et al. 1986a, b) but is not accompanied by the other inflammatory changes seen in pilchards. The pathological findings in pilchards also were not consistent with exposure to an algal bloom: there was little or no lamellar oedema or epithelial cell degeneration (Jones et al. 1982, Roberts et al. 1983, Endo et al. 1985, Toyoshima et al. 1985); the inflammation and hyperplasia was more severe than that which occurs in response to ichthyotoxic algae (Chang et al. 1990); algae that cause asphyxiation were not present in the interlamellar spaces or attached to secondary lamellae (Jones & Rhodes 1994); and there was no excess

mucus, oedema, haemorrhage, epithelial cell necrosis, siliceous fragments or foreign body reactions and proliferating epithelium did not retain polygonal morphology as is seen in mortality events due to physical irritation by siliceous diatoms (Ferguson 1989, Speare et al. 1989, Yang & Albright 1992, Kent et al. 1995). The absence of relevant pathological findings was consistent with the lack of evidence of an environmental insult. No single oceanographic disturbance was reported across Australia and New Zealand (Griffin et al. 1997), the spread of the disease was not impeded or assisted by prevailing currents or storms and it is impossible to conceive of a man-made toxin affecting such a wide region of the sea.

The epizootiological pattern was consistent with an infectious aetiology. Furthermore a herpesvirus was consistently present in affected fish, was found associated with early lesions several days before the onset of mortality and was absent from healthy fish ahead of the mortality front. Other candidate pathogens were not present in early lesions, were found inconsistently or were present in low numbers (Table 1). Therefore the herpesvirus is proposed as the aetiological agent. The nature and morphogenesis of this virus are described in the companion paper (Hyatt et al. 1997). Many herpesviruses have been identified in fishes and the majority have been associated with epithelial proliferations (Wolf 1988, Hedrick & Sano 1989). Some appear to have caused epizootic mortality (Iida et al. 1989, McAllister & Herman 1989) and at least one has been associated with hyperplasia of the branchial epithelium (Watson et al. 1995).

The nature of the gill pathology also enabled microbial agents other than the herpesvirus to be excluded. Filamentous bacteria were observed in some severely affected gills but they were present in insufficient numbers to warrant consideration of bacterial gill disease. This condition was also ruled out by the absence of mucous cell hyperplasia, epithelial oedema, degeneration and necrosis (Speare & Ferguson 1989, Speare et al. 1991). Although large numbers of amoebae were present on the gills of some affected fish, the lesions in these fish were not consistent with amoebic gill disease in which there is typically mucous cell hyperplasia (Kent et al. 1988, Roubal et al. 1989, Munday et al. 1990) with a multifocal or segmental and nodular epithelial reaction (Ferguson 1989, Roberts 1989).

The epizootiological data suggest that the disease spread from an origin near Anxious Bay in South Australia at a rate of about 30 km d⁻¹. Spread was often in directions contrary to prevailing currents. While northward migrations of pilchards along the New South Wales coast are known to occur in winter and spring (Fletcher 1990), tagging studies of Chilean pilchard *Sardinops sagax* have shown a speed of

about 5.4 km d⁻¹ (Torres et al. 1985). Thus it is not possible for currents or pilchard movements to account for the direction or rate of spread of the mortality front, which suggests that the spread in Australia involved a vector such as a piscivorous mammal or bird. Although there may have been separate foci of disease in New Zealand, the spread in the northeast also cannot be explained by movements of pilchards or currents alone. In both Australia and New Zealand there is overlap in the distribution of pilchards with that of some seabirds such as shearwaters but particularly the Australasian gannet *Morus serrator* (Blakers et al. 1984, Marchant & Higgins 1990). Many observers noted birds feeding from or near affected schools of pilchards during the epizootic in both Australia and New Zealand. Several viruses of fishes are known or suspected to be transmitted by piscivorous birds through regurgitation of infected fish or passage of viable virus in faeces (Olesen & Vestergard-Jørgensen 1982, Peters & Neukirch 1986, Whittington et al. 1996). The pilchard comprises a significant proportion of the diet of gannets in both Australia and New Zealand (Wingham 1985, Norman & Menkhurst 1995). Gannets may have been responsible for some of the spread of the disease in New Zealand as they were seen eating pilchards along the mortality front and arrived in Wellington Harbour a few days before the pilchard mortalities began there. Gannets were also seen in Western Australian waters associated with the mortality event but were not noticed during sampling off the coast of New South Wales at Port Stephens or Iluka.

The lack of history of large-scale mortalities in pilchards in Australian waters, the focal origin of the outbreak, and the dramatic spread of the disease around continental Australia are consistent with the theory that the epizootic was due to an infectious disease agent that was not present before in Australian pilchard stocks. The recognition that discharge of ballast water from commercial shipping and the importation of baitfish are possible mechanisms for the introduction of putative exotic microbial pathogens into Australian coastal waters predates the pilchard epizootic (Munday et al. 1994, Humphrey 1995). Coincidentally over 10 000 tonnes per annum of *Sardinops sagax* and a mix of bycatch species including other clupeoids have been imported without quarantine inspection from California, Peru, Chile or Japan and fed out to sea-caged southern bluefin tuna in South Australia. Meyers et al. (1994) have already suggested that frozen herring bait is a potential source for the dissemination of viral haemorrhagic septicaemia virus, which survives 2 conventional freeze-thaw cycles, and suggested that the European strain of virulent VHSV arose through the habit of feeding frozen bait to hatch-

ery fish. Bacterial fish pathogens are also known to survive in frozen clupeoids (Yasunaga 1982). As noted above migratory birds may also be a means of introduction of exotic microbial fish pathogens into Australian waters.

The possibility that the epizootic was due to activation of a latent herpesvirus infection in Australian pilchards is remote given the lack of evidence of a primary disease event or an environmental trigger. Acquisition of infection from another marine host also seems unlikely given that herpesviruses are mostly host specific. There is also the possibility of a new/emerging virus disease. These issues are discussed further in Hyatt et al. (1997). Work is underway in Australia to purify the herpesvirus from affected gills and prepare specific reagents for its detection, while in New Zealand an attempt will be made to transmit the virus experimentally to healthy pilchards.

The economic impact of this epizootic in pilchards is not yet known. There is a small but significant pilchard fishery in Western Australia and single pilchard fishers in New South Wales and New Zealand. The ecological impacts are also uncertain. The data suggest that some schools may have escaped the effects of the disease but it is reasonable to assume that the loss of pilchard biomass will cause, at least temporarily, measurable effects in populations of birds, mammals, fish, squid and other aquatic organisms that include the pilchard as an important dietary component.

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