Environmental and biological aspects of the mass mortality of pilchards (Autumn 1995) in Western Australia

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Fisheries research in Western Australia
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Executive Summary

The first pilchard deaths were reported in South Australia during March 1995 in the eastern region of the Great Australian Bight. Subsequently, dead pilchards were found both east and west of this point source moving in a 'bushfire-like' front at an average of 30 km/day. By early May the fronts had reached Albany in Western Australia (WA) and Bass Strait in Victoria. They continued moving up both coasts, reaching Carnarvon (WA) and Noosa Heads (Queensland) by the end of June, thereby affecting pilchards throughout their entire Australian distribution (6700 km). In early June, a similar pattern of deaths began to occur in the north of New Zealand.

The pattern of deaths at all locations was similar with only adult pilchards (> 10 cm, 13 cm in WA) affected. No other species or even juvenile pilchards were found dead; moreover, neither predators nor scavengers died as a result of their consumption. Fatalities lasted for only a few days at any one location, but the intensity did not appear to diminish with time or distance from the origin. Subsequently, no further deaths were observed.

Originally it was thought an upwelling event that occurred in South Australia during early March could have been the cause by lowering water temperatures and/or giving rise to a toxic phytoplankton bloom. The list of possibilities was later expanded to include clogging of gills by non-toxic phytoplankton, the impact of stress from altered environments, and finally the possibility that the deaths were caused by some exotic pathogen. To examine these theories, numerous biological and environmental samples were collected, particularly of dead and dying fish, along with healthy fish taken before the front and unaffected fish behind the front. Samples of phytoplankton and environmental measurements were also obtained. Similar investigations were conducted at many institutions around the country, with a Pilchard Mortality Task Force created under the auspices of the Consultative Committee for Exotic Animal Diseases (CCEAD) which organised a number of teleconferences to help disseminate information and coordinate activities.

Affected pilchards, aside from being dead, were in good physical condition with many having advanced gonad stages. Thus, in Esperance, significant levels of egg production were found in an area where many fish were dying. The dead fish usually had open mouths and gills that were pale in colour. Histopathology showed that the gills had epithelial hyperplasia (multiplication of the surface cells), synechiae of the secondary gill lamellae tips (they had stuck together), sloughing of epithelial cells and oedema. Thus, the cause of death was asphyxiation.

Identical patterns of damage were seen at all locations where the deaths occurred, indicating a common cause. Consequently, unless a single theory could explain the deaths in all areas, it was unlikely to be correct. Possible causes of the gill damage include contact with toxic and non-toxic phytoplankton. It was initially suggested (incorrectly) that adult pilchards were the only phytoplankton feeder in the region and may therefore have been the only affected group. Alternatively, this damage could have arisen from the pathogenic effect of some specific virus or bacterium to which only adult pilchards were susceptible.

The data collected in WA clearly showed that the pilchard mortalities had no relationship with phytoplankton. Toxic phytoplankton were not involved in the deaths; few were seen in the phytoplankton samples and no toxic substances were found in any affected fish. There were no blooms of non-toxic phytoplankton anywhere along the WA coast during the times when the mortalities were occurring. The composition of the phytoplankton that was present varied greatly between sites, independently of whether dead and dying pilchards were in the region. Most sites where deaths were recorded had very low densities of phytoplankton. Furthermore, examination of the affected gills by SEM showed no evidence of clogging or mechanical damage consistent with phytoplankton being responsible.

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The stomach contents of the affected pilchards varied greatly. In addition, many had empty stomachs. The slight difference in feeding capabilities between adult and juvenile pilchards is insufficient to support the total lack of impact on juveniles. Similarly, the lack of an effect seen on the other coexistent, filter-feeding clupeoids (e.g. anchovies, *Sardinella*) is a further rejection of the hypothesis that this phenomenon was somehow caused by phytoplankton blooms. Finally, the passage of the front of dying fish moved in the opposite direction to the prevailing currents and continued even after several severe storms. None of these observations are consistent with phytoplankton being involved.

The gill damage seen in the dead pilchards collected in WA was always associated with the presence of a Herpesvirus. This virus was not present in fish sampled ahead of the deaths, and was not found in samples of survivors after mortalities stopped. Work at the CSIRO animal health laboratories at Geelong, the New South Wales Agriculture Department and the New Zealand National Institute of Water and Atmosphere (NIWA) also linked the presence of the virus with the deaths. An amoeba was associated with gills of many dead fish, but this was usually in insufficient numbers to account for the damage seen, and was not always present in fish with gill damage. Thus the virus was the only consistent factor in all of the kills in WA, Australia and New Zealand. Furthermore, the rate of passage of the fronts was within the limits of daily movement rates of adult pilchards.

The interim task force report (Anon., 1995) reported that the virus could have been endemic and triggered by stress caused from changes in temperature or the onset of spawning. Neither of these 'causes', however, have any support. Firstly, water temperatures in WA were not abnormal: they were mostly in the 18-21°C range, with no cold-water intrusions on to the shelf or subsurface currents of cold water as mentioned in some reports to support any 'stress' theory. Secondly, as the spawning season for pilchards varies greatly between regions, affected fish were at all stages of the spawning cycle.

The more likely alternative hypothesis is that the virus was recently introduced into Australia. Whilst involvement of the Herpesvirus in the mortalities has not been conclusively demonstrated by infection trials, the association of the virus with the gill damage, the severity of the impact on the population and the bushfire-like passage of the front are all consistent with a novel pathogen infecting a naive population. Furthermore, once infection had passed an area, reinfection did not occur, suggesting that the surviving fish were resistant to infection. No direct evidence was obtained to determine the possible method for any introduction.

The impact of the deaths on the stocks of pilchards was also determined. Counts of dead pilchards were made both on the sea surface and the bottom at a number of locations in WA. In addition, biomass estimates were calculated using the daily egg production techniques which showed that the impact to the pilchard stocks in WA was the death of approximately 10-15%, with this still representing many thousands of tonnes. Nonetheless, all WA pilchard fisheries recommenced fishing a few weeks after the deaths had ceased and have since not registered any major unexpected changes in catch rates. No impacts have been seen in other species that utilise pilchards and consequently no longer term effects are envisaged.

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The major conclusions drawn from the investigations conducted in WA are:

1. Pilchards were affected over their entire range.
2. Approximately 10-15% of the stocks were killed. ·
3. There was no involvement of phytoplankton either directly or indirectly in the deaths of the pilchards.
4. There were no large or small-scale environmental anomalies which could have affected the pilchards over any part of their range in WA.
5. The only consistent factor in the deaths at all locations was the presence of a previously undescribed Herpesvirus in the gills of the affected pilchards.
6. The pattern and severity of the impact suggest that the Herpesvirus was not a latent infection.

Thus:

CONCLUSION

The most likely cause of the massive mortalities of pilchards in Australia during early 1995 was from a novel Herpesvirus to which the Australian pilchard population was naive and whose origin was, therefore, most likely to be exotic.

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Section 1   General Introduction

1.1   Background

In March 1995, the first scenes of dead pilchards lining the shores of South Australia were broadcast on national news reports across Australia (Fig. 1.1). Originally dismissed as a local upwelling phenomenon, the deaths were subsequently repeated over the following six months across the entire lower half of Australia and the north and centre of New Zealand. These deaths produced international media and public interest and resulted in intense scientific effort and speculation. They generated a large amount of hysteria, political debate and lobbying, anger, embarrassment and occasionally even humour. They also posed a threat to Australia's multi-million-dollar pilchard fisheries and, because of the key position of pilchards within the food chain, other parts of the marine ecosystem were also potentially at risk. These issues were often lost in the wake of other agendas. In this report we describe the observations and research undertaken within Western Australia (WA). We examine how the results of these investigations related to the hypotheses proposed to account for the deaths and we provide estimates of the impact on the pilchard stocks in WA.

The vast extent of the deaths resulted in parallel research being conducted in most of the other states in Australia (and ultimately in New Zealand). Within Australia, this effort prompted the establishment of a national task force on the pilchard mortalities coordinated by the federal Department of Primary Industry and Energy (DPIE) under the auspices of the Consultative Committee for Exotic Animal Disease (CCEAD). Using the CCEAD structure, a number of teleconferences were organised (see Table 11.1) following which an interim report (Anon., 1995) was produced. It should be noted that many of the conclusions expressed within the Pilchard Mortality Task Force interim report (Anon., 1995) were not universally accepted, especially within WA and New South Wales, even at the time of their publication. Furthermore, because of the interim nature, some of the data presented were later found to be incorrect. It was, however, signalled at the time of publication of the interim report (June 1995) that the research from WA would be written independently. We will not be reporting the findings or observations made in other locations except at a general level where it has a direct bearing on our interpretations and general conclusions.

A number of problems beset the interpretation of data and hypotheses generated during such events. The large scale of the mortalities often meant that local incidents and data were treated in isolation to those collected elsewhere. This was exacerbated by there generally being insufficient regional and historical data against which to compare results. A further problem which can occur in such circumstances is that 'each specialist predict(s) their interest as the possible villain' (Williams and Bunkley-Williams, 1988) and the production of 'instant' experts.

Finally, the concept that needs to be remembered, when assessing the information associated with what was a single problem that covered a very large area over a long duration, was that unless all the data can be explained, then none of it was. The neglect of this principle probably resulted in the continued citing of some hypotheses long after they were scientifically untenable (e.g. Anon., 1995; Douglas, 1995).

1.2   Mass Mortalities

Reports of mass mortalities of fish and other sea life are not uncommon, but scientific investigations of mass mortalities of marine fish under natural conditions are relatively rare (Rohde, 1982). Diseases in marine species result from a complex and constantly changing series of factors involving individuals, the population and the environment (Sindermann, 1965). Thus, determining the cause of mass mortalities usually requires input from a range of scientific disciplines which are often unavailable, partially because of the local nature of many kills and the difficulty in mounting a detailed scientific study of the problem at short notice. Despite these problems there have been several well studied mass mortalities of clupeoid fishes.

Sindermann (1990) reviewed mass mortalities of clupeoids in North America and concluded that stranding induced by predators, weather conditions, physicochemical factors and *Ichthyophonus* (fungal)

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infections have all been responsible for one or more kills. However, the cause was unknown in many instances, such as the mortality of 1000 tonnes of Pacific herring (*Clupea harengus pallasi*) in British Columbia in 1949 (Sindermann, 1990).

More recently (1993-94) in Alaska, 15-43% of spawning Pacific herring had haemorrhages and external ulcers associated with viral haemorrhagic septicemia. This virus is now known to affect herring and other marine species throughout the Pacific Northwest (Meyers *et al.*, 1986; Meyers *et al.*, 1994). It may also have been responsible for mass mortalities associated with subcutaneous haemorrhage in Pacific herring and Pacific sardines (*Sardinops sagax*) in 1941-42 off British Columbia (Sindermann, 1990).

A mass mortality of the sardine, *Sardinella aurita* was observed in a shallow marine embayment in Greece in 1991 (Economidis and Vogiatzis, 1992). They concluded that a dramatic lowering of the air and presumably water temperature caused thermal shock. The deaths occurred over an area less than 100 km^2 with fewer than 100 tonnes of *S. aurita* observed to have washed up on beaches. Significantly, a number of other species of fish were also affected.

There are a few anecdotal reports of small-scale pilchard mortalities in Australia; one published account (Copas, 1982) records deaths which appear to have been caused by the impacts of fishing activities. In New Zealand there was a small mortality event in December 1993 due to an algal bloom in a small enclosed lagoon (Jones and Rhodes, 1994) and there are anecdotal reports of pilchard mortalities going back at least to 1900-1902 (Graham, 1974), but the cause of these earlier deaths is not known.

The situation described in this report differs from previous reports of clupeoid fish kills in a number of ways. First, the deaths only affected adult (i.e. sexually mature) pilchards over about 10 cm (13 cm in WA) in length, smaller pilchards and all other species being unaffected. Second, the deaths were not confined to a single estuary or stretch of coastline, but appeared to have moved in a systematic front or wave of deaths along the all the lower half of the Australian coast, usually against prevailing currents. Storm events which frequently interrupted sampling did not stop the wave of mortalities.

1.3   Possible Causes

The main possibilities that were formulated to explain these deaths included:

1.   Some direct effect of a change in the environment, particularly cold water from upwellings possibly causing anoxia or some type of stress.
2.   A phytoplankton bloom or some toxin carried within the water column (possibly related to 1).
3.   An infectious agent.
4.   Some combination of the three.

As a result of the intense media interest, a number of alternative, but interesting, possibilities were also thought of. These included the impact of

1.   meteor showers;
2.   dumping of toxic waste down used exploration well holes;
3.   leaching of fertiliser from the wreck of the *M.V. Sanko Harvest* (located off Esperance, WA);
4.   undersea volcanic activity;
5.   excrement from ships carrying live sheep;
6.   submarine activity; and
7.   a 'lemming effect' from an overly large population;

along with a number of more humorous 'causes'.

1.4   General Research Methods

In WA, we were in a unique position to conduct research on this problem. First, WA has the largest and most widespread pilchard fisheries in Australia, which enabled samples of pilchards to be obtained from a number of locations with relative ease (see Fig. 2.7). This large distribution also allowed the coordination of samples by the same research group over nearly the entire period when the mortalities were occurring in Australia (2.5 months). Some other regions only had a few days or weeks. Detailed expertise on pilchard biology and stock assessment, fish pathology, phytoplankton and oceanography were all available in Perth. Finally, a large program of pilchard research covering both the adult and

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planktonic phases had already been in progress for the previous six years which allowed meaningful comparisons to be made.

To distinguish between the possible scenarios, extensive and intensive sampling programs were undertaken to examine both live and dead pilchards, the toxicity of collected pilchards, and the distribution and abundance of planktonic organisms (especially phytoplankton) in the regions before, during and after the deaths had passed. Information on the oceanographic and environmental conditions throughout the area over the period of the kills was also collected.

To facilitate analyses, testable hypotheses were constructed during the kill event (see Tables 8.1 - 8.3). This task was aided by there being well documented and specific circumstances under which each hypothesis could occur. It was our task to ensure that we had sufficient information to either support or refute each of these.

Because of the importance of the WA pilchard fishery, an additional requirement of the research program was that estimates were required of the levels of impact these mortalities had on the stocks of pilchards. With these estimates, an assessment could be made with regard to the appropriate levels of fishing that could subsequently be sustained and some warning gained of whether other species could also be affected through a loss of prey.

1.5   Institutions and Personnel Involved

The personnel involved in the WA investigations came from the Fisheries Department Research Services Division; the phytoplankton unit of the Water and Rivers Commission; Agriculture WA; and the CSIRO Division of Oceanography (Marmion), with additional data obtained on the R.V. *Franklin*. Funding for the state research team came from a specific funding from the trust account of the Fisheries Department of WA and a Fisheries Research and Development Council (FRDC) grant. CSIRO funding paid for the *Franklin* cruise. We were also greatly assisted by fishermen in the provision of information, the supply of samples and in the use of their vessels. Operations staff of the Fisheries Department of WA helped substantially by conducting counts of dead fish on beaches, through liaison work and the use of their patrol vessels.
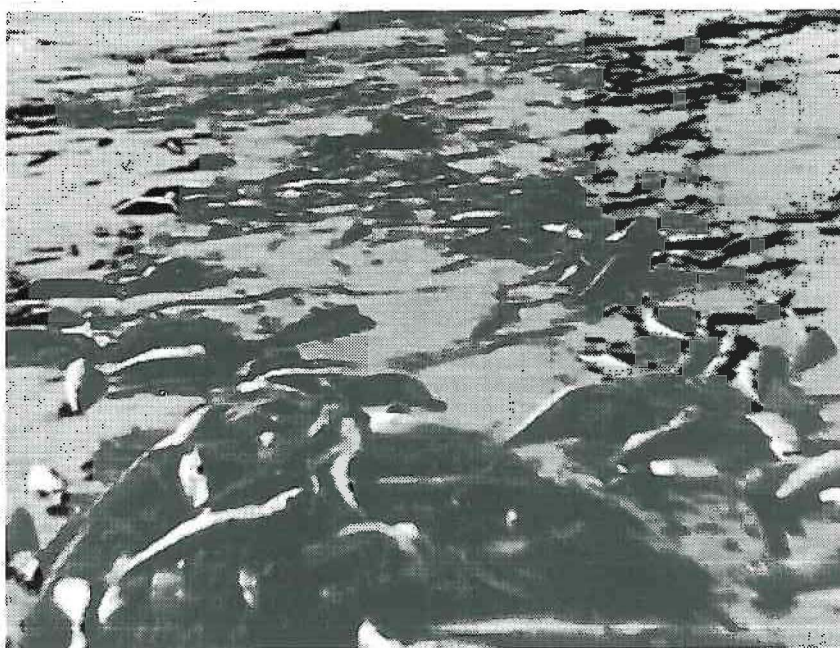
Figure 1.1   Video image from news report of dead pilchards washed ashore.

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Section 2   Biology of *Sardinops sagax* in Australia

2.1   Introduction

Some knowledge of the biology and ecology of pilchards is essential to fully comprehend what data were collected and the implications of this information. Documented variations in the biological characteristics of pilchards between areas and age groups along with measured differences and similarities between pilchards and other related species all influence which of the hypotheses, if any, remain tenable. The need to clarify these facts has been exacerbated given that a number of misconceptions, as well as out-of-date material, were used in some other discussions and media reports.

The biology and stock assessment of the pilchard (*Sardinops sagax [neopilchardus]*) in Australia and New Zealand has only recently been reviewed (Fletcher, 1990; 1991a). The following is, therefore, a summary of these reviews in addition to material, both published and unpublished, collected subsequently. Where relevant, information from populations of *Sardinops sagax* located elsewhere in the world and that of related species (e.g. anchovies) has also been included.

2.2   Taxonomy

The Australasian pilchard or sardine (*Sardinops sagax [neopilchardus]*) is a small pelagic fish of the family Clupeidae, which contains all the 'herring-like' species. This is the same species (*S. sagax*) that is found in California, South Africa, Japan and South America. Until recently, these were all considered separate species but an electrophoretic study (Parrish *et al.*, 1989) found insufficient genetic separation amongst the stocks to even justify elevation to subspecific status. A similar lack of genetic differentiation was also found between WA and South African pilchards by Dixon *et al.* (1993). Largely for convenience, the additional subspecific name (*neopilchardus*) has been retained in some publications from this establishment to differentiate the Australasian stocks. The current correct taxonomic name, and that used for the remainder of this report, is *Sardinops sagax* (Jenyns, 1842).

2.3   Distribution

The distribution of *S. sagax* in Australia covers the entire lower half of the continent, from Red Bluff near Carnarvon in WA, down and across the southern WA coastline, the Great Australian Bight, South Australia, Victoria and northern Tasmania and up the New South Wales coastline to Hervey Bay in southern Queensland (Fletcher, 1990; Figure 2.1). This is a total of 6700 km, which makes it the largest, in terms of linear distance, of all the *Sardinops* populations. The stock in New Zealand extends around the North Island and the northern part of the South Island (Baker, 1972).

Within Australian waters, pilchards are almost totally restricted to waters of the continental shelf. Results of trawl surveys for adults (e.g. Collins and Baron, 1981) and plankton surveys for eggs (e.g. Fletcher and Tregonning, 1992; Fletcher *et al.*, 1994) have found little evidence of pilchards beyond the 200 m isobath. Other populations of *Sardinops*, for example off Japan and Chile, can extend in excess of 1000 km from the shelf (e.g. Watanabe *et al.*, 1996).

Whilst larvae may sometimes be found in estuarine environments (Gaughan *et al.*, 1990), extensive sampling has found neither juveniles nor adults within these locations in WA (e.g. Potter *et al.*, 1983). Juveniles (< 80mm) have, however, been caught in oceanic regions along the south coast of WA, particularly near Esperance (Fletcher, unpublished data). On the east coast of Australia, Blackburn (1949; 1950) concluded that juvenile pilchards inhabited sheltered bays and inlets, albeit with little supporting evidence (Fletcher, 1990).

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2.4 Growth and Sexual Maturity

The sizes of individuals within Australasian populations of *Sardinops* are much smaller than are found at other locations around the world. Thus, the average and maximum sizes in the WA catch are 160 mm and 205 mm fork length (40-100 g) respectively (Fletcher and Blight, 1996). By contrast, the Californian catch has a mean length of 220 mm (150 g) (Barnes *et al.*, 1992) with a maximum length exceeding 300 mm (Clark, 1932).

Despite the difference in sizes attained, the pattern of growth appears to be relatively similar for all *Sardinops* populations (Fletcher, 1990). For the WA population, sexual maturity is attained during their second year at approximately 120-130 mm fork length and they live to a maximum of 9 years (Fletcher, 1995a; Fletcher and Blight, 1996). Females grow to larger lengths than males (L_∞ = 174 compared to 164) with the change in growth trajectories occurring after age 2 (Figure 2.2; Fletcher, 1995a; Fletcher and Blight, 1996).

Assigning ages to pilchards using 'annuli' located on either scales or otoliths is not a simple process due to secondary rings often being present (Fletcher and Blight, 1996). Routine ageing of samples for each of the pilchard fisheries in WA has been conducted using the otolith weight - age relationship (Table 2.1) which was determined and subsequently verified by Fletcher (1991b; 1995a).

2.5 Spawning Seasons

Pilchards are dioecious (separate sexes), synchronous multiple-batch spawners (spawn more than once per season) (Fletcher *et al.*, 1996a). The spawning seasons vary greatly between localities such that each month of the year is the main time of pilchard spawning somewhere in Australia (Fletcher, 1990). Thus, in the eastern region of WA and into the Great Australian Bight, the main time for spawning by pilchards is in the period April to July (Blackburn, 1950; Fletcher *et al*, 1996b). At Bremer Bay, there is only one main spawning period per year, in June-July (Fletcher *et al.*, 1994); whilst at Albany there are two periods of spawning, one in July and another in December-January (Fletcher and Tregonning, 1992; Fletcher *et al.*, 1994). There are also two periods of spawning on the west coast of WA, one in August and one in February-March (Fletcher *et al*, 1996b).

Elsewhere in Australia, pilchards spawn in February-March (South Australia) and in November (Victoria), whilst in New South Wales the season begins in the south during summer and moves later in the year with increasing distance up the coast (Fletcher, 1990a). Consequently, during the March-June period when the mortalities were occurring, pilchard populations in Australia and even in WA were at all phases of the spawning cycle: some were spawning (Esperance), some had recently completed spawning (west coast) and some had not yet begun spawning (Albany).

2.6 Feeding

This is not a well documented area of research for pilchard stocks in Australia with only limited data collected from a small number of research cruises (see Fletcher, 1990 for review). Little additional research has been done in WA due to the use of pollard (wheat husks) by fishermen on the south coast as a means of concentrating schools, which makes gut content analysis difficult.

The studies that have been done show that adult pilchards eat both zooplankton (particularly copepods) and phytoplankton (mostly diatoms) with the percentage of these varying according to their relative availability in the plankton (Fletcher, 1990). A worldwide review of clupeid diets by James (1988) concluded that both pilchards and the closely related anchovies were 'opportunistic foragers which select the largest particles available, especially herbivorous copepods; directed filter feeding on phytoplankton is of secondary importance'.

King and Macleod (1976) reported a size-specific change in the diet of both pilchards and anchovies in South Africa. They suggested that both pilchards and anchovies changed from being selective zooplankton feeders to filter feeders of phytoplankton, at sizes of 100 mm and 80 mm respectively. This, they believed, was due to the increased development of the gill raker structure. James (1988), however, has subsequently disputed this conclusion saying that the filtering mechanisms of both

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Sardinops and *Engraulis* 'were sufficiently well developed to trap diatoms at fish lengths well below those stated'. Scofield (1934) also concluded that Californian sardines of only 70 mm length would be capable of straining diatoms from the water. Even the data presented within King and Macleod (1976) showed that at least 10% of the diet of small-sized pilchards and anchovies was phytoplankton. It is only the larval stages, whose gill rakers have yet to develop, that are incapable of filtering phytoplankton (Scofield, 1934). Recent research in WA (see Section 6) confirms this opinion with the discovery of well developed gill rakers and phytoplankton in the stomach contents of very small (50-70 mm) Australian pilchards. Any change in dietary preference between size classes may be more to do with energetics than any functional difference in raker morphology (van der Lingen, 1995).

2.7 Schooling

Pilchards are a highly schooling species that often form large aggregations or shoals (*sensu* Pitcher, 1983). Such behaviour makes them highly vulnerable to purse seining types of capture methods. Schools vary in size from a few kilograms up to several hundred tonnes and these appear to form and disperse over relatively short temporal scales (hours to days). In WA at least, during periods when they are not schooling, individuals have sometimes been seen streaming across the bottom, often in single file. The gut contents of many fish examined indicates that they may still be feeding during these times as the prey found in their guts are often of benthic origin.

Schools are usually made up of individuals that are relatively similar in size, but catches with mixed fish are not uncommon and schools with different sized fish may be in close proximity to each other.

The density of the fish within schools varies greatly but it is certain that they come into contact with one another at times. This is evidenced by the large frequency of scales that show regeneration (indicative of scale loss) which are most likely to have arisen from their removal by collisions with neighbouring individuals.

2.8 Migrations and Rates of Movement

There are no direct studies of the rates of movement or migration paths of pilchards in Australia. The only information available comes from the anecdotal observations of fishermen, historical records and from analyses of logbook data. In the Albany region, the speed of movement by pilchard schools was recorded in daily logbooks over a two-year period (Fletcher, unpublished). These data indicate that schools can move at speeds in excess of 5 knots and are even capable of outrunning boats trying to shoot a net. The average speed of movement for all schools recorded was 1.9 knots (over 3 km/h). This would equate to a total distance covered in a day of approximately 70 km.

There are anecdotal reports of migrations along the south coast of WA from east to west during autumn. Similarly, both Roughley (1916) and Whitley (1937) reported northward migrations of large schools of pilchards along the New South Wales coast in winter. Nonetheless, Blackburn (1949) doubted that any one individual moved more than a few hundred miles along the coast; he believed that there were mostly onshore-offshore movements.

Tagging data collected from South African pilchards (Newman, 1970) showed that individuals of this stock were capable of large-scale migrations (> 1000 km). It was noted that the shorter the time period at liberty, the faster was the maximum rate of daily movement (20 km/day for 10-20 days at liberty (DAL), 6 km/day for 20-200 DAL; and 4 km/day for those caught > 200 DAL). Similarly, individual *Sardinops sagax (caerulea)* tagged off California also travelled in excess of 1000 km, some in approximately 100 days (Janssen, 1938; Clark and Janssen, 1945) therefore averaging about 12 km/day for an extended period. Similar tagging trials conducted off British Columbia (Hart, 1943) found that pilchards could move great distances quickly with a minimum estimate of daily movement of 14.3 km/day. The potential distance that can be travelled on one day from these studies could not be determined in the above studies but it will certainly be greater than the long-term averages.

The swimming speed of *S. sagax* has also been assessed under laboratory conditions (Beamish, 1984; van der Lingen, 1995). Beamish (1984) found that even very small (total length [TL] = 6 cm) individuals of *S. sagax* could easily maintain speeds of 15 cm/s (2.3 lengths/s or 0.54 km/h) for periods of 12 hours

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(6.5 km) which were used as 'training periods' for experiments in which the speed was increased to 80 cm/s. Similarly, van der Lingen (1995) showed that the average non-feeding swimming speed of adults (TL = 25.6 cm) in aquaria was approximately 0.8 lengths/s with this rate increasing to in excess of 2 lengths/s when feeding (17–65 cm/s =16–55 km/day). The smaller *Engraulis capensis* (TL = 9.32 cm), however, had a normal swimming speed of 1.73 lengths/s and therefore still had absolute speeds of 22–50 cm/s (James and Probyn, 1989). Using these figures for the Australian pilchards with TLs approaching 21 cm, distances in excess of 30 km/day could be easily achieved.

2.9   Stock Structure

A large amount of work has been devoted to the determination of the stock structure of pilchards in Australia, particularly in WA. These studies have provided a plethora of data, not all of which are consistent, but a general picture has emerged whereby separation can be seen at a number of different levels.

Spawning times

As stated above, there is ample evidence of variations in timing between locations to support the notion of some functional separation between stocks.

Age structure

Variations in the overall rates of mortality between areas with different levels of fishing have also provided information on the level of population separation. In WA, catch-at-age curves for Albany and Bremer Bay have been determined for the past six years using the otolith weight method (e.g. Fletcher, 1995a). A number of years are also available for Esperance and Fremantle (Fletcher, unpublished).

At Albany, the total mortality rate is relatively high (Figure 2.3) which correlates with the long history of heavy fishing exploitation that has occurred in this region. At Bremer Bay, where exploitation has been much lower, the curves are also lower. Whilst at Esperance, where exploitation has only recently begun, the curves appear to merely reflect natural mortality ($z = 0.43$; Figure 2.4). If there was total free mixing of the adult stock along the south coast all the curves would be similar, or they would alter randomly from year to year. This is clearly not the case, suggesting that there is not a large degree of mixing of adults along the south coast of WA. There is, however, some evidence that the three south coast locations are linked in terms of variations in the level of juvenile recruitment.

Plankton

Plankton surveys have been completed in summer and winter on the south coast of WA for the years 1991–95 (Fletcher *et al.*, 1994; Fletcher *et al.*, 1996b). These studies have confirmed that there are a number of discrete spawning areas which are consistent in space and time (Figure 2.5), but the products of these are often mixed due to transport by the Leeuwin Current.

Samples have also been taken along the lower west coast during 1993–95 in which there has always been a large gap between south coast spawning areas and west coast spawning areas (Figure 2.5). There has also been surprisingly little evidence of movement of material from the west coast to the south coast despite the presence of the strong, southward-flowing Leeuwin Current.

Morphological studies

Two studies (Blackburn, 1951; Syahailatua, 1992) have used morphological features as a method of assessing stock structure of Australian pilchards. Both found significant differences between regions. Blackburn (1951) distinguished three regions: an eastern group (New South Wales), a south-eastern group (Victoria) and a south-western group (southern WA). Syahailatua (1992) extended these findings to add a western group (west coast, WA) and confirmed that all other south coast WA locations were morphologically similar.

Electrophoresis

Dixon et al. (1993) completed an allozyme study of the genetic structure of pilchards throughout Australia and concluded that there were 'a series of contiguous quasi-independent pilchard sub populations'. They delineated western, south-western, south-eastern and eastern populations, the boundaries of which possibly shift in response to variations in environmental conditions, particularly the Leeuwin Current. However, this separation was not shown to be complete.

Minor and trace elements

Results of the relatively new technique of examining the minor and trace elemental composition of otoliths as a method of stock delineation were, for pilchards, initially promising, showing clear separation amongst sites on the south coast but with no separation between west coast sites (Edmonds et al., 1995). Repeated sampling on the south coast showed, however, that the level of temporal variation was similar to the level of spatial variation. There do, nonetheless, appear to be at least two south coast stocks, the position of which may vary with time, and there is some overlapping at least on a minor scale at times.

$O_{16/18}$ isotope analyses

Further sampling of pilchard otoliths from different locations revealed very clear differences in isotope ratios between sites which were sustained over a full year of sampling (Edmonds and Fletcher, unpublished). Otoliths from west coast pilchards had ratios consistent with their having lived in water on average 1.5°C warmer than that inhabited by south coast pilchards. Those from Esperance were also about 0.5°C cooler than those from Albany and Bremer Bay, which again is consistent with the average difference in temperatures between the sites. These data support the notion that there is little movement between the south and west coasts by adult pilchards, and that there is even restricted movement of adults along the south coast.

Conclusions

In WA there appear to be two separate spawning stocks, the west coast and south coast stocks, with recruits functionally separated. Along the south coast there also appears to be a finer level of separation of adults, with at least two and probably three adult stocks along this region. The position of these stocks is variable and there is evidence for major flow between areas during the juvenile phases (< 3 years of age), but only minor movement as adults. In terms of fisheries management, the three south coast areas are treated separately. By contrast, none of the data collected on the west coast have indicated any finer level of stock separation, consequently this area is treated as one stock.

2.10 Fishery

WA has the largest pilchard fisheries in Australia (Fletcher, 1991a). Fishing for pilchards in WA began in the Fremantle area during the 1950s with the immigration of many fishermen from the Mediterranean region. The fishery did not develop until the advent of purse seining in the 1970s but since this time the areas of fishing and the catches have expanded greatly (Figure 2.6). There are currently seven management zones for pilchard fishing in WA (Figure 2.7), for all of which a Total Allowable Catch (TAC) has already been set or is currently being determined.

A total of over 40 boats have endorsements to use purse seine gear within these zones and the total catch during the past five years has averaged approximately 9000 tonnes. This makes up over 75% of the total pilchard catch of Australia and equates to a landed catch value greater than $6 million per annum.

Until recently, the boats used in these fisheries have been relatively small, only 10-15 m in length. This was a consequence of the boat length restrictions which were in place before the output control methods were implemented. Most fishing has, therefore, been restricted to regions which were close to landing facilities because there were no on-board processing capabilities and the boats needed to fish in relatively sheltered water. This situation is likely to alter during the next few years.

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The prime market for WA pilchards is for blocks of angling bait. This currently comprises up to 60% of the landed product, but this has not always been the case and it may change in the future.

During the mid-1980s, the pet food market expanded rapidly, which was largely the impetus for the greatly increased catches during this period, taking over half the catch. The introduction of quotas in the early 1990s combined with the lower beach price paid for pet food has seen this percentage drop to only 25%. Two areas where the markets have expanded in recent years are for human consumption, both fresh and processed for supermarkets/restaurants, and as food for the caged southern bluefin tuna in South Australia.

2.11 Stock Assessment

The review by Fletcher (1991a) indicated that little was known about the stock size of pilchards in Australian waters. Since that time, considerable work has been done within WA to address this deficiency.

A computer model, based upon catch and effort information for the south coast pilchard fishery, was created in 1991 (Fletcher, 1992). This determined that the stock size in the Albany region was between 10 000 and 35 000 tonnes. Subsequent use of the model in conjunction with catch-at-age information, which has been collected yearly since 1989, has refined these estimates suggesting that the spawning stock size for Albany pilchards has varied during this period, due to fluctuations in recruitment, between 12 000 and 25 000 tonnes (Figure 2.8; Fletcher, 1994; 1995b). The model suggested that the spawning stock size in July 1995 should have been in the vicinity of 17 000 tonnes.

The fisheries-independent, daily egg production method of biomass calculation (DEPM) has been used in the Albany area in 1991-95 (Fletcher et al., 1992; 1996; see also Section 7). These estimates, based upon the plankton tow data and adult spawning parameters, have varied between 15 000 and 31 000 tonnes.

The stock at Bremer Bay has also been assessed using yearly catch-at-age information since 1989 and DEPM surveys in 1992-94. These both indicate that the stock in this area has also been in the vicinity of 20 000 tonnes.

2.12 Conclusion

A large amount of information is known about the biology, fishery and stock sizes of pilchards in WA. The information presented has direct relevance to a number of the scenarios that were suggested, including differential feeding by adult pilchards and stress from spawning. This will greatly narrow the list of possible causes and provides the necessary background to assess the results of the samples taken during the mortality phenomenon.

Table 2.1   Otolith weight – age relationship of WA pilchards (from Fletcher, 1995a)

Age	Mean Weight (mg)	Upper Value (mg)
2	0.96	1.14
3	1.25	1.38
4	1.50	1.63
5	1.74	1.85
6	1.95	2.05
7	2.15	2.25
8	2.35	

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Figure 2.1 Distibution of pilchard, *Sardinops sagax* in Australia. Except for Carnarvon and Hervey Bay (the northern limits of distribution), the locations named are the major regions where fishing occurs, modified from Fletcher,1990.

Pilchard Growth Curves
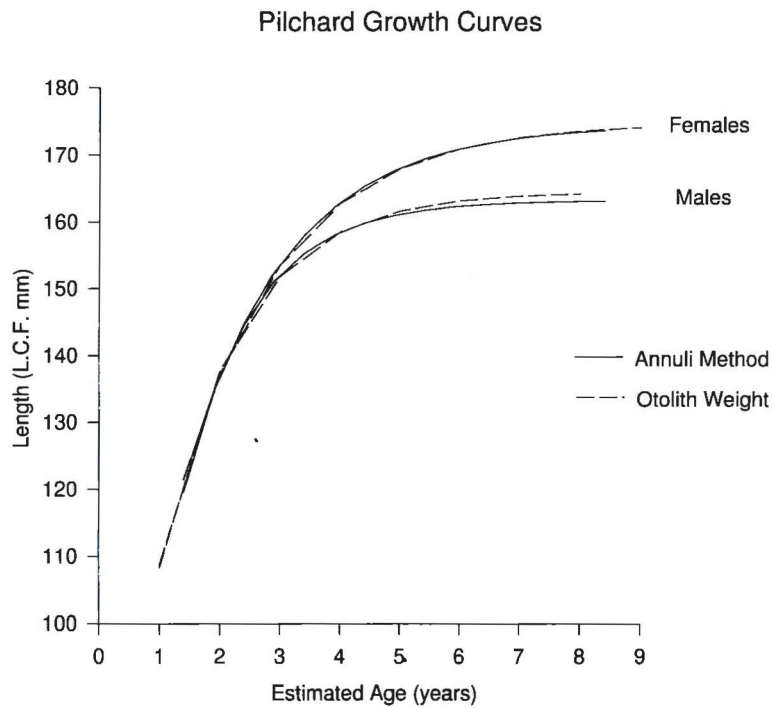
Figure 2.2 Growth curves for male and female pilchards in WA (modified from Fletcher, 1995 and Fletcher & Blight, 1996)

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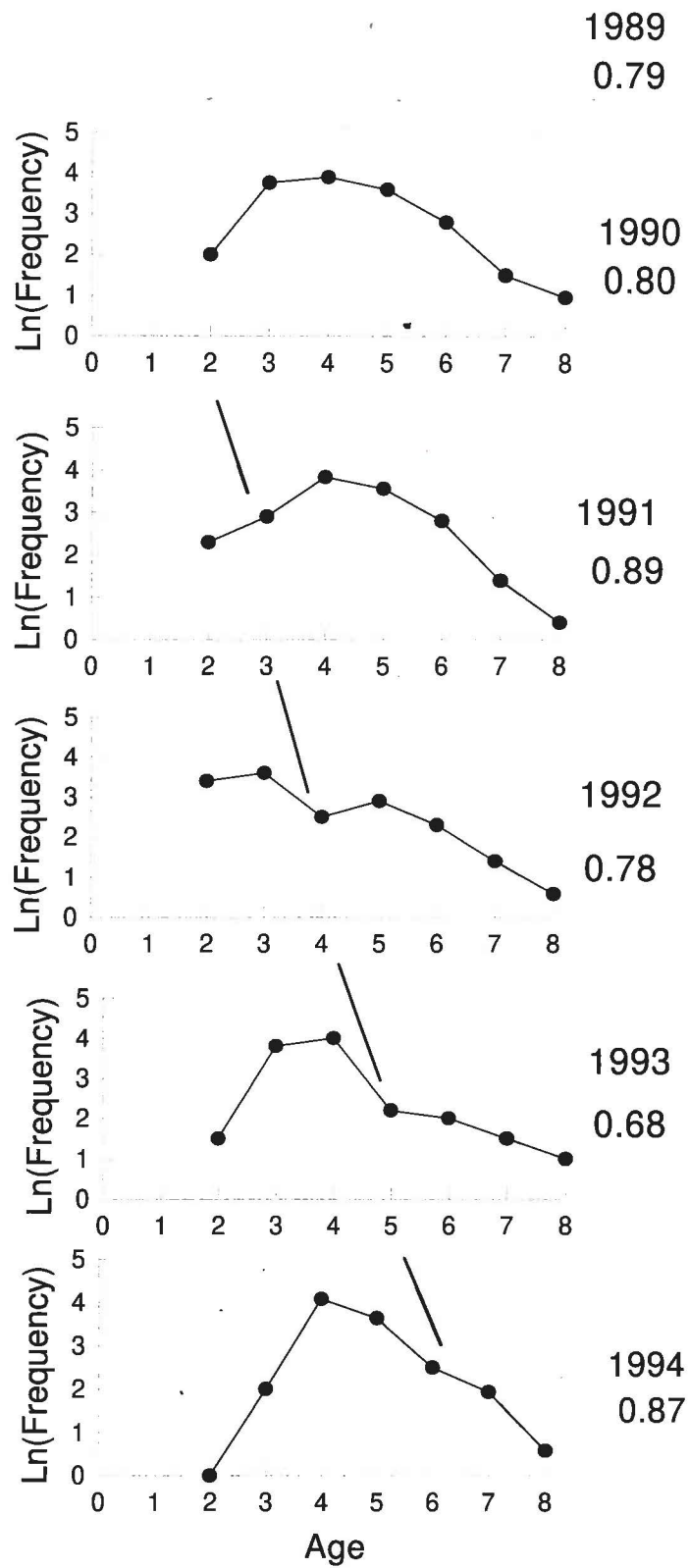
Figure 2.3 Yearly catch at age curves for the Albany pilchard fishery (modified from Fletcher, 1995a)

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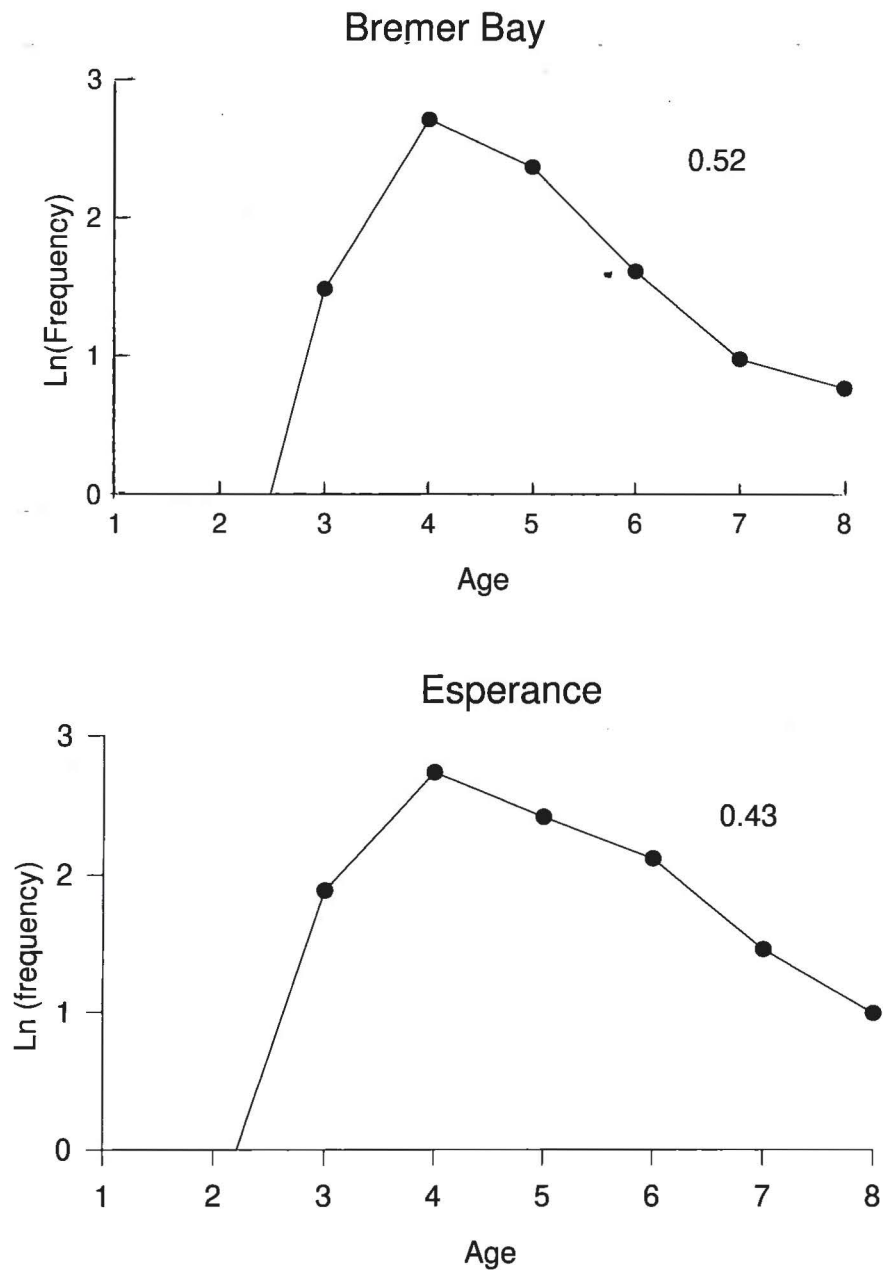
Figure 2.4   Catch at age curves for the pilchard fisheries at Bremer Bay and Esperance with the total annual mortality rate, Z, calculated as -1 x slope of the right hand limb of the curve.

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Generalized distribution of Pilchard Eggs
on South-West Coast of Australia
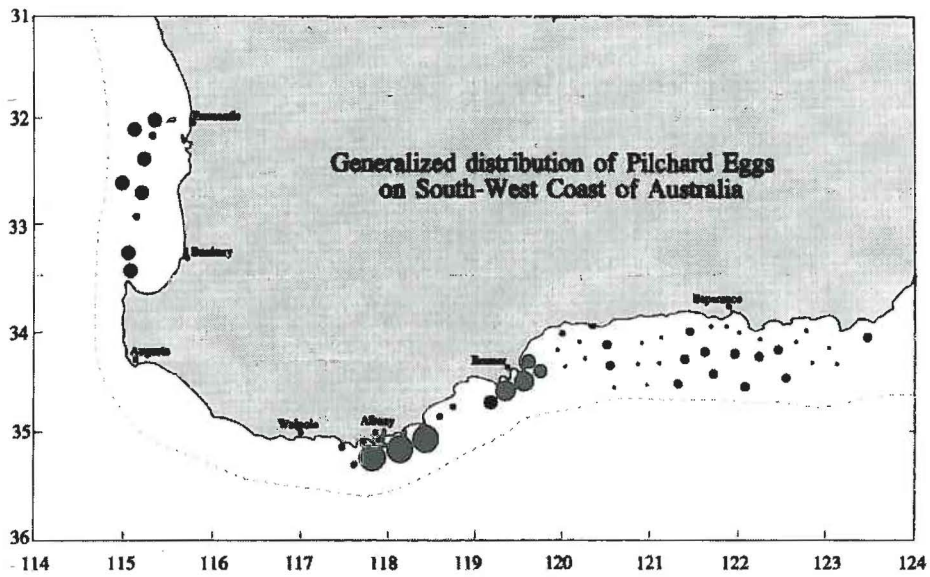
Figure 2.5   Summary of the distribution of pilchard eggs during July and December/January periods.

WA Pilchard Catch
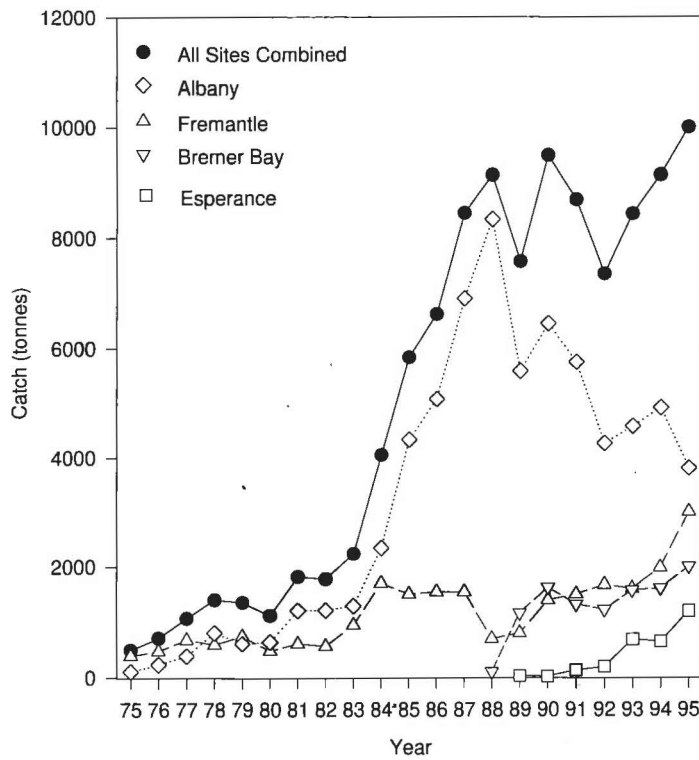
Figure 2.6   Catch of pilchards in each of the main fishing areas of WA.

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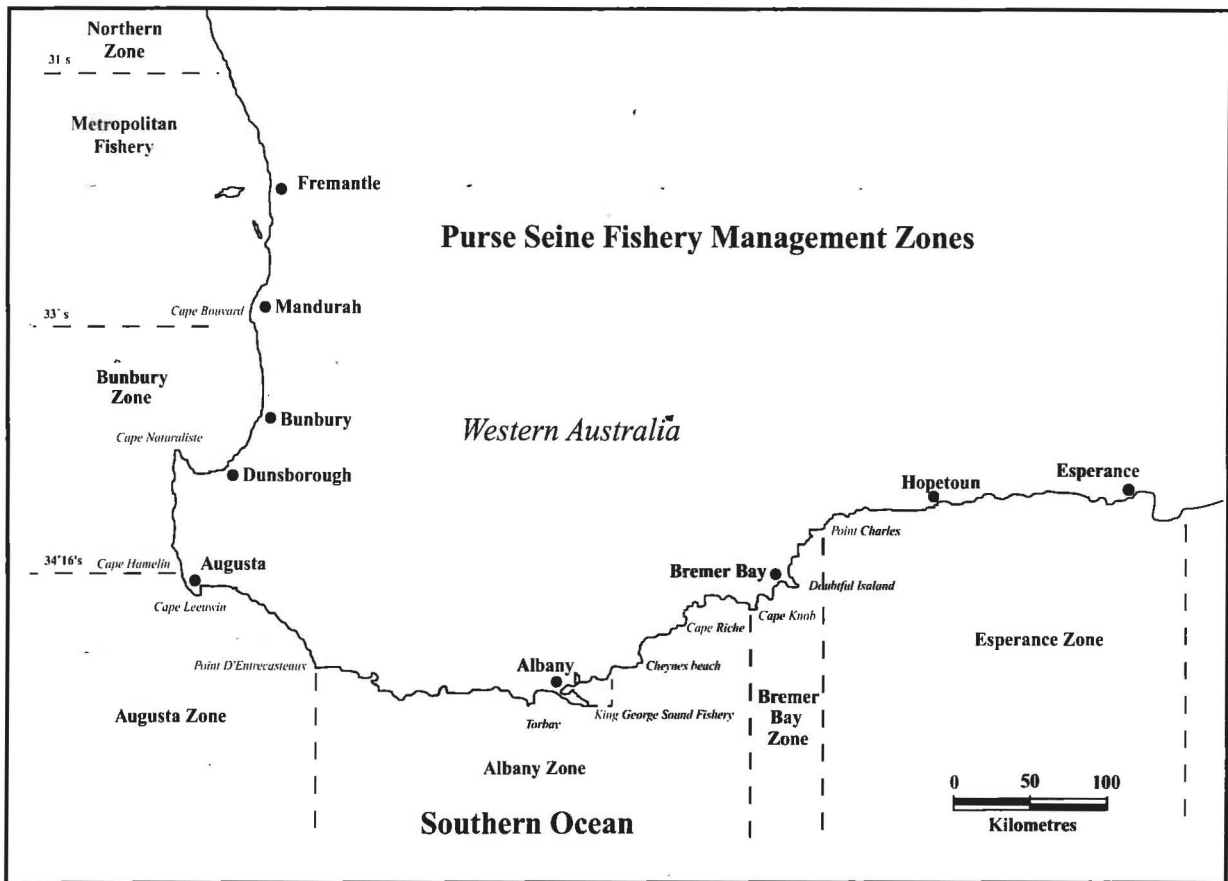
Figure 2.7 The management zones for the pilchard fisheries in WA. The northern zone fishes mainly for the tropical sardine, *Sardinella lemuru*.

Albany Pilchard Model
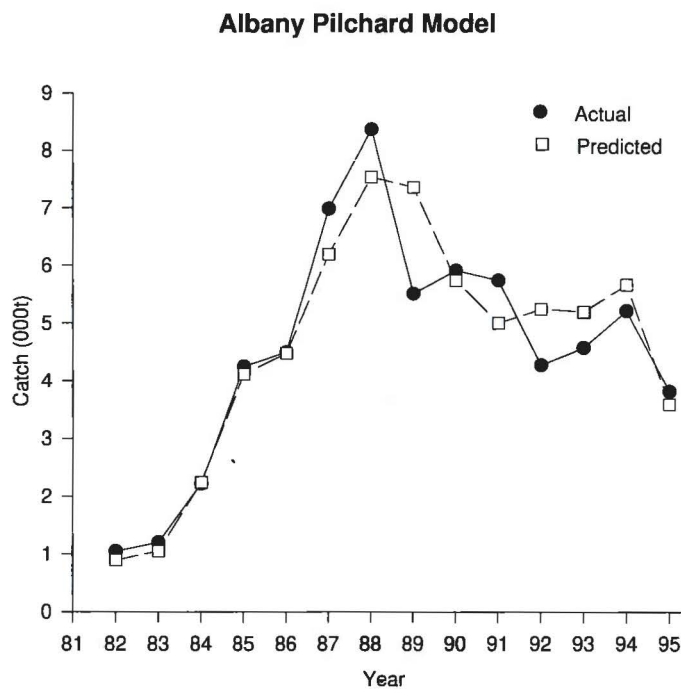
Figure 2.8 The predicted and actual catch of the Albany pilchard fishery using the model created by Fletcher (1992) modified by the yearly recruitment indices (see Fletcher, 1995a for details). Note, the TAC for the fishery was reduced from 5500 tonnes to only 3800 tonnes for 1995.

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Section 3   Epidemiology

3.1   Introduction

The first records of the pilchard kill were news reports in late March 1995 of the mortalities off the Eyre Peninsula (South Australia) which were attributed in the media to a toxic algal bloom. On 18 April the first reports of deaths in WA waters were received; these were confirmed by Fisheries Department of WA personnel on 25 and 26 April. The occurrence and spread of the disease in WA was subsequently monitored by commercial fishermen, Fisheries Department personnel and members of the public. Mortalities were observed to move as a 'front' or 'wave' along the coast in a westerly direction. Fish ahead of the front were alive; behind the front, dead fish were found floating, whilst at the front itself, pilchards in the throes of death were observed. The deaths at any one location only lasted one or two days and no deaths were reported behind the front. The dates at which mortalities were first observed at each location in WA are shown in Figure 3.1.

3.2   Methods

The distance and elapsed time of the disease front from its origin were calculated as the shortest distance in kilometres by sea from the place where dead pilchards were originally found (in the vicinity of Anxious Bay, South Australia, on 15 March 1995) and the time in days from the date of origin to the first date at each site in WA at which dead pilchards were seen, respectively. Data analysis was carried out using SYSTAT®.

3.3   Results

A plot of the elapsed time of the mortality front by its distance showed that the rate of spread of the disease to the west was highly correlated (Figure 3.2). The Spearman Rank Correlation between date of die-off and distance was significant ($r = 0.940$, $n = 22$). Linear regression through the points was attempted, but the pattern of the residuals was cyclic (Figure 3.3). Therefore, to estimate the rate of spread of the disease after 18 April 1995 when the mortalities reached WA, distance was divided by the number of elapsed days at each site. By this method the front had a median rate of 30.4 km/day (range 3.3 to 43.2 km/day, skew – 1.36).

3.4   Discussion

The relatively constant rate of movement allowed the prediction of when the front would occur in locations which enabled sampling to occur before, within, and behind the front. Storms on 29 April, 9 May, 17 May, 22 May and 6 June 1995 were expected to halt or interrupt the movement, but did not appear to do so. Likewise, the convergence zone between the subtropical Leeuwin Current, flowing south down the west coast, and the Southern Ocean Drift along the south coast was expected to form a barrier. It did not do so and deaths of pilchards continued up the west coast, eventually petering out off Carnarvon at the northern limit of the pilchard distribution in WA. The mortalities also moved to the east from South Australian waters (Whittington et al., 1997).

The autocorrelation plot of the residuals showed a cyclic trend which was removed when the residuals were differenced (values replaced by the difference between each value and the previous value). If each point in a series relies on the previous point's value, then differencing would remove that dependence (Wilkinson, 1988). It is also worth noting that the group of residuals corresponding to < 25 days after first deaths in Figure 3.3 are South Australian records, the negative residuals above 25 days are south coast and the positive residuals are west coast records.

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If the mortalities were due to an infectious agent then autocorrelation would be expected since the daily distance travelled would be governed by the distance travelled the day before. Thus, these observations are consistent with an infectious disease spreading into WA from a focus of infection in South Australia.
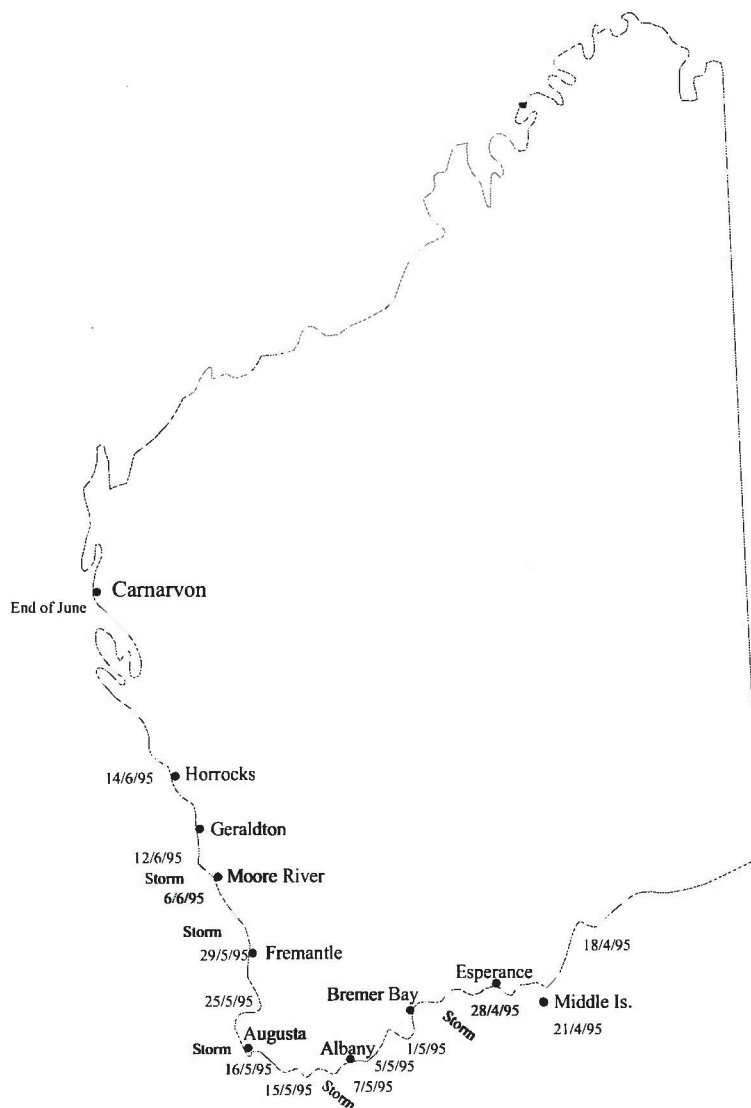
Figure 3.1   A map of Western Australia showing the dates at which mortalities were first observed.

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Western Australian Epidemiology
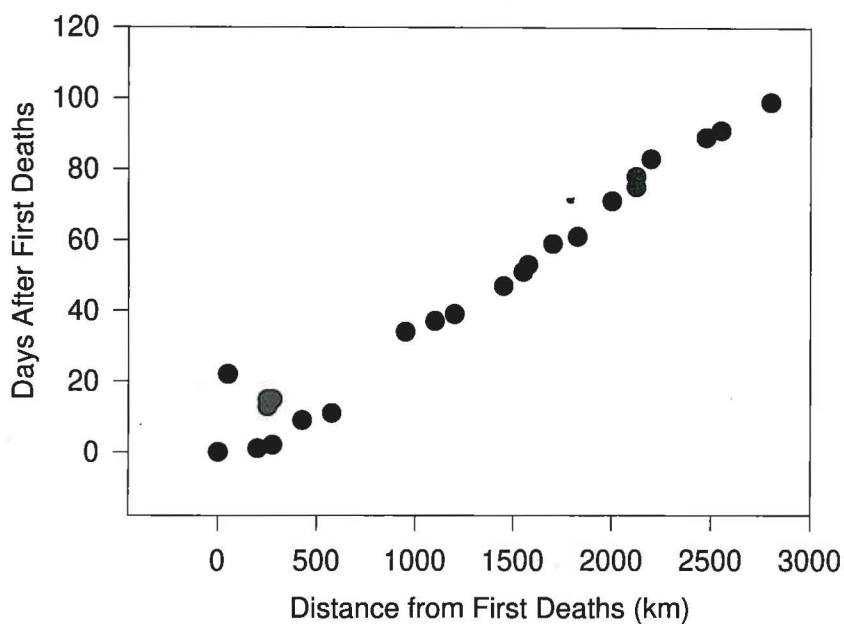
Figure 3.2   A plot of the elapsed time of the mortality front against the distance from the point source in SA. Data for South Australia were obtained from the web site at CSIRO and from that supplied by K.Jones SARDI.
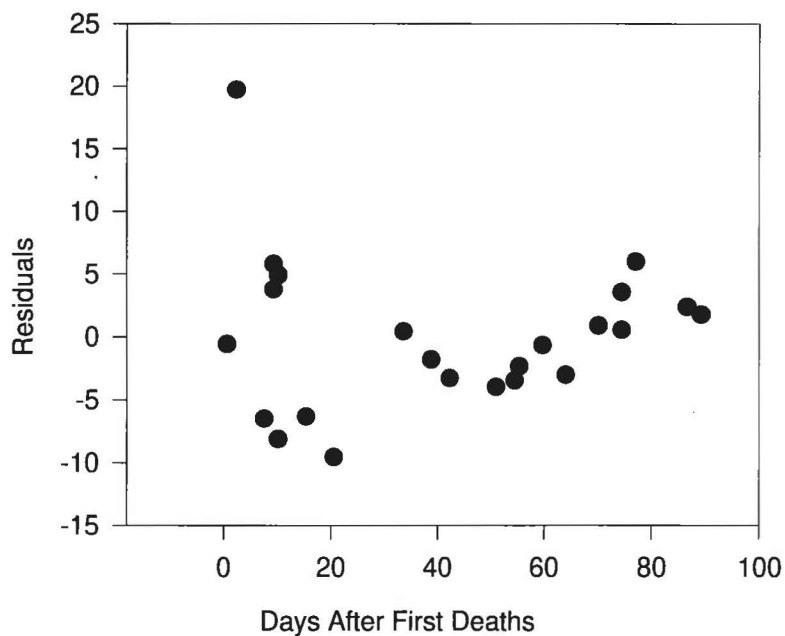
Figure 3.3   A plot of the residuals of the linear regression of elapsed time of the mortality front after the first death on distance from the point source in South Australia.

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Section 4   Environmental Data

4.1   Introduction

Physical processes which could have potentially played a role in the pilchard mortality include unusually strong upwelling, alongshore advection by currents, anomalously low or high water temperatures, or some nutrient variability leading to phytoplankton blooms. The main features of the wind field, shelf circulation and water properties along the WA coast are described here using historical data from which the possibility of alongshore advection being responsible for the progression of the pilchard mortality along the west coast can then be assessed. In addition, relevant data available for the time near to the mortalities are examined against this historical background.

Four coastal regions will be analysed: the upwelling area off South Australia, the south coast (Esperance to Albany), the south-west corner (Cape Leeuwin to Cape Naturaliste) and the west coast (Bunbury to the Houtman Abrolhos Islands).

4.2   General Circulation

The dominant ocean current off south-western Australia is the Leeuwin Current, a stream of relatively warm low-salinity water which flows southwards down the west coast and then eastwards into the Great Australian Bight. It is a unique eastern boundary current in that it flows *polewards* (in contrast with the cool *equatorward* Humboldt and Benguela Currents) (Pearce, 1991), and there is no large-scale upwelling off Western Australia (again in contrast with the strong seasonal upwelling of cold nutrient-rich waters off the west coasts of southern Africa and South America).

While the currents on the continental shelf are generally southwards during winter (when the Leeuwin Current is flowing strongly), they reverse to a net northward flow during the summer months (Figure 4.1). The regional circulation will be described in more detail below.

4.3   Regional Analyses

4.3.1   South Australian upwelling area (Eyre Peninsula, 10–13 March)

The pilchard mortality was first observed off the Eyre Peninsula during the second week of March 1995 (Fig. 3.1). While coastal upwelling has been described along the Bonney coast east of the South Australian gulfs by Lewis (1981) and Schahinger (1987), there is also an upwelling region along the western coast of the Eyre Peninsula (Griffin *et al.*, 1997). During the summer months, winds from the south-east blowing along the coast move the surface waters offshore, and a compensatory onshore flow of cool subsurface water rises on to the continental shelf as an upwelling plume; this may increase the nutrients on the continental shelf, potentially leading to blooms of phytoplankton and enhanced coastal productivity.

The wind regime at Port Lincoln and Elliston on the South Australian coast has been analysed in some detail by Oceanique Perspectives (1995). The proportion of winds from the south-east (rather than the wind strength as such) in January 1995 at these sites was higher than in any other month between January 1993 and March 1995, probably resulting in stronger than average upwelling off the Eyre Peninsula (anecdotal information supported by evidence of cool upwelling plumes in satellite imagery; Oceanique Perspectives, 1995; Griffin *et al.*, 1997). After carefully examining all the available data, however, Griffin *et al.* (1997) pointed out that in other years the winds were even stronger, concluding that 'there is no evidence to suggest the upwelling off the Eyre Peninsula in February–March 1995 was primarily responsible for initiating the mortalities by stressing the fish extraordinarily'.

4.3.2 South coast (Esperance to Albany, 20 April to 4 May)

Winds

Coastal winds have been recorded at the Seaframe site at Esperance by the National Tidal Facility since mid-1992. The east–west (alongshore) component of hourly winds from this site shows a few-day reversing pattern with wind component speeds of the order of 5 m/s and peaks of perhaps 10 m/s (Figure 4.2). The strength of the alongshore wind component in late April 1995 was no different from that in April of 1993 and 1994, although (as off the Eyre Peninsula) westward winds were more persistent in early April 1995 than in the other two years. It is unlikely, however, that this small difference could have contributed to the westward propagation of the mortality.

Leeuwin Current

Along the south coast, the Leeuwin Current tends to flow eastwards along the mid to outer shelf, although (as on the west coast) large 'warm offshoots' (Cresswell and Peterson, 1993) peel away from the coast and can temporarily disrupt the alongshore flow (Griffiths and Pearce, 1985). There are very few historical current measurements in this region: Acoustic Doppler Current Profiler (ADCP) records from the R.V. *Franklin* in June 1987 showed eastward currents of between 0.5 and 1 m/s right across the continental shelf and upper slope south of Albany (Cresswell and Peterson, 1993). Similar strength easterly currents were also found during a *Franklin* cruise across this region in July 1994 (Fletcher, unpublished).

Estimates of the westward propagation speed of the 'kill front' are 30 km/day (0.3m/s) (see Section 3 and Whittington *et al.*, 1997), and the eastward propagation from the Eyre Peninsula towards the New South Wales coast was only slightly faster (Whittington et al., 1997). Satellite imagery in early May 1995 clearly indicated the Leeuwin Current to be flowing eastwards on the outer continental shelf between at least Cape Leeuwin (116°E) and Bremer Bay (119°E). Although interpretation of thermal satellite images in terms of coastal currents is not perfect, there is no indication of any westward flow near the coast (Figure 4.3) which could transport the pilchards, or some pathogen, towards the west coast, and (with Griffin *et al.*, 1997) this suggestion can be dismissed.

Water temperatures

Esperance

A temperature recorder located on Esperance wharf, which is located only a few miles from where recently dead pilchards were found, showed very little variation in temperatures during the entire month of April 1995. This period encompasses the times before, during and after the kills had passed this location (Figure 4.2). Comparison of the temperatures at this site during the month of April in 1993, 1994 and 1995 (Figure 4.2) indicates that sea surface temperatures (SSTs) in April 1993 and 1995 were between 18° and 20°C (April 1994 was perhaps a degree warmer). There was no evidence of a sudden decrease in temperature towards the end of the month when the deaths occurred.

The surface temperatures taken on board the boats at Esperance during the period of the kills were not different to those expected at this time of the year (Figure 4.4). The temperatures at inshore locations were in the vicinity of 18°C and increased gradually in offshore regions to approximately 20°C. There was no difference between days, with no change from warm water to cold measured in areas where the kills occurred (Figure 4.4).

Albany

Measurements of the SSTs at Albany were all above 20°C during the entire period when the mortalities were occurring (Figure 4.4). These temperatures were also measured in a transect that ran from Fremantle to Albany ahead of the deaths.

The only slight temperature front (and this was less than 1°C) was associated with the Leeuwin Current which appeared to be flowing close to the coast west of Torbay, but moving offshore to the edge of the shelf at Albany with associated increased temperatures close to 21°C (Figure 4.3). This is a common feature at this location at this time of the year (Fletcher *et al.*, 1994).

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<u>Reynolds sea surface temperature (SST) analyses</u>
On a larger scale, satellite-derived SSTs (Reynolds and Smith, 1994) for two 1-degree latitude/longitude squares south of Albany show that the water was up to 1°C cooler than the long-term mean between March and June 1995 (Figure 4.5). Nevertheless, there have been many such periods over the past 14 years, some with even stronger anomalies, and it is therefore unlikely that they could be related to the pilchard mortality.

<u>Naval XBT data</u>
XBT (eXpendable BathyThermograph) records from the Royal Australian Navy during two excursions across the Great Australian Bight during the months relevant to the kills are shown in Figure 4.6. In the continental shelf waters of south-west WA, the water temperature was mostly greater than 20°C. There was no stratification in the water column in these regions, with a difference of more than 1°C between the surface and bottom measurements at only one of the eight stations. All other stations had differences of less than 0.2°C, thus there was no evidence for any subsurface current of cold water moving against the Leeuwin Current which could have played a role in the deaths of the pilchards.

4.3.3 South-west corner (between Cape Leeuwin and Cape Naturaliste, c. 20 May)

Leeuwin Current

As the Leeuwin Current approaches Cape Naturaliste from the north, it tends to ride up on to the continental shelf and flow closer inshore, before rounding Cape Leeuwin and heading eastwards largely as a shelf current (Figure 4.1). This provides the mechanism for the transport of tropical marine organisms to the south coast (Maxwell and Cresswell, 1981). As the Leeuwin Current weakens in late spring, however, it tends to move away from the coast between Cape Naturaliste and Cape Leeuwin, to be replaced by a cooler northward countercurrent recently named the Capes Current (Phillips *et al.*, 1991; Cresswell and Peterson, 1993; Pearce *et al.*, in prep.).

Coastal currents

Current measurements along the mid and outer shelf off Cape Mentelle (34°S) in April–May 1987 showed predominantly southward flow between about 0.2 and 0.8 m/s (Boland *et al.*, 1988; Smith *et al.*, 1991). However, the northward Capes Current was probably closer inshore than the innermost current mooring and therefore not evident in the current data. Anecdotal information from fishermen confirms the concept (derived from satellite imagery) of a relatively strong northward current between the Capes during the summer months of (say) October to March, and a transition to persistently southward flow by April of most years.

The Capes Current could provide a potential conduit for water (and, accordingly, the pilchard mortality) to be transported northwards past Cape Leeuwin and up the west coast. Satellite imagery at the time that the 'kill' passed Cape Leeuwin, however, confirms that the Capes Current was no longer flowing in May, the Leeuwin Current in fact being relatively close inshore in the Capes region (Figure 4.7) and effectively preventing any northward water movement.

Water properties

Temperature and salinity have been sampled monthly by the Fisheries Department at the puerulus collection site near Cape Mentelle (34°S, midway between Capes Leeuwin and Naturaliste) since 1984. Although the sampling interval is relatively poor, a large anomaly from the average could indicate abnormal conditions in this area. The temperatures for January and February 1995, as well as for mid-year, were below the mean, but during March, April and May they were right on average (Figure 4.8), supporting the evidence from the south coast that April–May 1995 was not unusual.

4.3.4 West coast (Busselton to Geraldton, 30 May to 15 June)

Leeuwin Current

Along the west coast, the Leeuwin Current tends to flow along the outer continental shelf, but periodic large meanders and eddies can transport the tropical water over 200 km offshore (Legeckis and

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Cresswell, 1981; Cresswell, 1980; Prata and Wells, 1990). Along the inshore current boundary, there are also smaller tongues of Leeuwin Current water penetrating across the continental shelf towards the coast, representing active cross-shelf mixing events (Pearce and Griffiths, 1991). Cross-shelf current speeds recorded by current meters are frequently up to 0.2 m/s (Pearce and Phillips, 1994). The southward flow is generally strongest during the autumn, winter and early spring months, being effectively retarded by the strong equatorward wind stresses during summer (Godfrey and Ridgway, 1985).

The current is relatively narrow (50–100 km) and shallow (200–300 m), and there is an equatorward undercurrent below about 300 m (Thompson, 1984; Smith et al., 1991). Although mean speeds in the current may be taken as 0.5–1 m/s (1–2 knots), net alongshore movement over a week or two may be less than this (Hutchins and Pearce, 1994). On the other hand, speeds exceeding 1.5 m/s have occasionally been observed in the trajectories of drifting satellite-tracked buoys (Cresswell, 1980).

Currents on the continental shelf between Fremantle and the Abrolhos Islands were measured using the Acoustic Doppler Current Profiler (ADCP) from the R.V. *Franklin* between 13 and 16 June 1995 while investigating the pilchard mortality along the west coast (Griffin, 1995). The flow was almost entirely southward across the shelf, with speeds generally between 0.1 and 0.3 m/s and rising to 1 m/s in the Leeuwin Current near the shelf break (Figure 4.9).

Monthly and annual mean coastal sea levels have been used by Pearce and Phillips (1988) and Pattiaratchi and Buchan (1991) as an index of the strength of the Leeuwin Current. Monthly mean sea level at Fremantle in the autumn of 1995 was slightly higher than in the previous two years but lower than most autumns over the period 1986 to 1995, a result of the enduring El Nino – Southern Oscillation (ENSO) event of the early 1990s (Figure 4.10). Again, the sea level does not indicate any anomalous conditions at the time of the pilchard mortality.

Coastal currents

Current measurements south of the Abrolhos Islands have shown that the net flow along the Western Australian continental shelf tends to be southwards at about 0.2 m/s in winter (April to August) and northwards at about half that speed in summer (November to March) (Cresswell et al., 1989). Superimposed on these mean drifts, however, are wind-driven current reversals during which the flow changes direction and peak speeds of up to 0.5 m/s (1 knot) may be experienced.

A year-long program of current measurements near the coast off Point Peron, just south of Perth, displayed a clear seasonal pattern, with the net flow in winter (May to August) being southwards at a mean speed of about 0.1 m/s and in summer (November to April) northwards at the same speed, the transition months being March–April and September–October (Steedman and Associates, 1981). Similarly, currents measured off Bunbury (about 33°20'S) during May 1975 were largely southwards (Steedman and Associates, 1980; Hearn, 1983; summarised in Wallis, 1983; Pearce, 1992). By May, therefore, when the 'kill front' passed this area, the inshore currents would normally be southwards and not assist any effective northward advection.

Water properties and nutrients

Surface and subsurface temperatures measured at sea along the west coast during the incident were similar to those found in the Albany region, with 18°C water inshore and over 21°C water in the offshore regions where the deaths were occurring (Figure 4.11). These temperatures were approximately the same in early May, about one month before the front had arrived on the west coast, with temperatures of 21–23°C recorded in offshore areas, and in late May and early June, during the time of the kills, with temperatures of 18–20°C (Table 4.1). Thus, there was no accompanying change in temperature along this part of the coast during the incident.

Dissolved oxygen readings were also taken on the west coast before and during the kills (Table 4.1). The values both at surface and in subsurface regions, before and during the kills, were in the vicinity of 4.7–8.4 mL/L. There was no evidence of any drop during this period.

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Profile temperature and salinity measurements at a CSIRO coastal monitoring station west of Rottnest Island in 1995 were essentially the same as the long-term (1970-94) means, although Griffin et al.(1997) have pointed out that salinity at this site has been rising steadily over the past two decades. The nitrate concentration in late May 1995 rose substantially (Griffin et al.'s Figure 6) but there were no indications of, for example, phytoplankton blooms which could have been responsible for the pilchard mortality (Section 5).

Hourly sea temperatures measured at the Seaframe monitoring site at the Hillarys marina, just north of Perth, show that there have been some substantial changes in temperature in other years not associated with mass mortalities: for example a drop of 3°C in a day or so in mid-May 1994, and a rise of about 2°C in a day in early May 1995 (Figure 4.12). Average temperatures in May 1994 were about 1°C higher than in May 1993 and 1995, but the data show no unusual features in 1995 which could be related to the pilchard mortality.

Satellite-derived SSTs for the 1-degree squares off Perth (Figure 4.13) indicate that (in contrast to the cool anomalies off Albany) the water was somewhat warmer than average off Perth in early 1995. However, the same conclusion is reached: there have been other periods with similar warm anomalies since 1982, and (in the absence of any other information) the conclusion is drawn that this is also unrelated to the pilchard mortality.

4.4 ASP and PSP Testing

Material sampled
Freshly dead pilchards were collected during the fish kill event from waters off Torbay (35°08.6'S, 117°39.64'E) on 7 May 1995. These were submitted to the Chemistry Centre of WA for amnesiac shellfish poison (ASP) and paralytic shellfish poison (PSP) algal toxin determination by high-pressure liquid chromatography (HPLC). The test was negative.

In addition, dead and dying pilchards which had been collected from the sea in Bremer Bay in the vicinity of the fish kill on 1 May 1995 and frozen until 15 May were tested for toxicity using a mouse test performed by the Animal Health Laboratories of Agriculture WA.

Fremantle sardines were collected from a commercial catch on 15 May. No deaths had been reported on the west coast at that time. These were also tested for toxicity using a mouse test.

Results
All but one of the mice tested negative; the one dead mouse was injected with a very high dose of Bremer Bay pilchard and could have been affected by toxins from the dead fish.

4.5 Conclusions
A plot of the numbers of dead pilchards observed on the surface against the surface temperature of the water in which they were located shows no significant relationship (Figure 4.14). The largest counts of dead fish occurred in water > 20° C with the only trend present being a slight positive slope, which is the reverse of the theory that cold water was the 'trigger'. Instead, the data collected showed that the deaths of the pilchards in WA could in no way be attributed to any abnormal deviation in the temperature of the water. A supposed intrusion of subantarctic waters on to the shelf off Esperance and Albany during the time of the kills, reported in the task force report (Anon., 1995) and media reports (O'Neil, 1995; Douglas, 1995), is incorrect.

At WA locations, there was either little or no drop in temperature during the times that preceded the kills. This pattern was observed at a number of locations around the coast using a variety of methods. Consequently, there is no evidence to support the hypothesis that there was a passage of some small environmental feature along the coast triggering the deaths.

From a variety of observations around the time when the pilchard mortality was occurring along both the west and south coasts of WA, it is clear that environmental conditions were well within normal

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ranges and are unlikely therefore to have contributed to the phenomenon. This conclusion is in line with the analysis by Griffin *et al.* (1997).

Table 4.1 Environmental measurements taken before and during the period when pilchards were dying on the west coast of WA.

	Mandurah (before)	Mandurah (during)	Fremantle (before)	Fremantle (during)	Bunbury (before)	Bunbury (during)
Temp (°C) Offshore (surface)	20.1-20.4	20.5-20.8	19.2-21.6	20-21.6	21.5-22.5	21.3
Temp (°C) Offshore (subsurface)	19.7-19.9	20.5	18.2-19.0	20.4-21.4		21.5
Temp (°C) Inshore	18.2-18.4		18.2-18.5			
Salinity (ppt) (surface)			36.3-36.9			
Salinity (ppt) (subsurface)		36.1	36.3-36.9	36.1-36.7		
Diss. Oxyg. (mL/L) (surface)	4.5-5.7	4.7	4.8-5.8	4.85-5.15	7.8-7.9	8.4
Diss. Oxyg. (mL/L) (subsurface)	4.2-5.9	4.72	4.8-5.0	4.9-5.03		
pH				7.02-7.11		
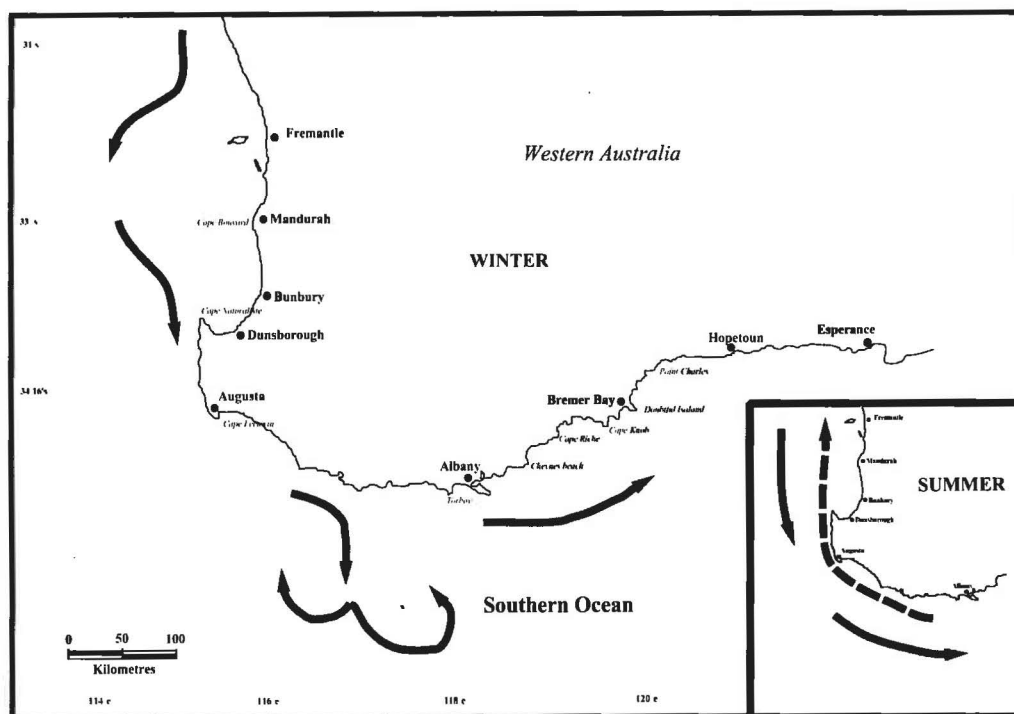
Figure 4.1 Diagrammatic representation of the major oceanographic currents off south Western Australia.

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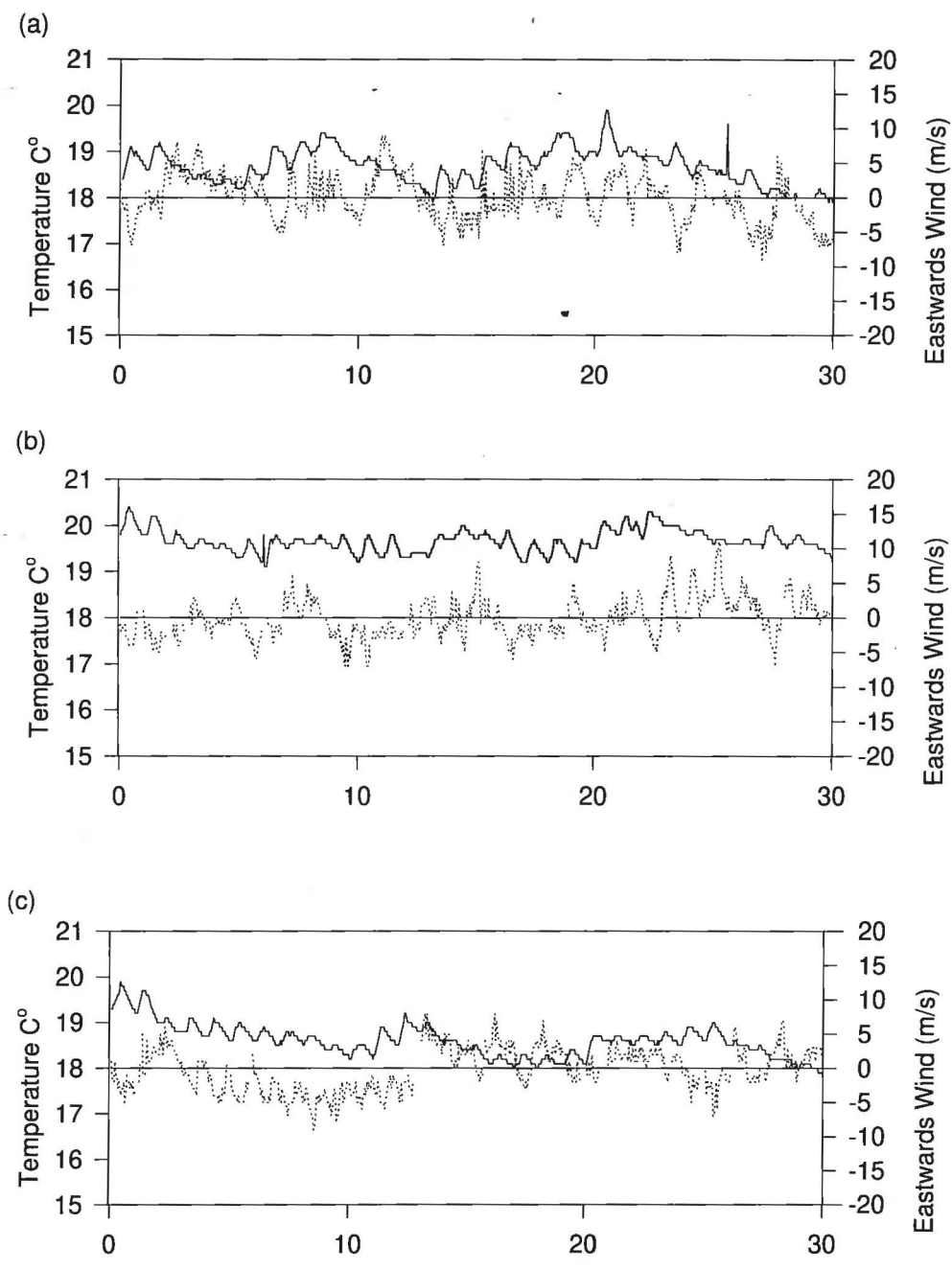
Figure 4.2   Hourly sea temperatures (solid line) and eastward wind components (dotted line) at the Seaframe recording site at Esperance in April of (a) 1993, (b) 1994 and (c) 1995.

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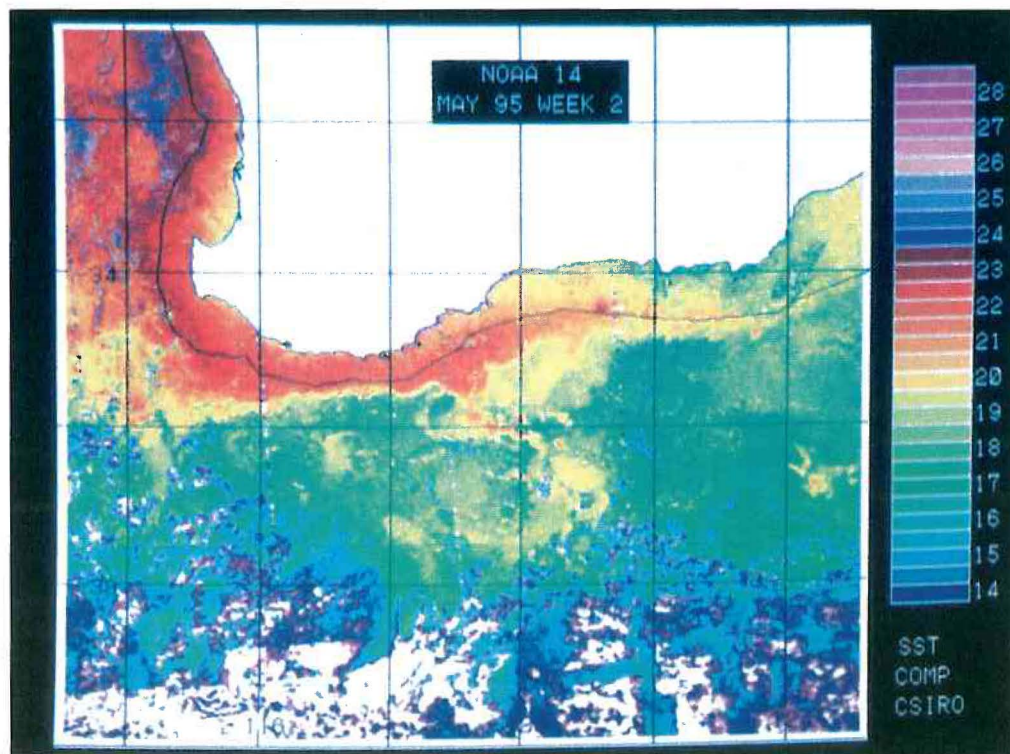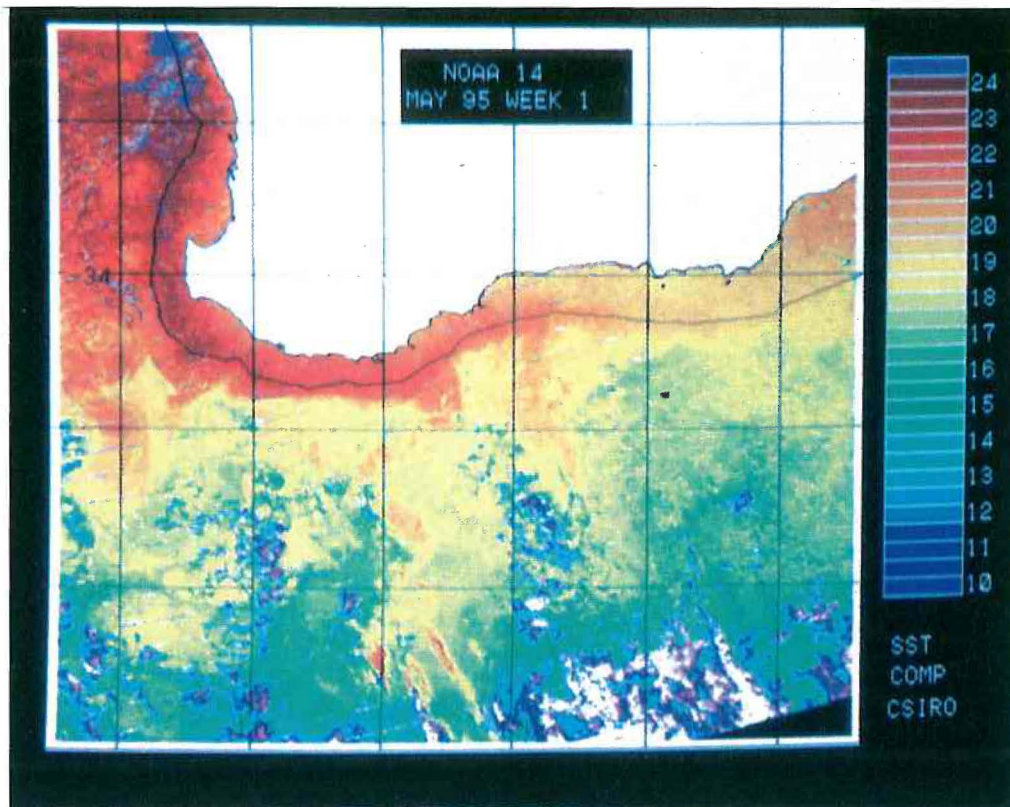
Figure 4.3　NOAA/AVHRR satellite images of the south coast, early and mid- May 1995. Warmest water (the Leeuwin Current) is shown in red/yellow; the black line marks the approximate position of the shelf break. White areas are cloud.

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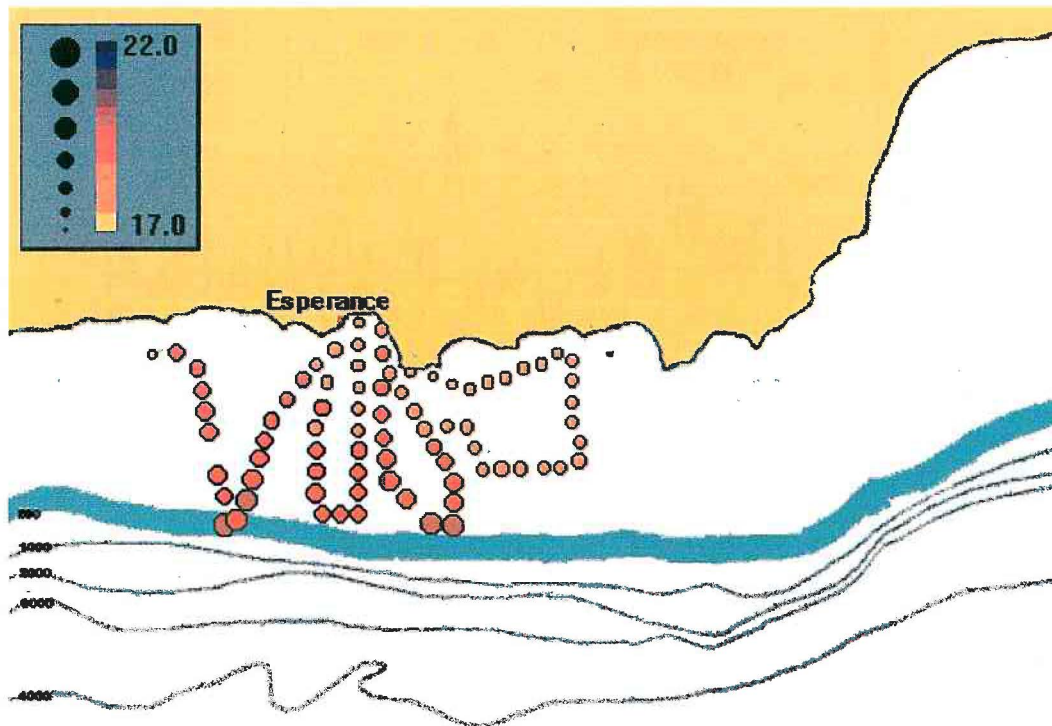
April/May 1995 - Sea Surface Temperature
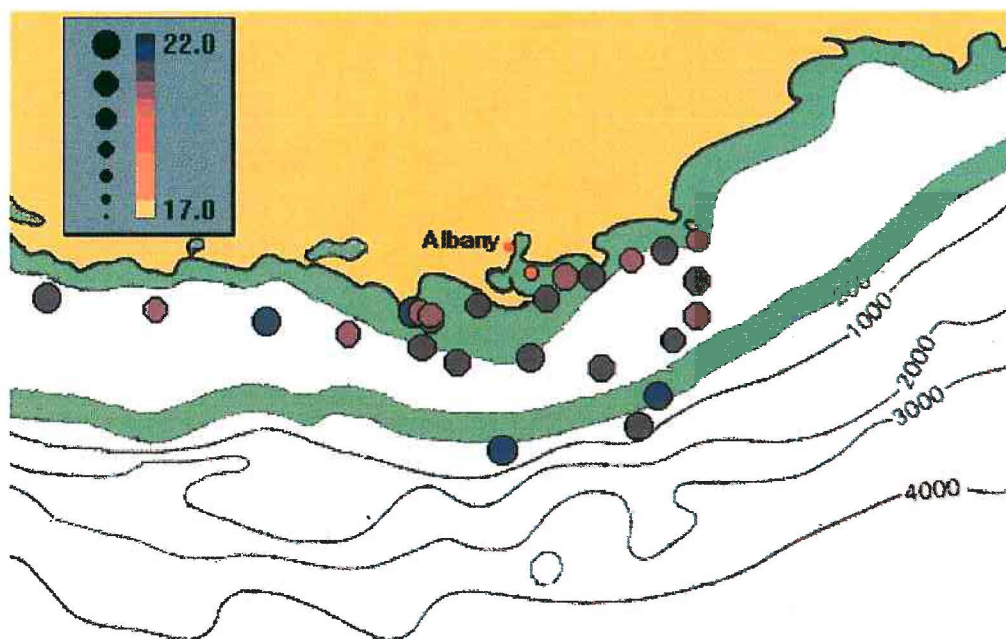
May 1995 - Sea surface Temperature

Figure 4.4   Sea surface temperatures recorded during surveys conducted off Esperance and Albany during the period when the pilchards were dying in each area.
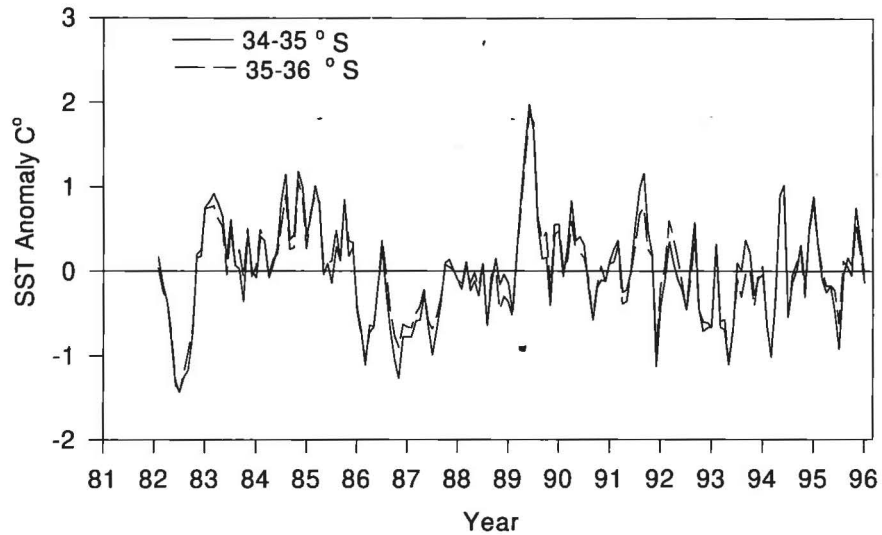
Figure 4.5 SST anomalies derived from thermal satellite data for the squares 34° to 35°S and 35° to 36°S (118° to 119°E). Each point is the difference between the individual monthly temperature for that month and the longterm mean.
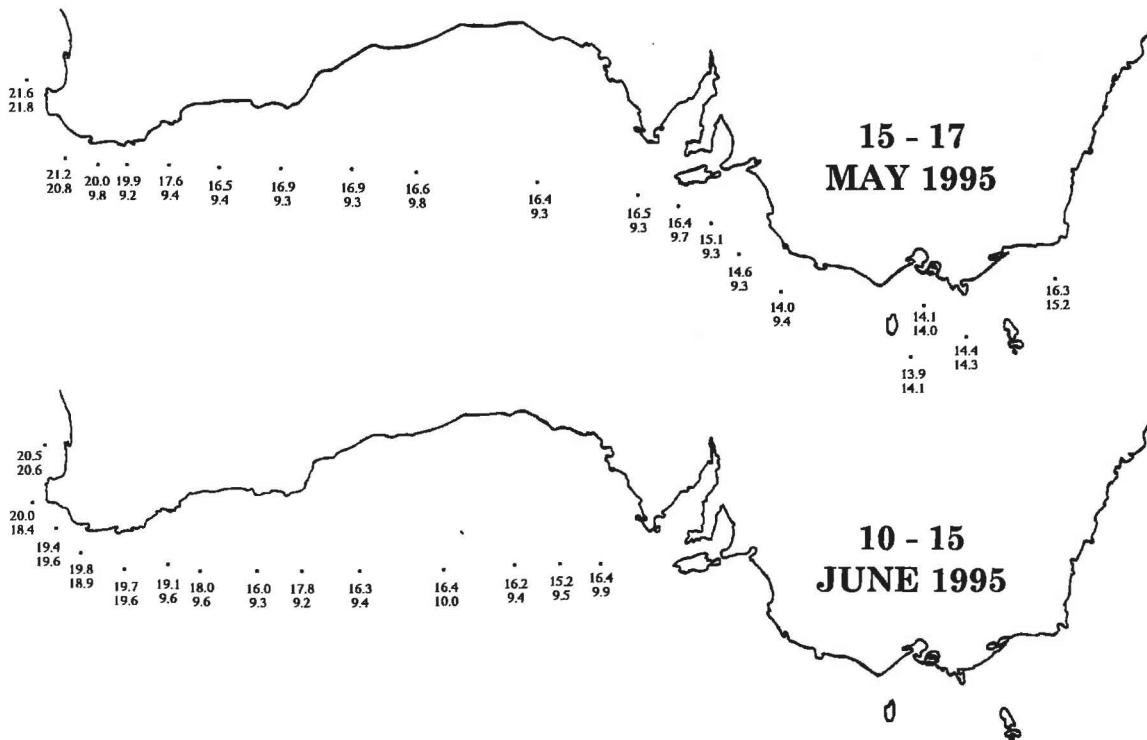
Figure 4.6 XBT records form the Royal Australian Navy (top number = surface temp; bottom number = temp at 200m depth).

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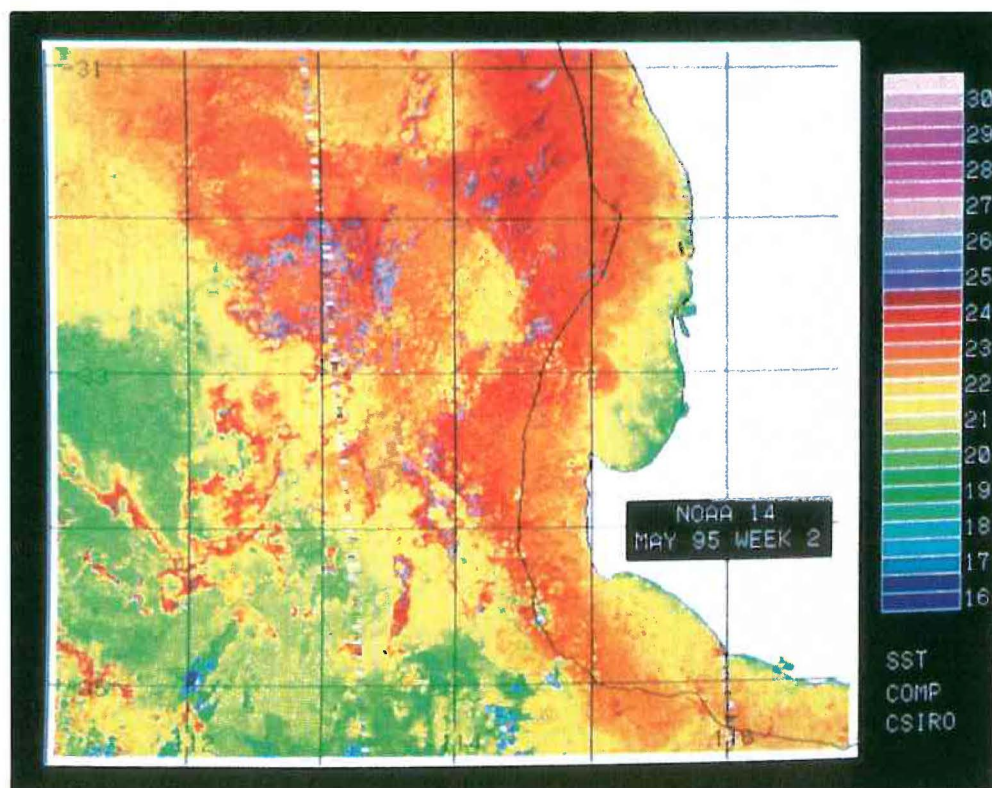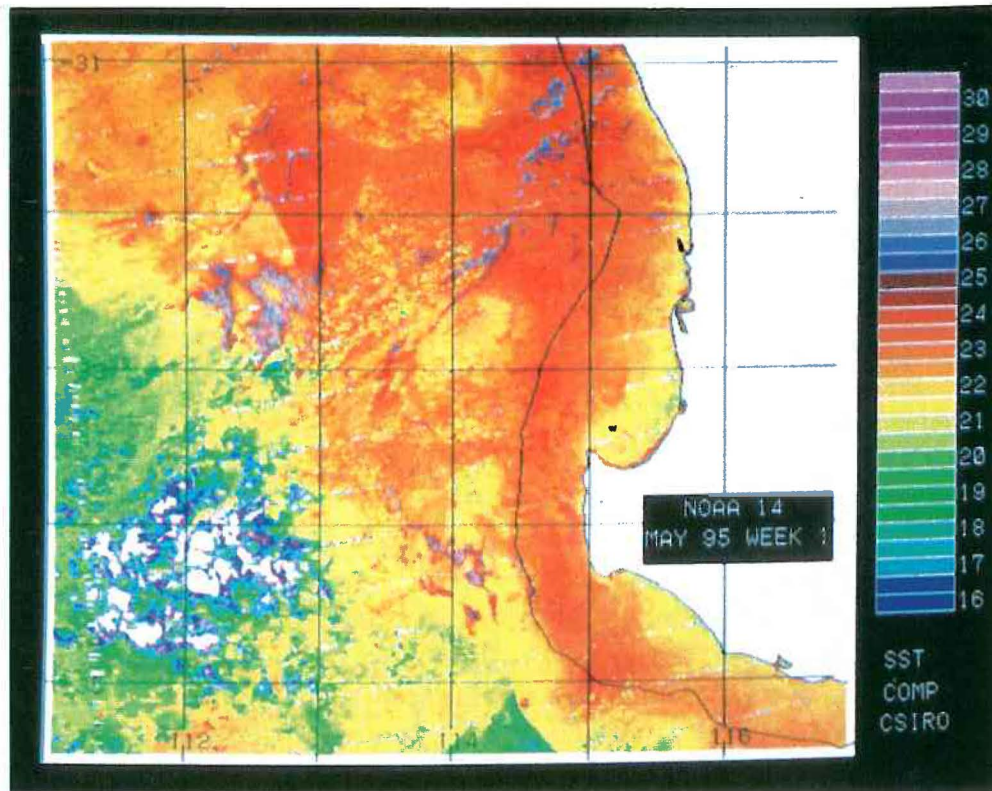
Figure 4.7 NOAA/AVHRR satellite images of the Capes region, early and mid- May 1995. Warmest water (Leeuwin Current) is shown in red, cooling through yellow to the coolest water in green; the black line indicates the approximate position of the shelf break.

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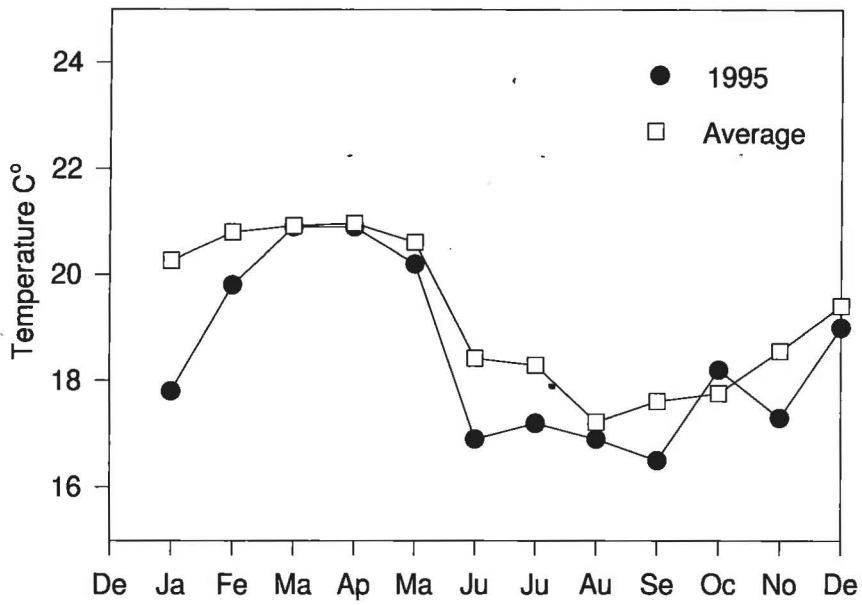
Figure 4.8 Monthly temperatures for 1995 (filled circles) compared with the 1984 to 1994 averages (open squares) at the puerulus collection site near Cape Mentelle.

Corrected Currents [b] at 15m
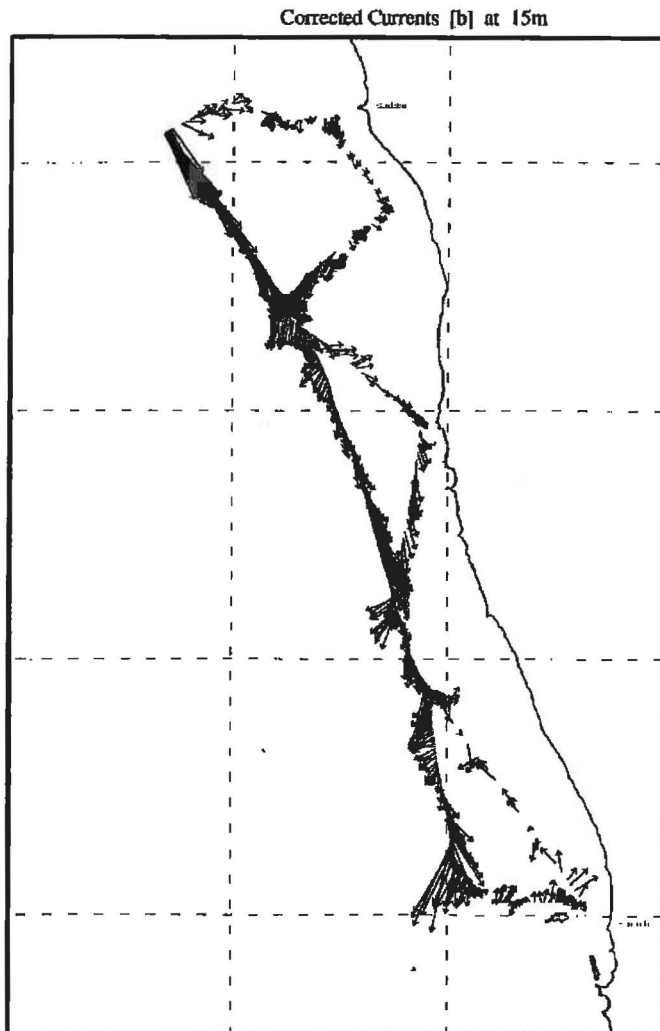
Figure 4.9 ADCP current profile data from the RV Franklin cruise in June 1995. Arrows indicate the relative speed and direction of current flow.

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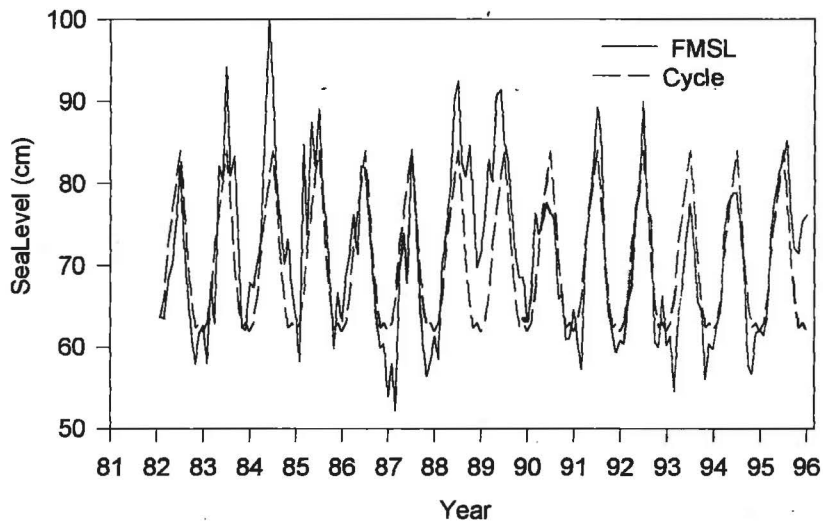
Figure 4.10 Monthly mean sealevels at Fremantle between 1986 and 1995; these have not been adjusted for atmospheric pressure. The solid line is the Fremantle sea level, the dotted line is the cycle.
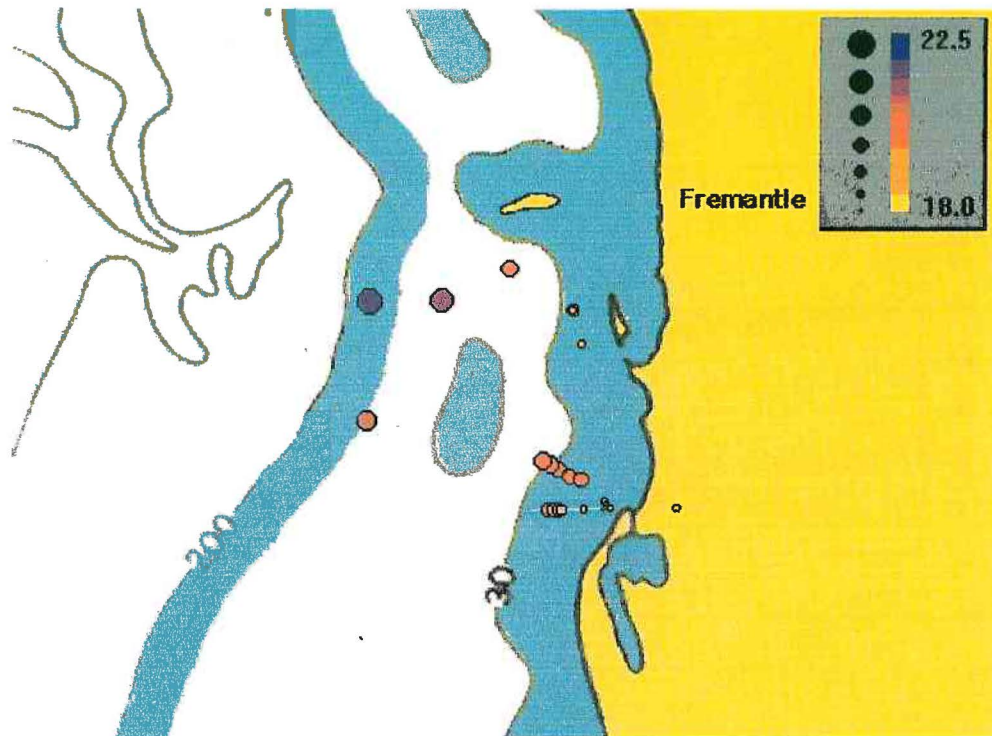
Figure 4.11 Sea surface temperatures recorded during surveys conducted off Fremantle during the period when the pilchards were dying in each area.

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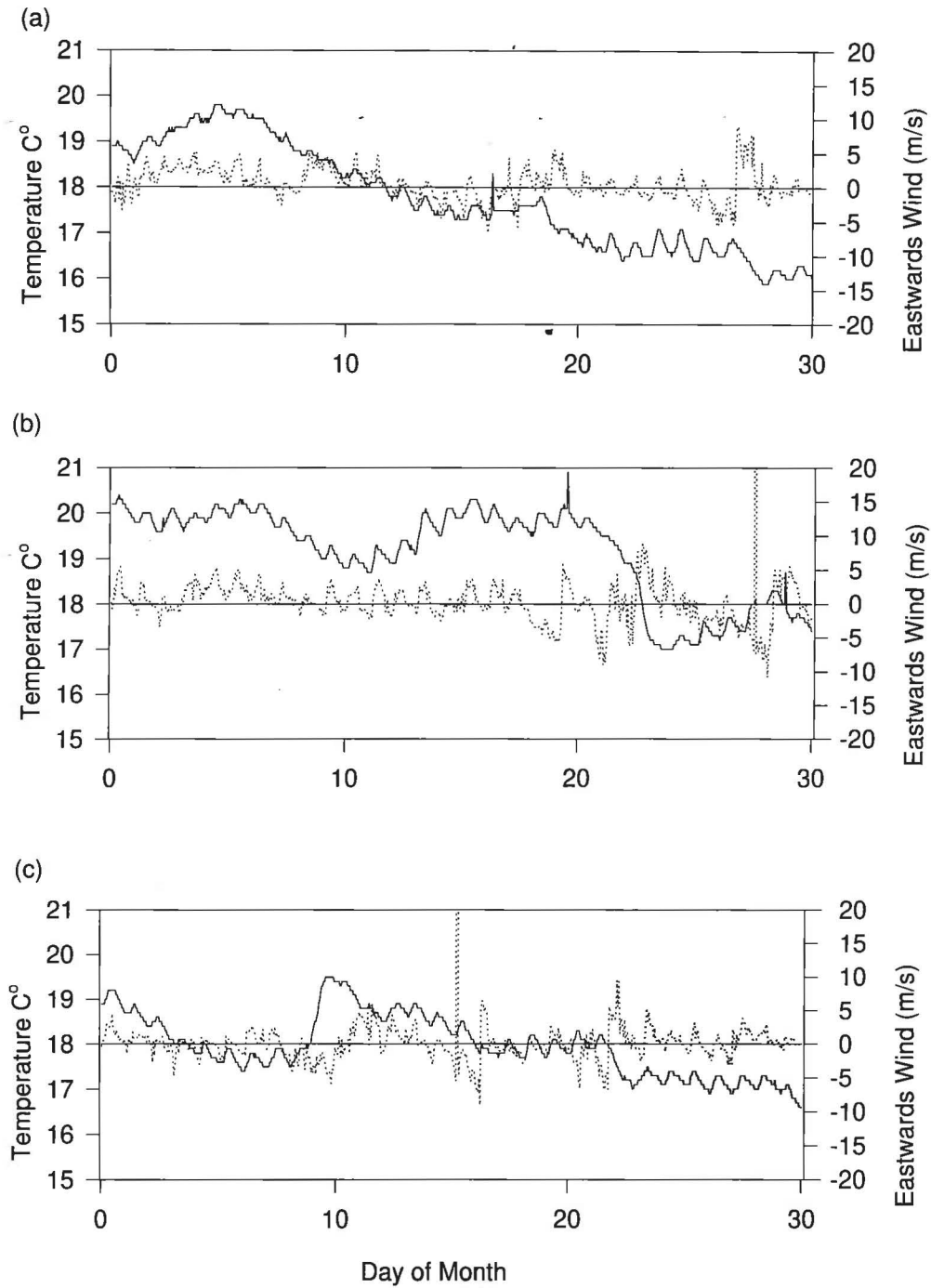
Figure 4.12 Hourly sea temperatures (solid line) and eastward wind components (dotted line) at the Seaframe recording site at Hillarys in May of (a) 1993, (b) 1994 and (c) 1995.

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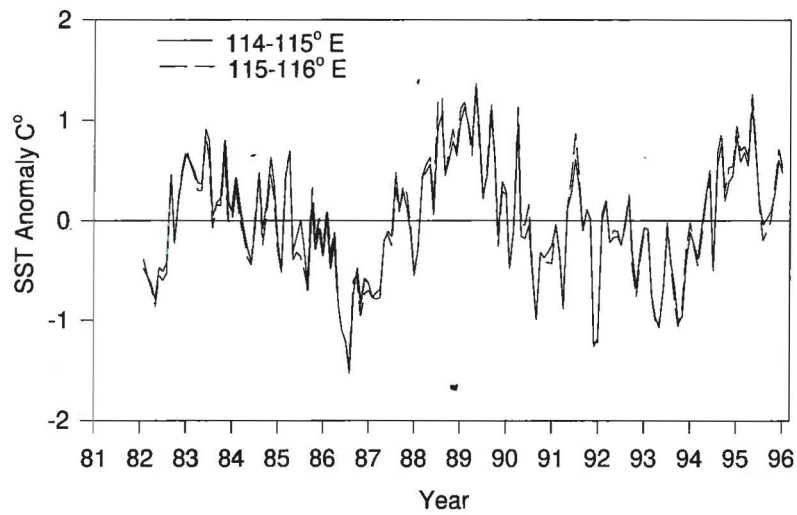
Figure 4.13 SST anomalies derived from thermal satellite data for the squares 114° to 115°E and 115° to 116°E (31° to 32°S). Each point is the difference between the individual monthly temperature for that month and the longterm mean.

Relationship between Temperature and Deaths
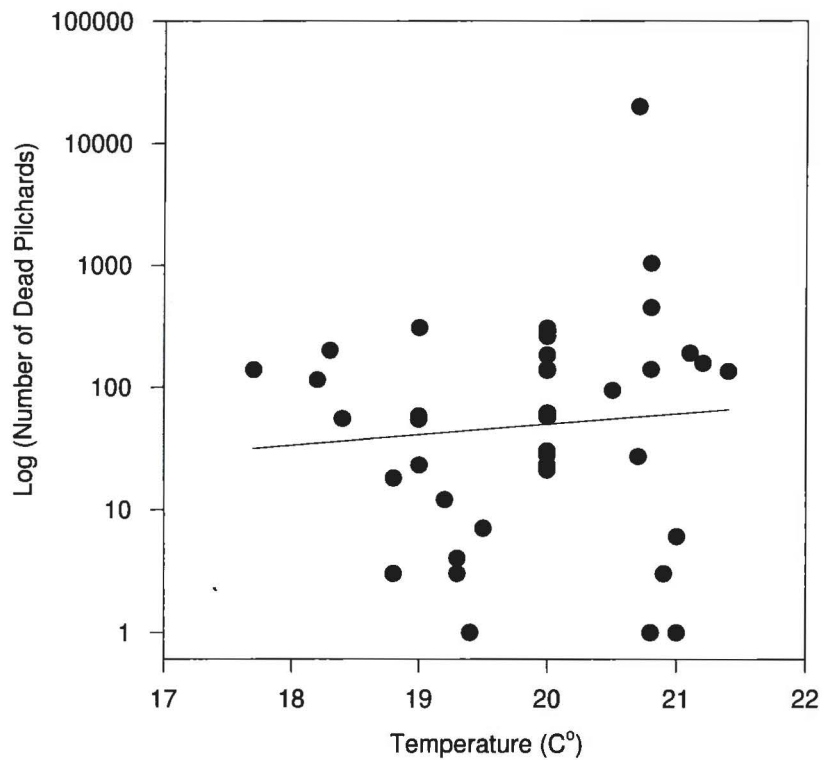
Figure 4.14 Relationship between the number of dead pilchards observed floating in an area and the sea surface temperature in the region.

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Section 5   Phytoplankton Densities and Pilchard Feeding

5.1   Introduction

In early March 1995, the first of the 1995 fish kills occurred off South Australia and these were associated with blooms of the dinoflagellate *Gymnodinium mikimotoi* (Hallegraeff, pers. comm., 1995). In this early instance a number of other species were also affected (South Australian Research and Development Institute, unpublished data). Consequently, when the first pilchard deaths were reported off the south coast of WA in April 1995, phytoplankton samples began being collected in front of, within, around and behind the areas containing dead and dying fish. It was anticipated that if the deaths of WA pilchards were caused by a phytoplankton bloom or a significant number of a harmful phytoplankton species (as was originally found in South Australia), then the samples should show a clear pattern.

Phytoplankton have often been responsible for fish mortalities (Table 5.1) but with the deaths caused from a variety of impacts. Thus, death can be caused through alterations in the physical environment from the breakdown of phytoplankton causing anoxic conditions, but some blooms result in oxygen supersaturation. Phytoplankton can cause physical injury to fish gills from spines, or they may physically clog gills. Toxic phytoplankton can also cause fish deaths through chemical reactions.

5.1.1   Physical conditions

In WA estuaries and rivers, it is not unusual for anoxic bottom water layers to develop annually over kilometres of an estuary without any fish being affected. Wild fish are apparently able to avoid these deeper anoxic areas.

Species of otherwise harmless phytoplankton, if dense enough, can cause indiscriminate kills of fish and invertebrates due to oxygen depletion in sheltered bays (Hallegraeff, 1993). In WA, fish kills in estuaries and canals have been attributed to anoxic conditions in the water column following the rapid collapse of dense phytoplankton blooms (Hosja, unpublished data). It has been shown above that dissolved oxygen concentrations measured both at the surface and at depth at a number of sites both in and outside fish kill areas had saturation levels not less than 56%.

Frequently blooms of phytoplankton in rivers and estuaries can result in supersaturated dissolved oxygen levels around 200%. In WA waterways, fish deaths have never been associated with the presence of a supersaturated water column.

5.1.2   Phytoplankton density

Information on phytoplankton density in southern and western coastal WA waters is sparse. While dense blooms of *Trichodesmium* appear almost annually along the WA coastline (Creagh, 1985), there is no evidence of fish deaths being attributed to these in WA coastal waters.

Phytoplankton sampling in Cockburn Sound, Warnbro Sound and the Sepia Depression off the west coast in 1991–92 showed that interannual phytoplankton densities can peak in summer at between 200 000 cells/L and 775 000 cells/L with diatoms being the dominant component (Cousins, 1991). Phytoplankton densities increased from the month of May with peaks of silicoflagellates of between 120 000 cells/L and 250 000 cells/L in June to August.

Dense blooms on the surface of harmless phytoplankton species occur in many WA estuaries. No fish kills have occurred in dense chlorophyte blooms even at surface densities as high as 5×10^9 cells/L. However, in the Peel-Harvey Estuary blooms of the toxic cyanobacteria *Nodularia spumigena* at integrated densities approaching 1×10^9 cells/L have affected fish and crab distribution in these areas.

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5.1.3 Phytoplankton species

Wild finfish stocks are better equipped to avoid noxious algal blooms than caged fish (Hallegraeff, 1991) because they are usually capable of moving out of affected areas. Nonetheless, even wild fish can succumb indirectly when trapped in natural environments with anoxic water resulting from the decomposition of massive blooms of harmless species (Hallegraeff, 1991). Furthermore, non-toxic phytoplankton flagellate species have been found to clog pilchard gill spaces leading to suffocation (Jones and Rhodes, 1994).

Table 5.1   Summary of some phytoplankton species which have the potential to affect fish.

Dinoflagellates	Impact on fish	Densities cells/L
Alexandrium tamarense	fish farm kills	
A. excavatum	Salmon, Rainbow Trout	*100,000-500,000
Gymnodinium mikimotoi	fish farm kills	
Gymnodinium nagasakiense	Tilapia, Etroplus, Chanos; fish farm kills	**400,000,000
†G. breve	fish kill	
†G. galatheanum	fish kill	
†G. sanguineum	fish kill	
†Pyrodinium bahamense var. compressum	fish kill	
Cochlodinium spp.	wild fish kills	
Noctiluca scintillans	toxic to fish	
Diatoms		
Chaetoceros convolutus	fish farm kills Salmon	*7,000-18,000,000
Leptocylindrus minimus	cultured salmonids	**35,000,000
Prymnesiophyceae		
Prymnesium parvum	fish farm kills Salmon	*2,500,000
Chrysochromulina polylepis	fish farm kills	*80,000,000
Chrysochromulina leadbeateri	Salmon	*2,000,000-10,000,000
Phaeocystis pouchettii	possible effect on fish migration	
Raphidophyceae		
Heterosigma akashiwo	fish farm kills NZ Salmon	2,000,000-45,000,000
Dictyophyceae		
Dictyocha speculum	fish kills Trout	130,000-1,300,000

(from Hallegraeff, 1991; Larsen and Moestrup, 1989; *Red tide Newsletter; **Harmful Algal News; †Steidinger, 1993.)

Intense blooms of Raphidophyceae have been implicated in kills of caged fish (Hallegraeff, 1991). The effect on fish has been shown to be due to toxins and/or mechanical damage to delicate organs, such as the gills of fish (ICES, 1992). These species of phytoplankton are delicate and readily rupture on preservation, no longer resembling the live organism.

Blooms of *Heterosigma* (c. 143 x 10^6 cells/L) have occurred in WA estuaries with no observable effect on wild fish populations (Hosja and Deeley, 1994). *Heterosigma* densities of approximately 6 x 10^6 to a maximum 45 x 10^6 cells/L have been associated with deaths of caged salmon in New Zealand (MacKenzie, 1991). Mortalities of fish have been attributed to densities in excess of 10^6 cells/L.

Dense layers of benthic stages of other Raphidophyceae (cf. *Chattonella*) have also been reported covering the shallows of the Wilson Inlet, Nornalup Inlet (Bastyan, pers. comm.) and Cockburn Sound (Scrimshaw, pers. comm.) without observed effect on wild fish populations. Toyoshima *et al.* (1987) showed that gas exchange across gills of yellowtail (*Trachurus*) was inhibited when exposed to *Chattonella antiqua*.

Dense blooms of the cyanobacterium *Nodularia* (c. 500 000 - 800 000 cells/mL) affect the distribution of fish in the Peel Harvey Estuary. Dense accumulations of *Trichodesmium* are regular occurrences along the WA coastline. A significant bloom of *Trichodesmium erythraeum* was reported in the Albany region on 3 May 1995 (Albany Waterways Management Authority, unpubl. data). In India, harmful effects have been observed in sheltered bays from the anoxic conditions caused by decaying *Trichodesmium* blooms (Hallegraeff, 1991). Nonetheless, this species also appears to provide an important food source for sardines in some circumstances (Chacko, 1942; Ramamurthy, 1970 in Creagh, 1985).

The thecate dinoflagellates of the genus *Alexandrium* are PSP toxin producers which are known to affect fish. In the Faroe Islands a brown bloom (c. 10^7 cells/L) of *A. excavatum* was believed to have caused fish mortalities of rainbow trout and salmon. Their gills showed acute histopathological damage of necrosis and sloughing of the epithelial layer of secondary lamellae (Mortensen, 1985). In WA, *Alexandrium minutum* occurs, but has only been found at densities less than 1000 cells/L (Hosja and Deeley, 1994).

The naked dinoflagellate species *Gymnodinium galatheanum* is also believed to be toxic to fish. Other naked species such as *Gymnodinium mikimotoi* and *Cochlodinium* spp. are toxic to fish, producing mortalities at between 1.7×10^6 and 6.6×10^6 cells/L (Yuki and Yoshimatsu, 1987). *Noctiluca scintillans* is also toxic to fish but has yet to be reported in WA waters. The naked dinoflagellate *Gymnodinium breve* contains potent neurotoxins called brevetoxins which have been associated with widespread fish mortality in red tides (ICES, 1992). Low densities (< 1000 cells/L) of *Gymnodinium breve*-like dinoflagellates have been found in a south coast estuary. Morphologically similar species may not necessarily, however, produce toxins (Steidinger, 1993).

The naked stages of a silicoflagellate have been implicated with fish kills in Danish waters (Larsen and Moestrup, 1989). Densities of the silicoflagellate *Dictyocha speculum* of between 1.3×10^5 and 1.3×10^6 cells/L have been associated with the deaths of farmed trout.

The Haptophyceae are well known to be toxic to fish especially *Prymnesium parvum* in brackish water. Densities of 2.5×10^6 cells/L have been associated with the deaths of farmed salmon. This species occurs in the Vasse Wonnerup Estuary in south-west WA. *P. calathiferum* was associated with a fish kill in New Zealand waters in 1983 (Chang, 1985). The prymnesiophyte *Chrysochromulina polylepis* is toxic, affecting fish and a wide variety of invertebrates including polychaetes, gastropods, bivalves and echinoderms. The toxin of *Chrysochromulina polylepis* is believed to be similar to that of *Prymnesium parvum* (ICES, 1992). Cells of *Phaeocystis pouchettii*, which form mucilaginous colonies, have been reported to affect herring migration (Hallegraeff, 1991).

Blooms of the diatom *Leptocylindrus minimus* exceeding 1×10^7 cells/L have recently been associated with fish kills in Chile. The cause was attributed to mechanical damage (Clémente, 1994).

Chain-forming diatoms with barbed spines, especially *Chaetoceros convolutus* and *C. concavicorne*, are associated with deaths of fish by gill damage at densities as low as 5000 cells/L (Hallegraeff, 1991). Mechanical damage to gills leading to asphyxiation has been the attributed mechanism for deaths of some farmed fish (ICES, 1992). These barbed species are present in WA coastal waters (Hosja and Deeley, 1994).

5.2 Phytoplankton Sampling

No routine or emergency phytoplankton sampling strategy was in place when the fish kill occurred off the WA coast. The fish kill progressed quickly requiring the rapid recruitment and deployment of resources including those made available from other government departments. Initially the samples were screened qualitatively for the presence of potentially harmful phytoplankton species. This was later changed to quantification of all cells present to clarify the situation.

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5.2.1 Methods

Samples of water for phytoplankton examination were collected by three main methods: surface samples, composite samples using water bottles, and integrated net samples using a 10 μm phytoplankton net. Different methods were used at the south and west coasts sites (Table 5.2). Shortfalls and limitations of methods used are recognised and recommendations for future sampling methods have been made.

<u>South coast</u>

Samples of surface water were collected from the Esperance area on 24 April, 26 April and 3 May 1995 and between Fremantle and the Albany area on 5 May and 7 May 1995. The phytoplankton cells in the samples were concentrated by passing 30 litres of the water through a 25 μm mesh.

<u>West coast</u>

Composite samples from water collected by bottle samplers from three depths at two sites were collected in the Garden Island area on 17 May 1995; five sites were sampled in the Mandurah region on 18 and 19 May and two sites in the Fremantle region on 19 May 1995. These were all before the fish kill. Samples were also collected at the same time at some sites using the vertical-haul 10 μm phytoplankton net from a depth of 10 m. This enabled a comparison of the two methods to be made.

Integrated 10 μm mesh net hauls from 10 m were collected from the Dunsborough region on 18 May 1995, before the passage of the fish death front. Net samples were also taken in the Bunbury region on 27 May 1995, the Fremantle/Mandurah regions on 29 and 30 May and the Dongara region on 13 and 14 June, which were all in or near the region where dead and dying pilchards were located.

The preserved phytoplankton samples were examined using an Olympus BH-2 microscope. Cell enumerations were carried out on a Sedgewick Rafter counting chamber (1 mL volume).

Table 5.2 Summary of methods used and dates of sampling to obtain phytoplankton samples during pilchard mortality.

Site	Surface	Bottle	Net
Esperance	26/4–3/5		
Albany/Sth Coast	5/5–7/5		
Fremantle/Garden Is.	5/5	17/5–19/5	29/5–30/5
Mandurah	5/5	18/5–19/5	18/5–19/5 29/5–30/5
Bunbury/Duns.	5/5		18/5 27/5
Dongara			14/6–15/6

5.3 Results Phytoplankton

5.3.1 Methods

For many harmful species it is preferable to use live samples for microscopic examination because flagellate morphological information required for identification is either destroyed or masked with many preservatives (ICES, 1992).

It was apparent that some of the sampling techniques employed had limitations. The use of a 25 μm mesh filter in the early sampling may have missed smaller phytoplankton. This could include fragile or small species such as *Chrysochromulina polylepis*, *Heterosigma akashiwo* and *Aureococcus anophageffferens* (ICES, 1992). Similarly, sampling of surface water meant that phytoplankton species present at greater depth may have been missed.

Vertical net hauls with a plankton net (20-30 μm mesh) provide a non-quantitative method for detecting some species present at low densities (ICES, 1992). The only phytoplankton net available for

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vertical net-haul sampling in the fish kill area was a small 10 μm mesh net. The most significant problem was that the use of integrated (10 μm) net vertical-haul samples resulted in an underestimation of phytoplankton density. A comparison of samples collected from three depths by the bottle method and the 10 μm mesh vertical net haul from the Mandurah area revealed a huge discrepancy in estimated phytoplankton cell densities by the latter method (Table 5,3).

Table 5.3 The comparison of density and percentage of total cells estimated by the bottle at 3 depths and the 10 μm vertical net-haul method in the Mandurah area.

Site number	Integrated bottle density over 3 depths (cells/L)	Integrated net-haul density (cells/L) with percentage [%] of integrated bottle density	Maximum density (cells/L) and percentage [%] of integrated bottle density
1	5,875	225 [3.82%]	14,736 [251%]
2	6,555	136 [2.08%]	8,941 [136%]
3	8,012	27 [0.34%]	1,801 [22%]
4	28,406	287 [1.01%]	18,793 [66%]
6	59,253	313 [0.53%]	20,541 [35%]

From the above table it can be seen that the use of the 10 μm net vertical hauls for enumeration produced a dramatic underestimation of cells. A more plausible total phytoplankton density value was achieved by assuming that the volume of water which passed through the net was minimal. Therefore, for the samples where vertical net hauls were used, phytoplankton densities were based on the maximum possible densities to avoid underestimation of cells present.

5.3.2 Phytoplankton

5.3.2.1 Esperance area

<u>Kill area 26 April 1995</u>
Surface samples collected from within the area of dying fish on 26 April 1995 had total phytoplankton densities ranging from 1000 cells/L to 121 000 cells/L with diatoms the dominant group (Table 5.4). The density of barbed *Chaetoceros* diatoms (Figures 5.1 and 5.2) present within the kill area were low with a peak density of 1480 cells/L (Table 5.4). Mucilaginous diatoms (*Thalassiosira* spp.) (Figure 5.3) were moderately abundant at some sites in the kill area with a peak density of 55 831 cells/L and an average of 14 000 cells/L. *Pseudonitzschia* spp. were present at most sites with a peak surface density of approximately 3400 cells/L. Other diatoms were dominant in the kill area with a range of 515 cells/L to 64 245 cells/L. Dinoflagellates, cyanobacteria and silicoflagellates were all present at less than 1000 cells/L.

<u>Kill area (following day) 27 April 1995</u>
These samples were from the area where the pilchards were seen dying the previous day. The density of total phytoplankton was similar, ranging between 2247 cells/L and 35 965 cells/L. Diatoms were again the dominant group, consisting mainly of *Pseudonitzschia* and *Thalassiosira*. Densities of barbed *Chaetoceros* (between 14 and 76 cells/L) were lower than the previous day. There was, however, a significant increase in the density of the cyanobacterium *Trichodesmium* (Figure 5.4) compared to the previous day.

<u>Post-kill area 3 May 1995</u>
Follow-up sampling a week after the kill event in the Esperance region showed a higher and more uniform phytoplankton density across sites. The average of 62 000 cells/L was again mostly dominated by harmless mixed diatom species with low densities of barbed *Chaetoceros* and *Pseudonitzschia* spp. present. The density of mucilaginous diatoms *Thalassiosira* spp. was still variable and moderate.

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5.3.2.2 Fremantle to Albany

In front of kill area 5 May 1995

Samples 1–13 were collected on 5 May 1995 between Fremantle and Albany in this region ahead of the advancing front. The phytoplankton cell densities ranged between 2695 cells/L and 165 000 cells/L (Table 5.5). The average phytoplankton density for all sites was 34 960 cells/L.

A mixed composition of harmless diatom species was dominant at most sites. *Pseudonitzschia* spp. were common but only at densities up to 6820 cells/L. Site 10 was dominated by *Trichodesmium erythraeum* at a density of 148 975 cells/L.

In and around kill area 6 May 1995

On 6 May 1995 sampling was carried out in the Albany region in a number of areas, some of which contained almost no dead fish while others contained very large numbers of dead and dying fish (Table 5.5). Total phytoplankton densities were variable with no obvious trend between areas with and without dead and dying fish. Thus, the average density of phytoplankton was low and was similar in areas containing either few or many dead fish, at 7450 cells/L and 4635 cells/L respectively.

Amphidinium spp. (Figure 5.5) were detected in concentrated residue from Sample 14 from the Albany area. Multiple cells of this species occurred in a mucilage though this was rare, being sub-visible during counting techniques.

In and around kill area 7 May 1995

On 7 May 1995 the average density of phytoplankton in areas where no dead fish were detected was higher (29 050 cells/L) than in areas containing significant numbers of dead fish (10 560 cells/L). *Trichodesmium erythraeum* was present as a significant proportion of the total phytoplankton. The potentially harmful *Dinophysis fortii* (Figure 5.6) was present at low numbers at a few sites.

5.3.2.3 Garden Island area 17 May 1995

These samples were from 10 days prior to the kills beginning here, but had the second highest total phytoplankton density of 126 250 cells/L (Table 5.6). Harmless diatom species were dominant. Silicoflagellate densities were the highest found for all sites at 3788 cells/L but these were still low compared to densities found previously for Cockburn and Warnbro Sounds (Cousins, 1991).

5.3.2.4 Mandurah region 18 and 19 May 1995

Samples collected from the Mandurah region prior to the fish kills on 18 and 19 May 1995 were similar with average densities of 21 620 cells/L and 42 205 cells/L respectively (Table 5.6). Harmless diatom species were common on both days. The densities of dinoflagellates were similar over the two days with densities less than 7182 cells/L.

5.3.2.5 Fremantle region 19 May 1995

In this region before any fish deaths were observed, densities at both sites were low at between 11 360 and 20 515 cells/L. Harmless diatoms and dinoflagellate species were dominant (Table 5.6).

5.3.2.6 Dunsborough region 18 May 1995

Integrated net samples collected from four sites in the Dunsborough region 8 days before the passage of the death front had maximum cell densities of 6700 – 68 050 cells/L (Table 5.7). The species composition was variable though harmless diatoms, dinoflagellates and some cyanobacteria were common. Only the presence of *Trichodesmium* at a maximum density of 44 700 cells/L was noteworthy.

5.3.2.7 Bunbury region 27 May 1995

By using the maximum cell densities obtainable by the integrated net method of collection, densities would have been very low at between 820 cells/L and 3900 cells/L (Table 5.7). The cyanobacterium *Trichodesmium thiebautii* was present in the kill area.

5.3.2.8 Fremantle to Rottnest area 29–30 May 1995

The phytoplankton densities of the samples from 29 May (when the fish were dying) were low at between 2020 cells/L and 13 410 cells/L (Table 5.7). On 30 May, densities were similar at 19 100 cells/L. Diatoms were dominant and dinoflagellates were also significant.

5.3.2.9 Dongara area

The maximum density of these samples out of the kill area was 96 835 cells/L with an average of 26 240 cells/L. This average was similar to the maximum phytoplankton density found within the fish kill area at 25 100 cells/L (Table 5.7).

5.3.2.10 Total phytoplankton average densities all sites

There was no consistent pattern in phytoplankton densities within and outside fish kill areas (Table 5.8). Average phytoplankton densities were generally low with no substantial blooms of any species and no consistent composition amongst locations. *Thallasiosira*, which was implicated in other reports (Neira *et al.*, 1995) as a likely cause of the deaths, was only present in variable but low to moderate numbers on the south coast and was largely absent from the west coast.

Table 5.8   Summary of average total phytoplankton densities in and outside of fish kill areas.

Site	Average density all samples (cells/L)	Average density in kill (cells/L)	Average density outside kill (cells/L)
Esperance	45,189	40,102	48,386
Perth–Albany	19,327	6,740	31,734
Garden Island			126,250
Mandurah			31,913
Fremantle			15,939
Dunsborough*	24,029	9,562	38,496
Bunbury*	2,362	3,904	820
Fremantle–Rottnest*	11,906	19,126	7,093
Dongara*	29,079	25,105	31,490

* These concentrations are based on integrated net samples and therefore are the maximum possible density.

5.3.2.11 Sarcodinians

The sarcodinians and their spicules were observed in a number of samples (Figure 5.7a–d). No distribution pattern with respect to fish kills was able to be made. The highest density occurred in the Mandurah area on 18 May 1995, well before fish deaths occurred in the area.

5.4   Pilchard Feeding and Gut Contents

5.4.1 *Rationale*

One of the major reasons for the persistence of the phytoplankton hypothesis as the cause of the deaths of pilchards, given that no other species were affected, centred on a presumed difference in the feeding capabilities of adult pilchards compared to other species in the region and even compared with juvenile pilchards (< 100 mm) (Neira *et al.*, 1995). To examine this hypothesis, we conducted a qualitative investigation of the stomach contents of the dead adult pilchards and also of juvenile pilchards caught during the same time period. We also completed a quantitative investigation of the morphology of their gill rakers, which are the primary mechanism for filtering phytoplankton.

An alternative theory, that the density of lamellae on the gills of pilchards was such that only adult pilchards could be clogged by algae as had been found in New Zealand (Jones and Rhodes, 1994), was

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also investigated. Variations in the density and therefore the gaps between the secondary lamellae amongst age classes and species may have contributed to the differential susceptibility.

The hypotheses tested were:

1. Because the same effect was found everywhere, the stomach contents of all of the dead fish should contain a similar species composition, or at least one common component beyond normal levels.

2. The gill raker morphology of adults and juveniles should be vastly different such that the juveniles would not have been capable of sifting out phytoplankton even accidentally, i.e. they should have none in their stomach contents.

3. Gill filament morphology should be significantly different between age classes of pilchards such that differential lodgement of particles could occur (this should have also been obvious from histological work).

5.4.2 Methods

5.4.2.1 Gut contents

The gut contents of pilchards that were found dead at Albany, Bremer Bay, Fremantle and Bunbury were examined along with a sample of Californian pilchards obtained for comparison. The preserved gut was sliced open and agitated, and the freed contents then examined microscopically for major phytoplankton species. The material found was separated into the major groups of zooplankton, phytoplankton, spicules and unidentifiable material. Where possible, the phytoplankton material was identified to species level. The stomach contents of juveniles that were collected live from the Esperance region near the time of the mortalities were also examined.

5.4.2.2 Gill morphology

The morphometrics of the gill rakers and the gill filaments were determined for both small (filament length 59-65 mm) and large (filament length 155-165 mm) pilchards. For the large pilchards, both those collected live and dead were examined. We also examined the gill structure of the related tropical species *Sardinella lemuru*, which is coexistent with pilchards from Fremantle to Geraldton and which was not affected.

In each individual, a number of measurements and counts were made on the first branchial arch using the left side only, as was used by King and Macleod (1976), with measurements restricted to the ventral arch.

5.4.2.3 Gill rakers

(a) density of gill rakers (no./mm), assessed by counting the number of rakers within a known distance (usually 2.5 mm);
(b) length of the longest gill raker on that arch;
(c) density of the denticles, assessed by counting the number on one side of the longest raker over a known distance;
(d) the length of the longest denticle;
(e) average width of rakers, measured near the base (i.e. near the attachment to the gill arch) using an eyepiece micrometer;
(f) the average gap between gill rakers, calculated using the method modified from King and Macleod (1976) whereby the average gap = total gill arch length - ((no. of rakers-1)* raker width).

5.4.2.4 Gill filaments

(a) density of primary lamellae (no./mm);
(b) density of secondary lamellae (no./mm);
(c) length of secondary lamellae (mm).

5.4.3 Results

5.4.3.1 Gut contents

The contents of the stomachs of pilchards found dead at the four localities varied substantially with no species consistently found at all locations or even, to some extent, amongst individuals at the same location. Many of the fish examined had empty stomachs indicative that they had not fed recently prior to death, whilst others had relatively large gut contents. Thus, three of the five individuals examined from Albany were empty, as were three of five from Fremantle; two of the five were empty at Bunbury but all those from Bremer Bay had some material.

It was noted that in a majority of WA pilchards examined the stomach content volume was generally less than that of the Californian fish.

Albany
Fish from the Albany area had different volumes of stomach contents. Fish from site 16 had full stomachs with *Trichodesmium* the main food source (it should be noted that *Trichodesmium* was not present in plankton samples in the immediate vicinity indicating movement of these fish into the area). The other fish contained mainly diatoms. Fish from sample area 33 had very little material in their stomachs. Barbed setae of *Chaetoceros* diatoms were present in one stomach sample.

The fish guts from sample AK 3 contained mostly marine diatoms.

Esperance
The dead pilchards collected in the Esperance area contained many diatom species and a significant number of dinoflagellate species. Barbed setae from *Chaetoceros* were present in one sample.

Bremer Bay
The Bremer Bay fish stomachs contained no unusual phytoplankton species.

Geraldton
The Geraldton fish stomachs contained no unusual phytoplankton species.

California
The numbers of species and especially the density of phytoplankton in Californian stomachs were greater than in the WA pilchards. Many of the phytoplankton species in Californian are also present in Australian coastal waters. The Californian pilchards contained a significant number of *Dinophysis tripos* which is known to be a diarrhetic shellfish poison producer.

5.4.3.2 Gill rakers

The gill raker structure of pilchards is well developed from an early age with few obvious differences between juvenile and adult pilchards. In terms of the density of primary gill rakers, juvenile pilchards have a greater density than adults (Table 5.9), but *Sardinella* have an even higher density. The relative length of juvenile pilchard gill rakers is slightly longer than those of adults and again they are even longer in *Sardinella*. The density of the secondary projections (denticles) is also greater for juveniles but these projections lack the complex serrated nodules present on the end of adult projections. There are no such secondary projections on the rakers of *Sardinella*. The associated gap between gill rakers was similar in all groups at 0.14 mm for juvenile pilchards and 0.15 mm for adult pilchards but only 0.09 mm for *Sardinella* (Table 5.9).

5.4.3.3 Gill filaments

The density of both primary and secondary gill lamellae was greater for juveniles than adult pilchards with lower numbers on the gills of *Sardinella* (Table 5.9). The relative lengths of the lamellae were all, however, very similar.

A comparison of the secondary lamellae length on both the first and second gill arches between the dead fish and those that were alive demonstrated the gross impact of the pathogen on the gill structure.

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The length of lamellae from all parts of the gill were significantly shorter on the affected than on the non-affected fish (Table 5.9).

5.5 Discussion

5.5.1 Sampling methods

Potentially harmful phytoplankton species can be present as a discrete layer below the water surface. Even within shallow estuaries, flagellates continually vary their position in the water column during the day. Consequently, there are problems associated with most single sampling methods used to assess the presence and density of potentially harmful phytoplankton species.

The surface samples collected between Perth and Esperance in the April–May period were representative only of surface phytoplankton. The cell density data is likely to be representative only of larger phytoplankton cells > 20–25 μm because the smaller cells which are able to pass through the net mesh pores were likely to be underestimated.

Bottle sampling from three depths can miss species at discrete layers. However, the method is preferred as it increases the likelihood of detecting harmful species below the surface.

Comparisons of the integrated net sample and combined discrete depth samples from the same area showed that there was at least an order of magnitude difference between sampling methods. The integrated vertical tow (10 μm) net sample was therefore considered unsatisfactory for enumeration of cells. Nets should not be used for quantitative phytoplankton sampling because of their selective and non-predictable filtering properties (Tangen, 1978). The very low density of phytoplankton achieved by this method when assessed as the net having filtered all water from 10 m depth indicated that either the net became clogged much too rapidly, or the net needed to be raised more slowly. This could mean that phytoplankton nearer to the surface could have been excluded from the net samples. The use of the minimum volume filtered compensated for these deficiencies by calculating the maximum possible density.

In future, a combination of integrated (hose) sampling and bottle samples from depths along with 20 μm net hauls to collect moderate-sized cells would be preferred.

5.5.2 Phytoplankton total cell densities

The collection of surface samples on the south coast could mean that potentially harmful phytoplankton species deeper in the water column may have been missed. The highest surface total phytoplankton density of 165 388 cells/L was considered to be moderate. There was no bloom event associated with any of the areas in which dead pilchards were found. Inside and outside of fish kill areas, the average total phytoplankton density was variable and showed no obvious pattern.

5.5.3 Potentially harmful diatom species

Mucilaginous diatoms

Mucilaginous diatoms have been associated with clogging gills of farmed oysters. Related species have clogged fishing nets with slime (Hallegraeff, 1991). The highest surface density of mucilaginous diatoms *Thalassiosira* cf. *mala* occurred at a few sites in the Esperance area during the fish kill at 55 831 cells/L. Variations in the density of *T.* cf. *mala* in the Albany and Esperance area, however, showed no correlation with the presence of dead pilchards and this species was rare in all west coast samples, indicative that this species was not associated with the pilchard deaths in WA. Finally, integrated samples collected from the Swan River estuary at Fremantle in April 1995 showed apparently harmless integrated densities of *Thalassiosira* cf. *mala* exceeding 500 000 cells/L (Swan River Trust, unpublished data).

Chaetoceros with barbed spines

The densities of *Chaetoceros* spp. with barbed spines were generally low. No pattern of presence or absence in and out of kill areas was found. Furthermore, there was no evidence of barbed setae lodged

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in gill tissue (Section 4), but barbed spines were among the gut contents in some dead pilchards from the south coast (Figure 5.2).

The highest density of *Chaetoceros* with barbed spines found was 1600 cells/L at a site from Mandurah where no dead pilchards were present. In the kill areas which contained barbed *Chaetoceros*, the densities were < 1 cell/L. Thus, the low densities of barbed *Chaetoceros* collected indicate that this species was not implicated in the fish deaths.

Other diatoms
Marine planktonic diatoms (including *Pseudonitzschia* spp.) were generally the most dominant. The species composition was generally variable and no pattern in their presence or absence with fish deaths was found and hence they cannot be implicated.

Dinoflagellates
The total densities of dinoflagellates were less than 1000 cells/L between Esperance and Albany during the fish kill in that area. The highest total dinoflagellate density occurred in the Mandurah area at 7100 cells/L before the kills occurred. No bloom was associated with kill areas. Thus, dinoflagellates are not implicated in the fish deaths.

Potentially harmful dinoflagellates
There was no confirmed presence of *Alexandrium* in any of the samples (i.e. < 1 cell/L). Most of the thecate cells present were represented by *Protoperidinium* spp., *Dinophysis* spp., *Prorocentrum* and *Ceratium*, none of which are known to cause problems.

Naked dinoflagellates
Some of the harmful 'naked' dinoflagellates which are responsible for fish kills are at times difficult to identify in the preserved state. The potentially toxic dinoflagellate *Gymnodinium* cf. *breve* was identified in one surface sample from the area in front of the fish kill area at Albany at a very low density (< 1 cell/L). A few other Gymnodinoid species were detected in samples at low densities. A number of *Amphidinium* and cf. *Cochlodinium* cells were present at low densities (< 1 cell/L). The rarity of these potentially harmful naked dinoflagellates in the samples indicates that they were not involved in the fish kill.

Cyanobacteria
Trichodesmium erythraeum (dominant species) and *T. thiebautii* were present in many of the samples. *Trichodesmium* was dominant at two sites with a maximum density of 149 000 cells/L, but even this could not be considered a bloom. Furthermore, the species was absent from the kill area on a number of occasions.

Trichodesmium spp. have often been present as thick scums extending for kilometres without any observed effect on fish in WA waters. The species was not implicated in the fish kill.

Silicoflagellates
The silicoflagellates were more abundant in samples from the west coast. However, the maximum density of 3800 cells/L is considered low, being significantly less than a bloom observed in Cockburn Sound (Cousins, 1991). The naked stages of silicoflagellates have been associated with fish kills elsewhere. These stages are difficult to identify in the preserved state. Nonetheless, the silicoflagellates were not implicated in the fish kill.

Sarcodinians
The sarcodinians were observed in a number of samples. No distribution pattern with respect to fish kills was able to be made.

5.5.4 Gut contents
The gut content of WA pilchards was generally less than that of Californian fish. Gut volume of dead fish was variable and indicates that fish died at various stages of feeding.

The presence of potentially harmful phytoplankton species (*Pseudonitzschia, Dinophysis fortii*) and complete *Protoperidinium* dinocysts in the guts of Californian pilchards is of concern. It is conceivable that the Californian pilchard may also ingest other dinocysts from toxic species (e.g. *Alexandrium*) when

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feeding in highly productive areas. After thawing, cysts, spores or eggs of potentially harmful species may remain viable. Culture/germination experiments need to be carried out to see if the use of foreign frozen fish as bait fish poses a risk for the transfer of potentially harmful organisms into Australian waters.

5.5.5 Pilchard feeding

King and Macleod (1976) found that the species composition and abundance in the gut contents of both pilchards and anchovies reflected closely that found in the surrounding water. Thus, if there were some bloom of phytoplankton large enough to cause deaths, this would be evident in the stomach contents of the affected individuals. Some of the species of phytoplankton found by King and Macleod (1976) in the stomach content analyses included those observed in the present situation, notably *Thalassiosira* and *Chaetoceros*. Thus, these species may be present in an area and merely form part of the diet of pilchards.

The general morphology of the gill rakers of adult and juvenile pilchards was very similar. The impact of the density and length of the denticles on the effectiveness of the structure for filtering is, however, likely to be large with adult pilchards having a very effective mesh-like structure despite having larger gaps between rakers. Thus, whilst there were slight differences in the density of rakers, the effective gaps were similar to an extent that they were all capable of filtering out phytoplankton. Thus, if there was some species of phytoplankton that was in sufficient concentration to kill millions of adults, the feeding abilities of the juveniles would have been at least sufficient for a certain percentage of these to have been affected. Thus, the differential survival of adult and juvenile pilchards cannot be ascribed to differences in their potential diets.

Further evidence that the cause was not from phytoplankton blooms was the lack of any deaths being reported for coexistent and related species such as the anchovy *Engraulis australis* and, on the west coast, *Sardinella lemuru*, both of which have gill raker structures capable of filtering phytoplankton (James, 1988). Both of these species should, therefore, be just as susceptible to harmful blooms of phytoplankton as would pilchards.

5.6 Conclusions

5.6.1 Sampling phytoplankton

As more phytoplankton samples are analysed from WA coastal waters the number of new species found increases. Due to the lack of open ocean phytoplankton data in WA, there is a need for a long-term routine monitoring strategy. This would establish a database of information on potentially harmful species present and seasonal trends of phytoplankton for a number of strategic sites.

In WA, the Hazardous Algal Bloom Coordinating Committee is currently formulating a strategy for the cooperative routine monitoring and management of potentially harmful phytoplankton blooms in rivers, estuaries and wetlands that could have harmful effects on humans. The committee comprises representatives of the state Health, Environmental Protection and Fisheries Departments, Agriculture WA, the Water and Rivers Commission and the Chemistry Centre, together with experts from tertiary institutions.

A phytoplankton sampling protocol for future responses to emergencies such as mass fish kills needs to be formulated. A list of response teams along with human and logistic resources that can be readily accessed when required needs to be drawn up. These need to be dedicated with little or no disruption when an emergency arises.

The sampling strategy should employ sampling techniques which provide both quantitative and qualitative phytoplankton monitoring. The sampling techniques should facilitate the detection of potentially harmful species even at low densities.

The use of a 10 µm mesh phytoplankton net to collect potentially harmful species is to be avoided in future as it can result in a gross underestimation of phytoplankton densities if used without validation. The use of a 20–30 µm mesh phytoplankton net may be useful in concentrating larger potentially harmful species to support quantitative samples collected using sampling bottles.

5.6.2 Phytoplankton density

Phytoplankton densities did not exceed the moderate levels of 200 000 cells/L and most were much less. None of the results indicate that a phytoplankton bloom was associated with the deaths of fish. No consistent phytoplankton density distribution pattern in and out of fish kill areas was common between Esperance and Dongara.

5.6.3 Phytoplankton species

Most of the dinoflagellates found in surface samples were harmless thecate marine species. Although *Alexandrium minutum* occurs in WA waters, no toxic *Alexandrium* species were associated with the fish kill areas.

Though *Gymnodinium*, *Gyrodinium* and *Amphidinium* cells were found in low densities in concentrated samples, these were largely sub-visible in the enumeration process. The *Gymnodinium breve*-like dinoflagellate was found at one site off the south coast but it was extremely rare and sub-visible in surface samples. No cells resembling *G. mikimotoi*, which was the dinoflagellate species associated with the initial kill in South Australian waters, were identified so far in WA samples.

The mucilaginous diatom *Thalassiosira* cf. *mala* was only fairly abundant and then only in some areas. Greater densities of this species were recorded in the lower Swan River estuary without observable effects on fish or shellfish. It would be useful to compare WA *Thalassiosira* cf. *mala* density data with data from other areas of Australia.

The absence of significant densities of raphidophyte species suggests that they were not associated with the pilchard kills. These phytoplankton species, though small and easily ruptured, are still recognisable in the preserved condition.

With no evidence of gill damage by either barbed *Chaetoceros* diatom spines, sponge or sarcodinian spicules it is most unlikely that the fish had died as a result of encountering these potentially harmful agents.

Trichodesmium erythraeum has not been associated with fish kills in the other areas of WA. This species is a common bloom-forming species in WA (annually) without observed harmful effects on pilchards.

There is sufficient evidence presented here that the density and distribution of phytoplankton present in and around the fish kill areas was not the cause of, or even related to, the pilchard deaths in WA coastal waters between April and June 1995. It has, however, highlighted the need for a routine phytoplankton monitoring program in open coastal waters in WA.

5.7 Summary

The data collected in WA clearly show that the pilchard mortalities had no relationship with phytoplankton. There were no blooms of phytoplankton anywhere along the WA coast during the times when the mortalities were occurring. The composition of the phytoplankton that was present varied greatly between sites independently of whether dead and dying pilchards were in the region. Many sites off the west coast where deaths were recorded had very low densities of phytoplankton.

The stomach contents of the affected pilchards varied greatly. In addition, many had empty stomachs. The slight difference in feeding capabilities between adult and juvenile pilchards is insufficient to support the total lack of impact on juveniles. Similarly, the lack of an effect seen on the other coexistent, filter-feeding clupeoids (e.g. anchovies, *Sardinella*) is a further rejection of the hypothesis that this phenomenon was somehow caused by phytoplankton blooms.

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Table 5.4 Phytoplankton densities (cells/L) in samples from the Esperance region.

Site	Date	Depth	Fish kill status	Diatoms -others	Chaetoceros -barbed	Pseudonitzschia	Thalassiosira cf. mala	Gymnodinium	Alexandrium	Dinoflagellates - others	Trichodesmium	Blue-greens - others	Raphidophytes	Mucilaginous colonials	Silicoflagellates	Others	Spiny Sarcodina	Total phytoplankton
Esp W2	26/4/95	0m	Kill area	64247	0	281	55831	0	0	561	0	0	0	0	70	0	0	120990
Esp W6	26/4/95	0m	Kill area	11868	0	0	3062	0	0	379	0	0	0	0	0	0	32	15341
Esp W11	26/4/95	0m	Kill area	40139	1480	3396	11580	0	0	784	0	0	0	0	174	0	348	57901
Esp W16	26/4/95	0m	Kill area	515	61	106	76	8	0	98	0	0	0	0	0	152	15	1031
Esp W13	26/4/95	0m	Kill area	4419	25	455	141	25	0	25	505	0	0	0	51	0	6	5652
Esp W21	27/4/95	0m	Live fish	25730	14	0	303	25	0	455	6818	1212	0	0	0	0	5	34562
Esp W26	27/4/95	0m	Live fish	31417	76	1742	530	76	0	606	1515	0	0	0	0	0	0	35962
Esp W28	27/4/95	0m	Live fish	1566	51	581	0	0	0	25	0	0	0	0	25	0	2	2250
Esp E16	3/5/95	0m	Post kill	66206	0	19	4394	0	0	303	2273	0	0	0	152	0	0	73347
Esp E18	3/5/95	0m	Post kill	38784	20	28	8863	0	0	303	1515	0	0	0	152	0	5	49670
Esp E20	3/5/95	0m	Post kill	55676	63	240	0	0	0	455	121	0	0	0	2	0	6	56563
Esp E22	3/5/95	0m	Post kill	62570	17	14	0	0	0	530	0	0	0	0	2	0	0	63133
Esp E24	3/5/95	0m	Post kill	70220	0	227	0	0	0	606	424	0	0	0	0	0	2	71479

Table 5.5 Phytoplankton densities (cells/L) in samples taken between Fremantle and Albany before the kills, and in the Albany area during the kills.

Site	Date	Depth	Fish kill status	Diatoms -others	Chaetoceros -barbed	Pseudonitzschia	Thalassiosira cf. mala	Gymnodinium	Alexandrium	Dinoflagellates - others	Trichodesmium	Blue-greens - others	Raphidophytes	Mucilaginous colonials	Silicoflagellates	Others	Spiny Sarcodina	Total phytoplankton
Per-Alb 1	5/5/95	0m	Before	28533	84	4208	926	0	0	253	168	0	0	0	84	0	3	34256
Per-Alb 2	5/5/95	0m	Before	22989	12	6818	0	0	0	253	0	0	0	0	84	0	3	30155
Per-Alb 3	5/5/95	0m	Before	56897	168	842	0	0	0	337	0	0	0	0	0	0	2	58243
Per-Alb 4	5/5/95	0m	Before	5892	0	253	0	0	0	84	421	0	0	0	84	0	3	6733
Per-Alb 5	5/5/95	0m	Before	2188	84	0	0	0	0	421	2273	5611	0	0	84	0	0	10661
Per-Alb 6	5/5/95	0m	Before	42843	5	4882	5050	0	0	253	0	0	0	0	84	0	3	53116
Per-Alb 8	5/5/95	0m	Before	9809	57	673	421	0	0	84	0	0	0	0	0	0	2	11044
Per-Alb 9	5/5/95	0m	Before	3819	84	22	253	0	0	126	976	0	0	0	21	0	2	5301
Per-Alb 10	5/5/95	0m	Before	10773	1263	1852	2188	0	0	337	148975	0	0	0	0	0	15	165388
Per-Alb 11	5/5/95	0m	Before	6102	0	253	421	0	0	42	0	0	0	0	168	0	2	6986
Per-Alb 13	5/5/95	0m	Before	2567	2	42	42	0	0	42	0	0	0	0	0	0	2	2695
Per-Alb 14	6/5/95	0m	Few	3771	13	0	0	0	0	0	0	0	0	0	67	0	0	3851
Per-Alb 15	6/5/95	0m	Few	898	0	0	28	0	0	140	0	0	0	0	0	0	0	1066
Per-Alb 16	6/5/95	0m	Lots	253	0	28	0	0	0	0	0	0	0	0	0	0	0	281
Per-Alb 17	6/5/95	0m	Lots	2609	5	0	42	0	0	126	0	0	0	0	0	0	0	2783
Per-Alb 18	6/5/95	0m	Few	7252	13	505	5050	0	0	84	3788	0	0	0	0	0	0	16692
Per-Alb 19	6/5/95	0m	None	3271	15	168	126	0	0	42	673	0	0	0	0	0	0	4296
Per-Alb 20	6/5/95	0m	Few	7491	40	1178	0	0	0	0	0	0	0	0	0	0	0	8710
Per-Alb 21	6/5/95	0m	V Few	7743	32	84	0	0	0	0	0	0	0	0	0	0	0	7859
Per-Alb 22	6/5/95	0m	V Few	9427	19	421	0	0	0	0	0	0	0	0	0	0	2	9866
Per-Alb 23	6/5/95	0m	Lots	5811	2	561	1094	0	0	84	0	0	0	0	0	0	2	7552
Per-Alb 24	6/5/95	0m	Lots	8676	3	673	673	0	0	0	0	0	0	0	84	0	5	10110
Per-Alb 25	6/5/95	0m	V Few	3956	2	168	0	0	0	0	0	0	0	0	0	0	0	4126
Per-Alb 26	6/5/95	0m	Lots	926	3	0	0	0	0	84	0	0	0	0	0	0	2	1013
Per-Alb 27	6/5/95	0m	Lots	5833	59	0	0	0	0	16	38	0	0	0	118	0	0	6063
Per-Alb 28	7/5/95	0m	None	15234	8	1936	337	0	0	0	0	0	3367	0	84	0	3	20966
Per-Alb 30	7/5/95	0m	None	10353	5	926	505	0	0	0	6733	0	84	0	84	0	0	18690
Per-Alb 31	7/5/95	0m	None	13467	5	4545	3535	0	0	253	25671	0	0	0	0	0	2	47475
Per-Alb 33	7/5/95	0m	Lots	4250	7	126	42	0	0	253	3072	0	0	0	0	0	2	7750
Per-Alb 34	7/5/95	0m	Lots	5723	2	589	842	0	0	0	5892	0	0	0	337	0	2	13384

Table 5.6 Phytoplankton densities (cells/L) in samples taken off west coast before pilchard kills arrived.

Site	Date	Sample location	Fish kill status	Diatoms -others	Chaetoceros -barbed	Pseudonitzschia	Thalassiosira cf. mala	Gymnodinium	Alexandrium	Dinoflagellates - others	Trichodesmium	Blue-greens - others	Raphidophytes	Mucilaginous colonials	Silicoflagellates	Others	Spiny Sarcodina	Total phytoplankton
Gdn Isl	17/5/95	3 Depths	Before	1E+05	0	1263	0	0	0	2525	0	0	0	3788	0	0	3788	126250
MDH1	18/5/95	3 Depths	Before	2671	0	0	0	0	0	2671	0	0	0	534	0	0	0	5875
MDH2	18/5/95	3 Depths	Before	3642	0	0	0	0	0	728	0	0	0	0	2185	0	0	6555
MDH3	18/5/95	3 Depths	Before	3205	1602	534	0	534	0	1602	0	0	0	534	0	0	0	8012
MDH4	18/5/95	3 Depths	Before	24461	0	0	1578	0	0	0	0	0	0	789	1578	0	3156	28406
MDH6	18/5/95	3 Depths	Before	47283	0	2394	0	0	0	7182	0	0	0	599	1796	0	2394	59 253
MDH1	19/5/95	3 Depths	Before	8116	0	0	0	0	0	1353	0	0	0	451	0	0	0	9920
MDH2	19/5/95	3 Depths	Before	1435	0	0	0	0	0	1435	0	0	0	0	717	0	0	3587
MDH3	19/5/95	3 Depths	Before	49238	631	0	0	0	0	0	0	0	0	0	0	0	0	49869
MDH4	19/5/95	3 Depths	Before	88059	0	2841	947	0	0	1894	0	0	0	0	0	0	0	93741 -
MDH5	19/5/95	3 Depths	Before	44188	0	6186	884	0	0	2651	0	0	0	0	0	0	0	53909
FREM S1	19/5/95	3 Depths	Before	6944	0	0	0	0	0	3788	0	0	0	631	0	1894	0	11363
FREM S2	19/5/95	3 Depths	Before	14992	0	1578	0	0	0	3945	0	0	0	0	0	0	789	20516

Table 5.7 Phytoplankton densities (cells/L) in samples from west coast during the period when the kills were occurring.

Site	Date	Sample location	Fish kill status	Diatoms -others	Chaetoceros -barbed	Pseudonitzschia	Thalassiosira cf. mala	Gymnodinium	Alexandrium	Dinoflagellates - others	Trichodesmium	Blue-greens - others	Raphidophytes	Mucilaginous colonials	Silicoflagellates	Others	Spiny Sarcodina	Total phytoplankton
Dunsb S1	18/5/95	Max int*	Before	20366	0	0	0	0	0	993	44705	0	0	0	497	1490	0	68051
Dunsb S2	18/5/95	Max int	Before	5464	0	0	0	0	0	1490	0	0	0	0	0	1987	0	8941
Dunsb S3	18/5/95	Max int	Before	4843	0	373	0	0	0	1118	0	0	0	0	373	0	0	6706
Dunsb S4	18/5/95	Max int	Before	10431	0	993	0	0	0	0	0	0	0	0	993	0	0	12418
Bun S1	27/5/95	Max int	In kill	224	0	30	0	0	0	75	3576	0	0	0	0	0	30	3904
Bun S2	27/5/95	Max int	Near kills	447	149	0	0	0	0	224	0	0	0	0	0	0	0	820
S1 Frem-Rott	29/5/95	Max int	During	1171	0	0	0	0	0	0	0	0	0	0	0	852	0	2022
S2 Frem-Rott	29/5/95	Max int	During	8817	124	1863	0	373	0	1614	0	0	0	0	497	124	0	13411
S3 Frem-Rott	29/5/95	Max int	During	4470	115	344	0	0	0	802	0	0	0	0	115	0	115	5846
S4 Frem-Rott	30/5/95	Max int	During	16094	0	1341	0	0	0	1490	0	0	0	0	149	0	0	19074
S5 Frem-Rott	30/5/95	Max int	During	2327	0	2522	0	229	0	3783	9170	0	0	0	917	229	229	19177
Dong 1	14/6/95	Max int	None	73059	33	13660	0	0	0	4967	5116	0	0	0	0	0	75	96836
Dong 2	14/6/95	Max int	None	24367	0	1518	16557	0	0	552	0	0	0	0	0	0	0	42994
Dong 3	14/6/95	Max int	Near dead fish	21628	166	2070	414	0	0	828	0	0	0	0	0	0	33	25105
Dong 4	14/6/95	Max int	None	66	0	0	25	0	0	2732	0	0	0	0	8	0	25	2831
Dong 5	14/6/95	Max int	None	2835	99	310	0	0	0	414	911	0	0	0	138	0	33	4707
Dong 6	15/6/95	Max int	None	137	0	6	2	0	0	1	0	0	0	0	0	0	1	147
Dong 7	15/6/95	Max int	None	9107	0	414	0	0	0	0	0	0	0	0	414	0	33	9934

* Integrated net sample - maximum possible density.

Table 5.9 Comparative morphology of the gill structure of juvenile and adult pilchards and adult *Sardinella*. The two values indicate the actual measurements and a relative value based on their standard length.

	Juvenile Pilchards	Adult Pilchards	Adult *Sardinella*
Average standard length (mm)	61.0	161.0	166.0
1^0 gill raker			
Length (mm)	2.76	6.7	8.9
Length (mm in SL)	4.532×10^{-2}	4.16×10^{-2}	5.4×10^{-2}
Denticles			
Density (# per mm)	11.36	7.58	(not present)
Length (mm)	2.72×10^{-2}	5.61×10^{-2}	(not present)
Length (mm in SL)	$4.459 \times 10{-4}$	3.484×10^{-4}	(not present)
Distance between (mm)	8.803×10^{-2}	1.319×10^{-1}	(not present)
1^0 lamellae			
Density (# per mm)	9.22	3.16	2.56
Length (mm)	2.053	5.474	5.644
Length (mm in SL)	3.365×10^{-2}	3.4×10^{-2}	3.4×10^{-2}
2^0 lamellae			
Density (# per mm)	63.71	48.95	42
Length (mm)	1.471×10^{-1}	4.146×10^{-1}	3.103×10^{-1}
Length (mm in SL)	2.411×10^{-3}	2.575×10^{-3}	1.869×10^{-3}
Length of gill arch Seg. A (mm)			
total	5.027	13.85	15.60
in standard length	8.241×10^{-2}	8.6×10^{-2}	9.4×10^{-2}
Length of gill arch Seg. B (mm)			
total	3.512	6.665	10.63
in standard length	5.757×10^{-2}	4.14×10^{-2}	6.4×10^{-2}
Length of gill arch Seg. C (mm)			
total	3.694	8.082	13.28
in standard length	6.056×10^{-2}	5.02×10^{-2}	8.0×10^{-2}
Total length of gill arch			
total	12.233	29.317	39.51
in standard length	2.005×10^{-1}	1.776×10^{-1}	2.38×10^{-1}
1^0 gill raker			
Density (# per mm)	5.38 (s.d.=0.38)	3.08 (s.d.=0.05)	6.54
Distance between (mm)	1.37×10^{-1} (s.d.=1.41×10^{-2})	1.55×10^{-1} (s.d.=1.980×10^{-2})	8.63×10^{-2}
Average width	6.45×10^{-2} (s.d.=1.762×10^{-3})	1.98×10^{-1} (s.d.=1.833×10^{-2})	7.17×10^{-2}

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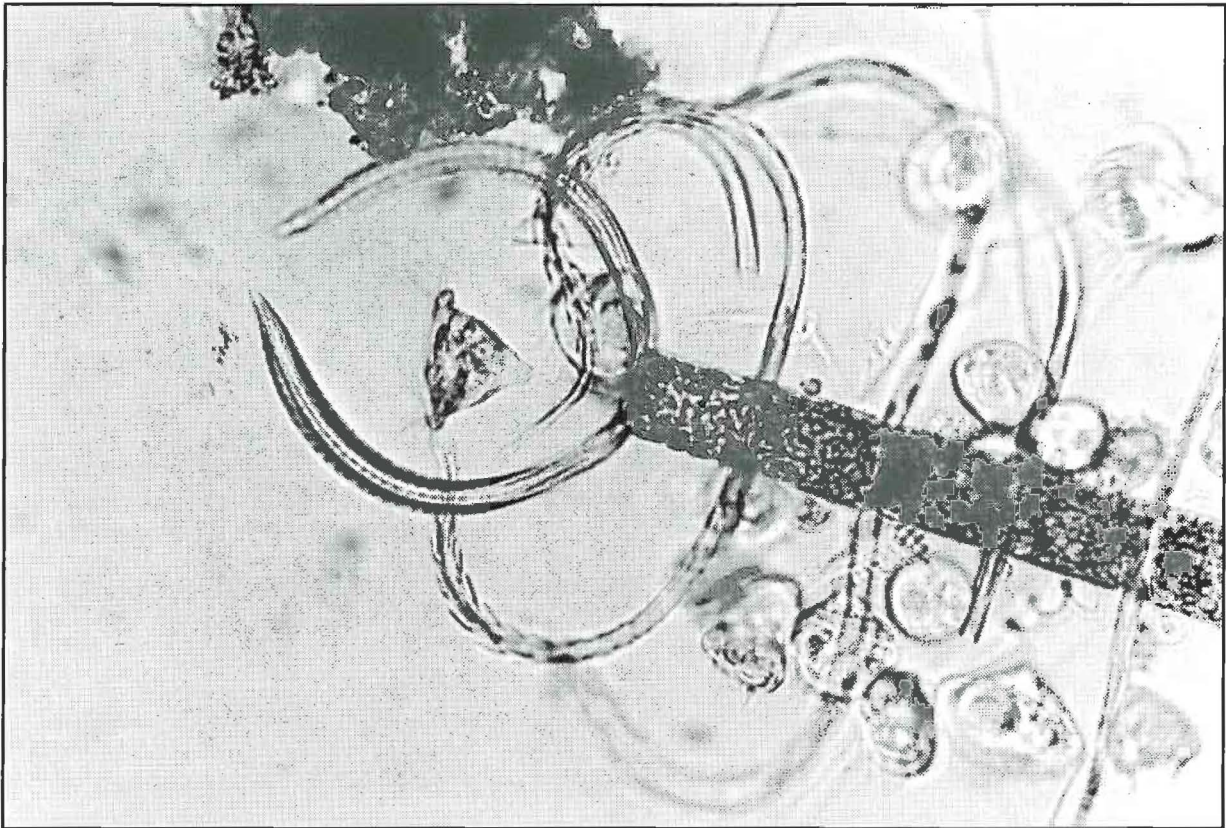
Figure 5.1 *Chaetoceros coarctatum*
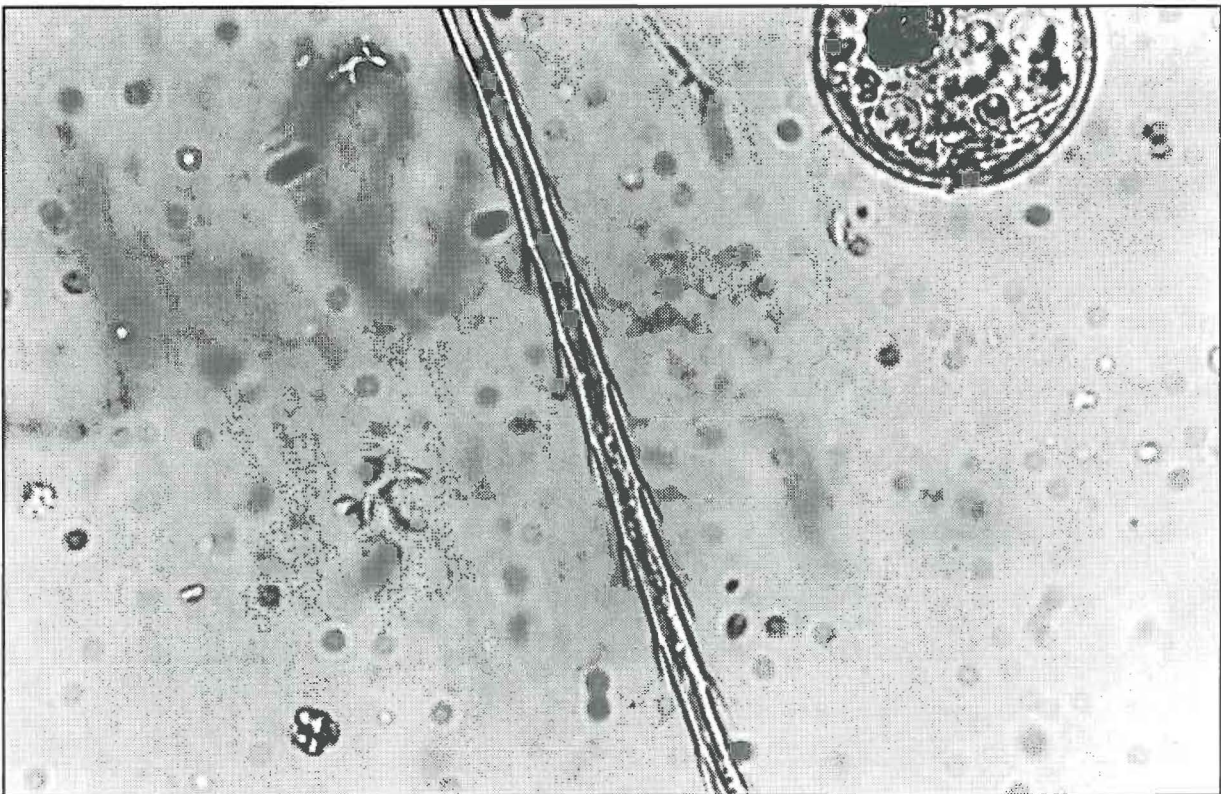
Figure 5.2 Barbed *Chaetoceros* spine.

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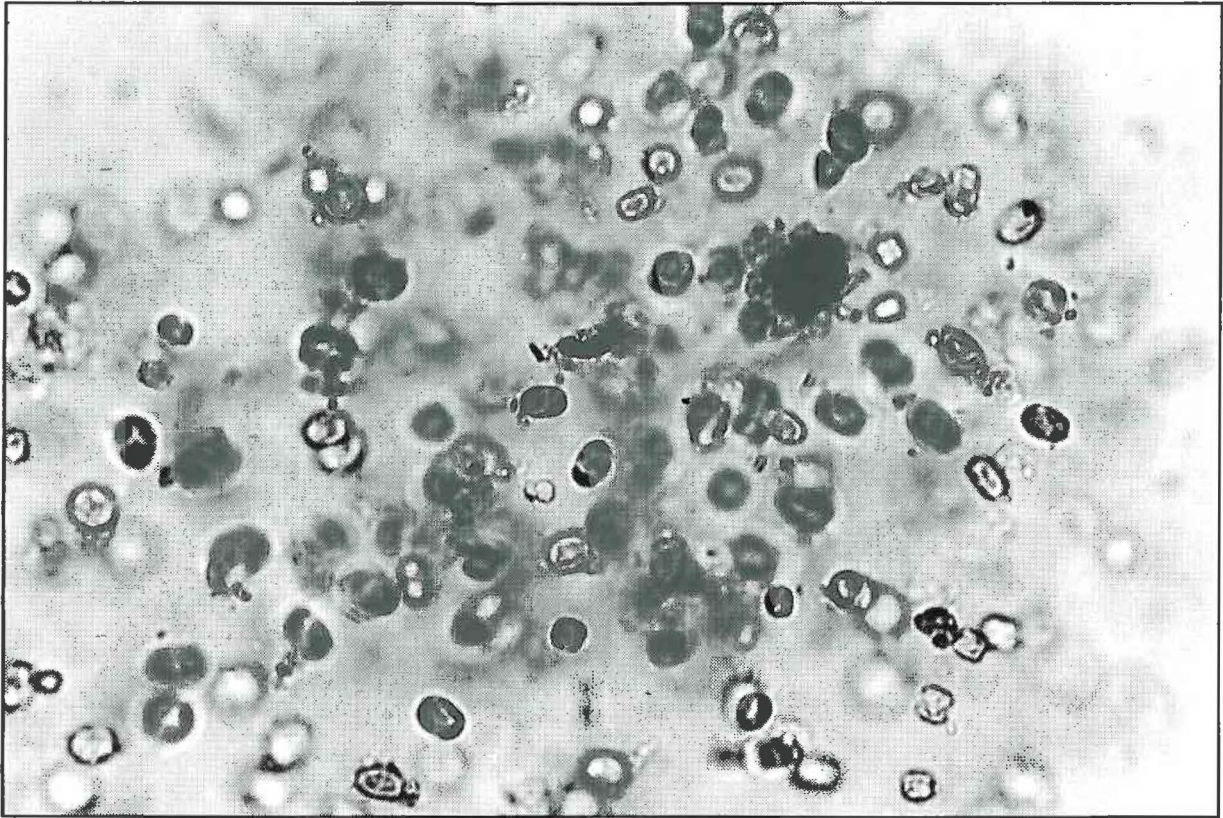
Figure 5.3   *Thallasiosira mala*
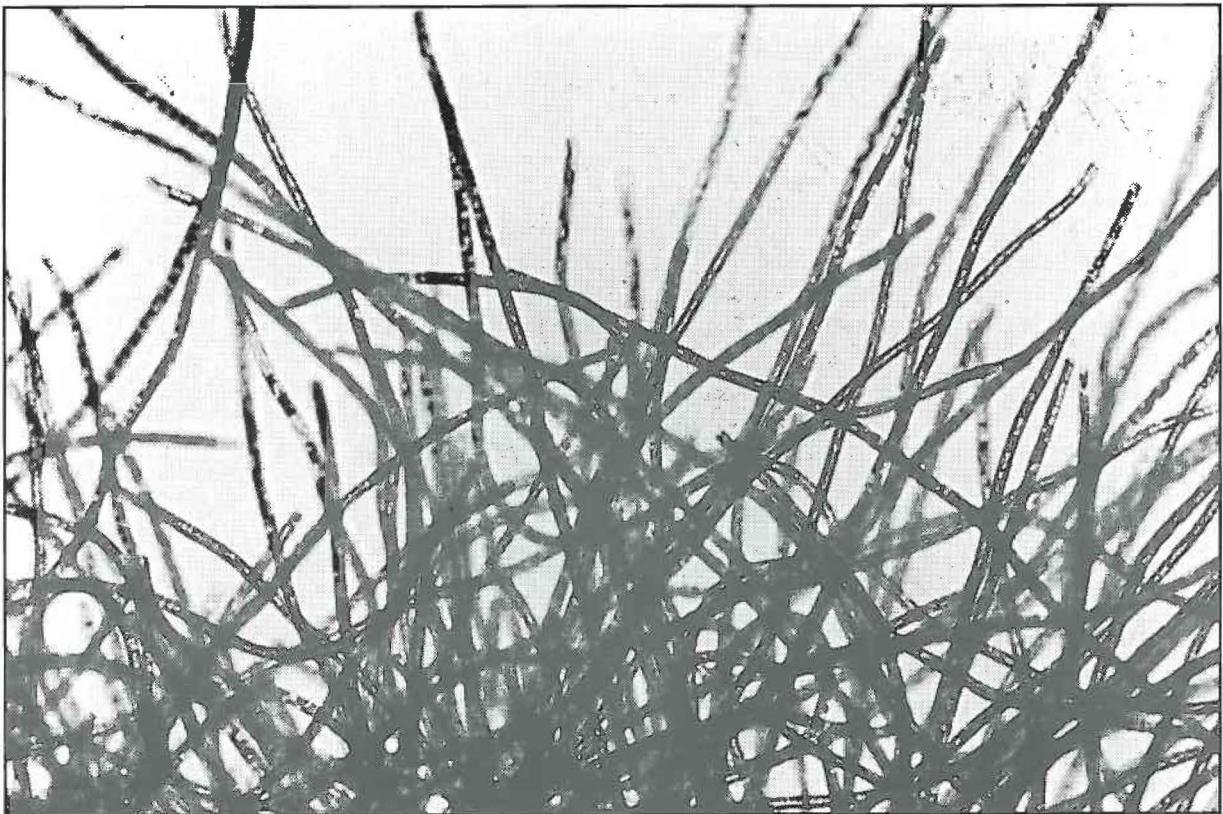
Figure 5.4   *Trichodesmium thiebautii*

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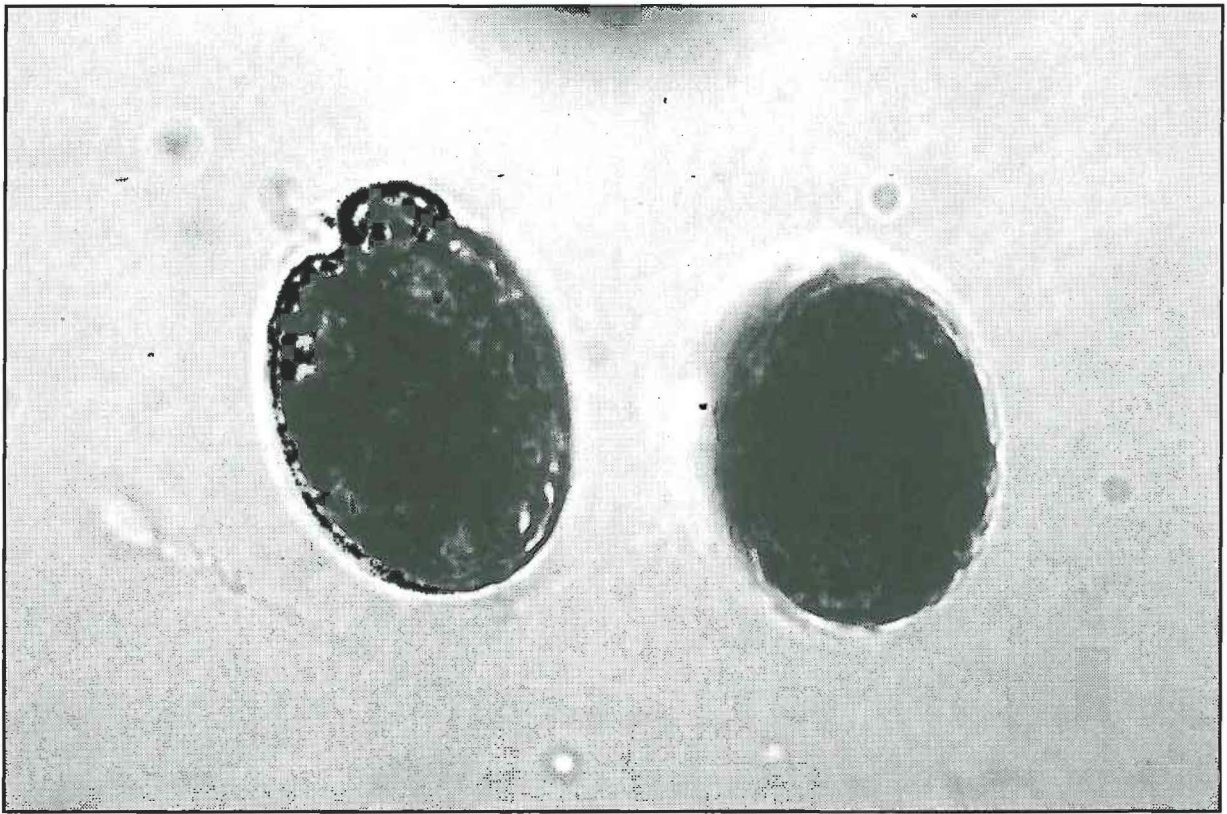
Figure 5.5 *Amphidinium* sp.

Figure 5.6 *Dinophysis* sp.

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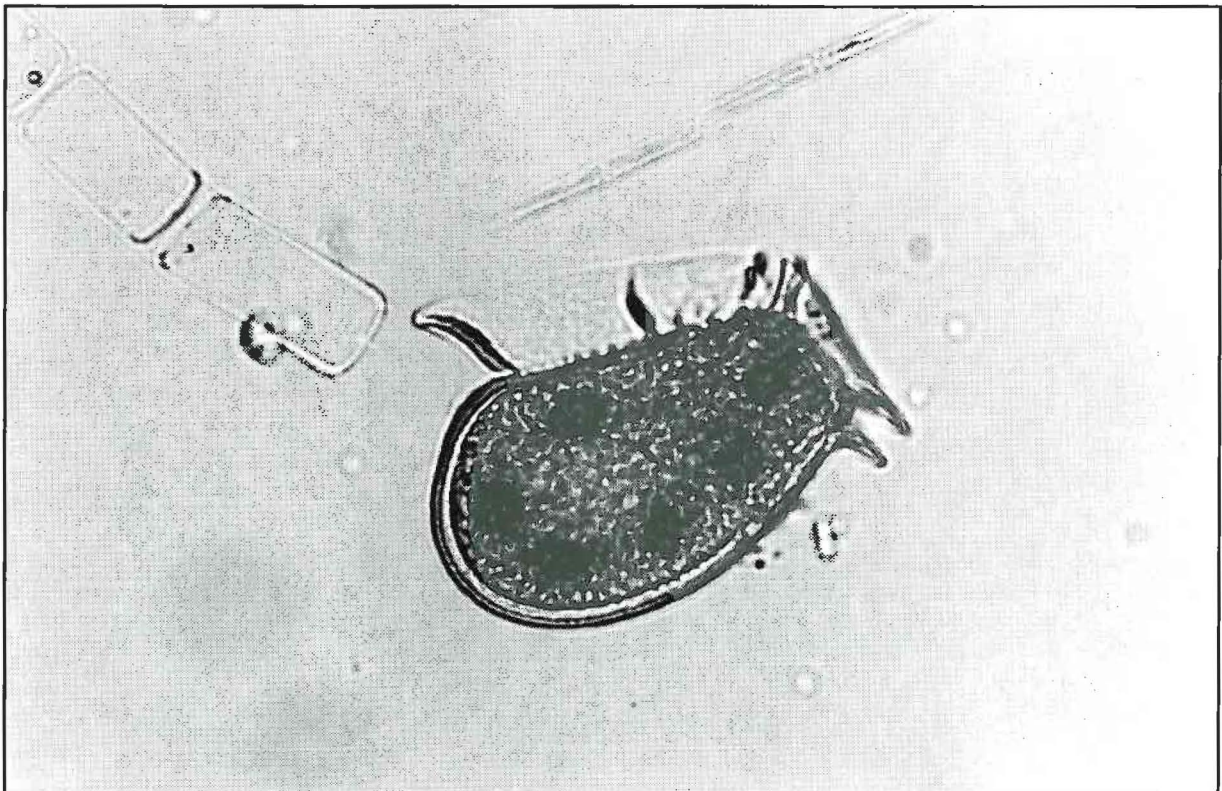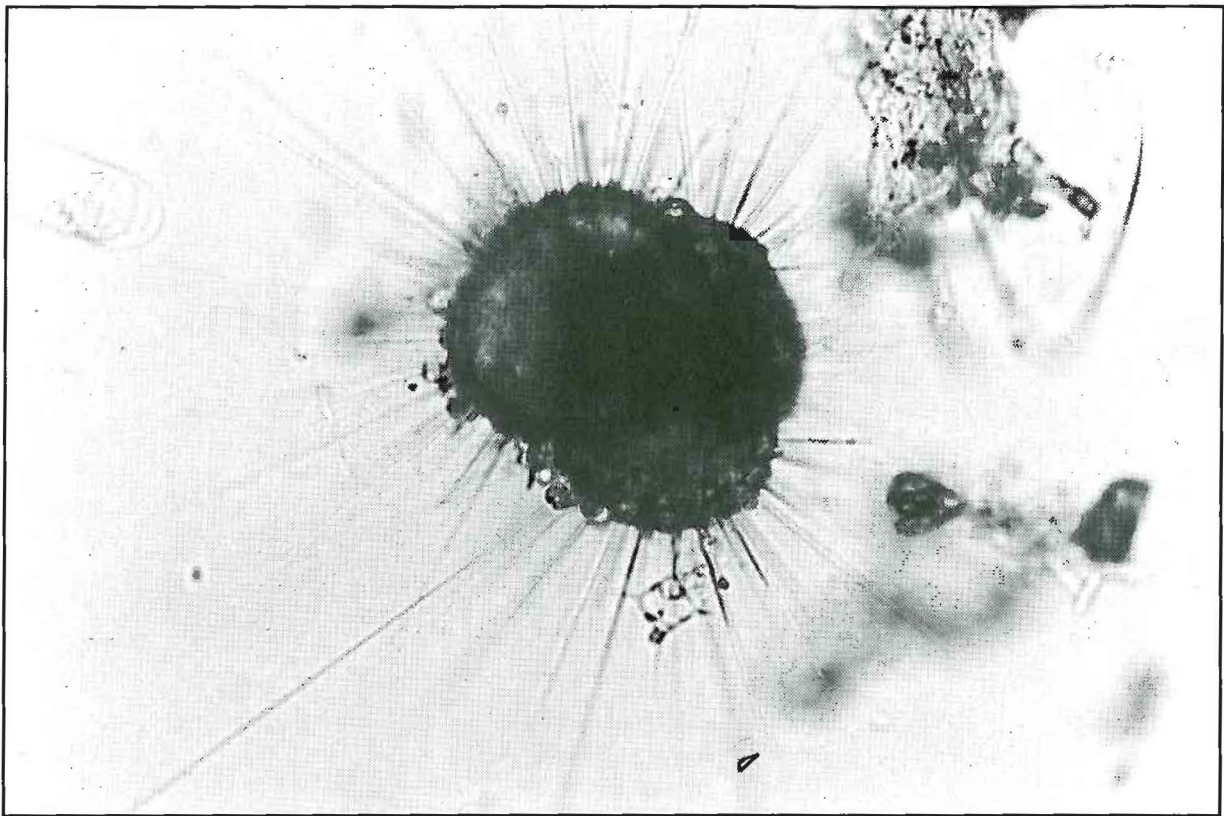
Figure 5.7(a)   *Globigerina* sp.
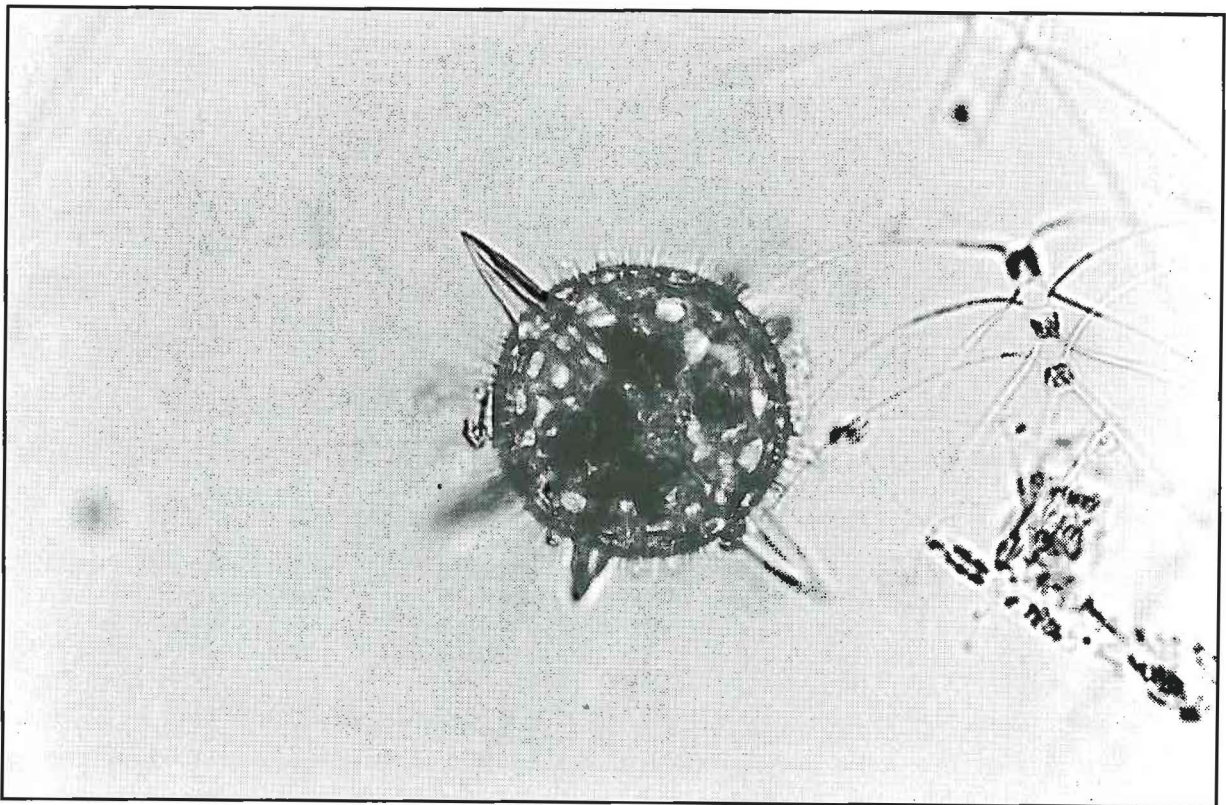
Figure 5.7(b)   cf. *Hexalonche* sp.

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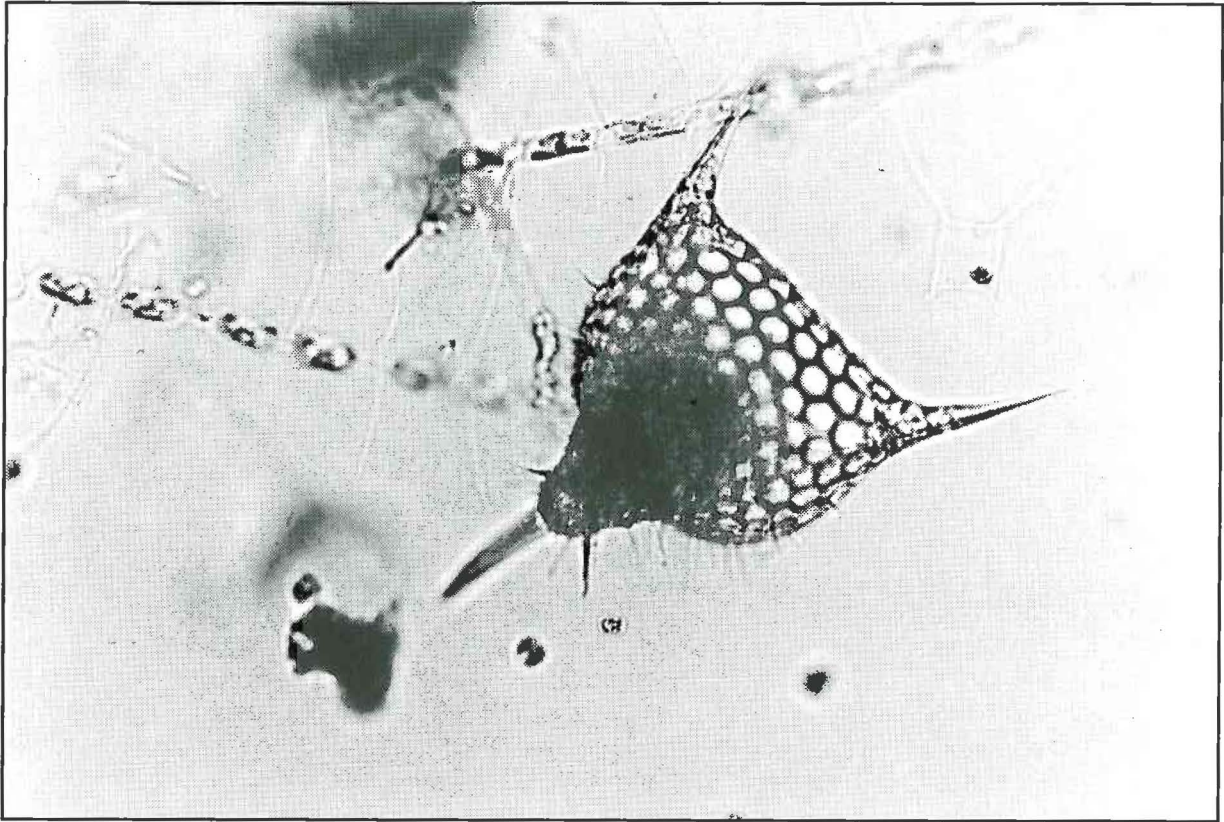
Figure 5.7(c) *Lychnocanium* sp.

Figure 5.7(d) *Sticholonche* sp.

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Section 6   Pathology and Aetiology

6.1   Pathology

6.1.1   Introduction

An essential first step in any mortality event is to have fresh samples examined by a pathologist. The condition of the tissues can provide evidence for a wide variety of problems other than those attributable to disease. Damage caused by anoxia (lack of oxygen), gill clogging by sediments and damage due to abrasive particles, toxins in the water and food, and algae, have all been well documented elsewhere (see Ribelin and Migaki, 1975).

Samples of fresh and formalin fixed pilchards were supplied to the Fish Health Laboratory as soon after the first reports of the mortality as the Fisheries Department of WA could arrange collection.

6.1.2   Methods and results

Fish were subjected to full necropsy. Fish appeared in good condition. Some had died with the mouth open and the operculae abducted. Gills of freshly dead pilchards appeared normal with no mucous apparent. No filamentous bacteria, fungi, protozoa, or metazoa were observed on the gills of affected fish from Albany or Rottnest. Gill scrapes and smears from five fish collected off Rottnest Island were specifically examined for amoebae, but none were found. Tissue samples were taken from the brain, skeletal muscle, gills, liver, spleen, kidney and liver and fixed in 10% seawater buffered formalin for histology.

Spleen liver and kidney were pooled for culture on rainbow trout gonad (RTG-2) cells using standard techniques (Hyatt *et al.*, 1997). Cultures were examined for 21 days, including passage on to fresh cell culture. No cytopathic effect (CPE) was observed.

Gills were dissected from frozen and thawed pilchards, crushed with a frozen (-20°C) sterile pestle and mortar. The gill material was added to phosphate buffered saline (pH 7.4) to make a 20% suspension. This was clarified by centrifuging at 2000 g for 15 minutes, then further clarified by centrifuging at 10 000 g for 30 minutes in a type TI 45 rotor. The clarified fraction was pelleted at 110 000 g for 2 hours in a type TI 45 rotor. 0.5 ml of resuspended pellet was floated on to a 35% sucrose gradient. Samples of the suspension layer were stained with 2% phosphotunstic acid at pH 7.0, layered on Formvar coated grids, and examined with a Philips CM10 transmission microscope.
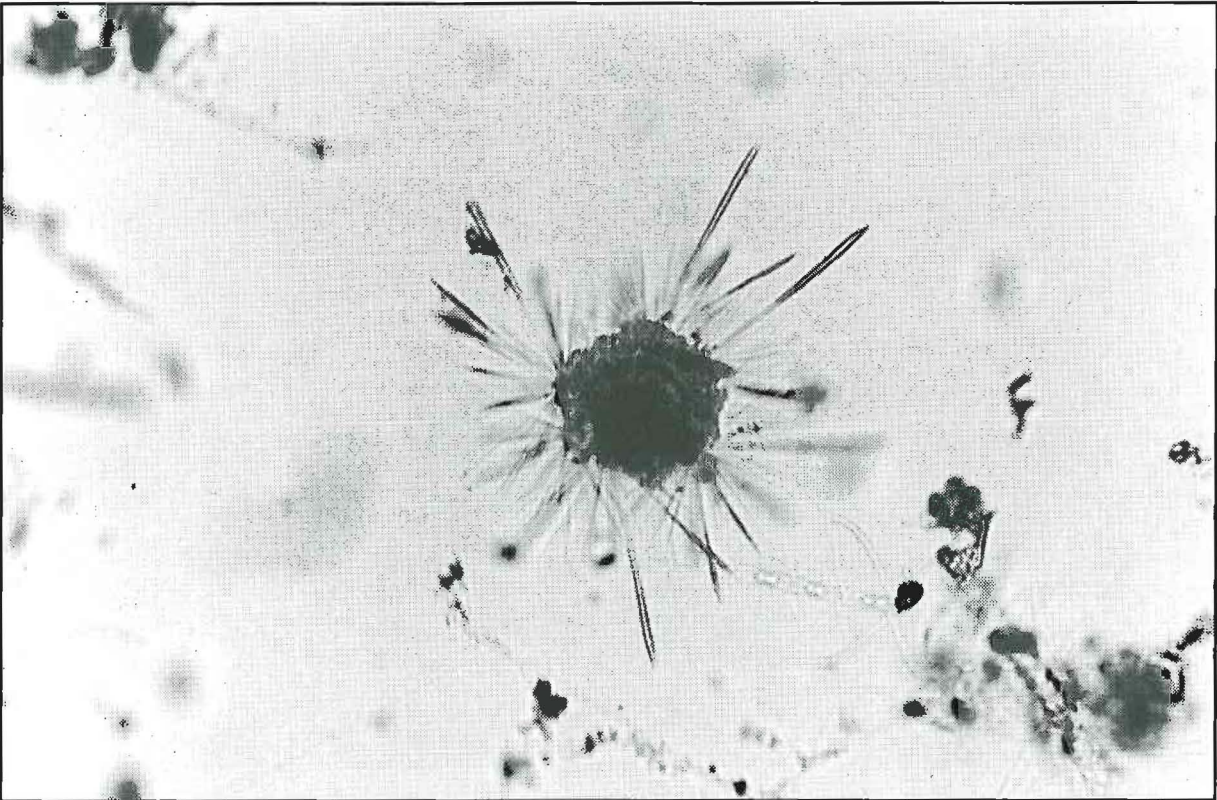
Transmission microscopy was also carried out on thin sections of 10% (v/v) seawater buffered formalin fixed gill tissue, washed (0.1M phosphate buffer, pH 7.2), postfixed with buffered 1% osmium tetroxide, dehydrated in graded alcohols and embedded in Epon. 812 epoxy resin. Thin sections were stained with 5% uranyl acetate and 5% lead citrate. Herpesvirus was purified from affected fish gills, and was also seen in the epithelial cells of affected fish (Figure 6.1, Table 6.1). Nuclei of affected cells were irregular in shape with a large ovoid granular inclusion. Electron dense bodies 50 nm dia were present along with viral capsids (approximately 100 nm dia). Enveloped virons (about 200 nm dia), capsids and nucleocapsids occurred in the cytoplasm (Figs 6.2, 6.3). Complete data are given in Hyatt *et al.* (1997).

Spleen and blood samples were also taken for bacteriology. No significant bacterial isolates were cultured on MSA using standard techniques.

Histological examination showed a variable degree of autolysis in all samples. There were no visible lesions in the brain, spleen or kidney. Spores of *Kudoa thyrsites* were occasionally seen in the musculature of some fish as previously reported by Langdon *et al.* (1992). A coccidian occurred in the liver of some fish, including frozen Californian pilchards. The oocyte diameter was approximately 20 microns and the spore size was 9-10 x 6 microns with a residuum, which is consistent with *Goussia clupearum*. A very small unidentified parasitic copepod was seen embedded in the base of the secondary lamellae on the gills of some fish from Bremer Bay.

The gills of normal pilchards have secondary lamellae of uniform length, straight and evenly distributed on the primary lamellae. Lamellae are covered with a single layer of squamous epithelium. Chloride cells were abundant on some primary lamellae, restricted to the bases of the interlamellar troughs. The

epithelium was without mucous cells, except at the tips of the primary lamellae in some fish (Figure 6.4a).

Fish collected immediately before the front from Albany showed various stages of infection from focal (Figure 6.4b) to extensive inflammation and epithelial cell hypertrophy (Figure 6.4c). Fish collected during the kill event had widespread hyperplasia with chloride cell lymphocytes and neutrophils in inflammatory exudates, with eosinophilic granulocytes being uncommon. The lesions were generalised in fish collected during the mortality event, the hyperplastic epithelium filling the spaces between the secondary lamellae, often leaving crypts with inflammatory exudate or amoebae within (Figure 6.4d). Amoebae were also found on the epithelium but were not numerous and were not present on all affected gills.

6.1.3 Conclusions
Gill lesions may be induced by a wide variety of environmental, nutritional or infectious causes, but the lack of mucous cell activity and any epithelial degradation or necrosis assisted in reducing the list of possible causes (Whittington et al., 1997). Amoebae were considered to be a possible candidate because of their association with epithelial hyperplasia and fusion of the secondary lamellae, but there was no mucous cell hyperplasia and amoebae were never seen in large numbers.

In addition, algal blooms (including *Thalassiosira* species blooms which were initially blamed for the deaths), microbial and metazoan pathogens were excluded as aetiological agents from their inconsistent pathology (Kent et al., 1995; Whittington et al., 1997) and by their absence from some of the areas where fish were affected.

The Herpesvirus was present in dying fish, not only in WA but also at other locations in Australia and New Zealand (Hyatt et al., 1997). It was not found ahead of the deaths, or in fish which were captured after the event. In addition, the changes in the epithelial cells, visible in the light microscope, were seen under the electron microscope to be due to the effect of the virus development.

Whittington et al. (1997) estimated that the time required for the gill lesions to develop was about four days. The focal inflammation followed by epithelial hyperplasia and hypertrophy resulted in the fish dying of a combination of asphyxia and osmotic stress (asphyxia is the result of the deprivation of oxygen causing anoxia (absence of oxygen) in tissues, not from anoxic conditions in the water).

Many Herpesviruses have been described from fish, and most are associated with epithelial proliferation (Hedrick and Sano, 1989) including branchial epithelial hyperplasia and osmoregulatory failure (Watson et al., 1995).

The focal origin of the outbreak, the spread around the coast of continental Australia and the consistent gill pathology are all consistent with the epizootic being due to an infectious agent for which the Australian pilchard population was naive. The only pathogen consistently isolated from moribund fish in Australia and New Zealand was the Herpesvirus (Hyatt et al., 1997; Whittington et al., 1997).

6.1.4 Behaviour of 'sick fish'
The movement of the epizootic from pilchard populations in cold southern waters into the warm Leeuwin Current where the infection passed to normally separate western pilchard stocks may be explained by the abnormal behaviour of sick fish. Fish suffering from infection have been shown to exhibit a 'fever response' by seeking warmer water (Reynolds, 1977; Covert and Reynolds, 1977; Reynolds et al., 1978; Duff and Durum, 1982). Thus it could be expected that infected pilchards would swim into the warmer inshore waters and into the Leeuwin Current (and the East Australian Current on the east coast of Australia).

6.2 Biological Characteristics of Affected Fish
6.2.1 Methods
Large samples of the dead and dying fish were obtained by Fisheries Department staff from Esperance, Bremer Bay, Albany, Bunbury and Fremantle. Smaller samples (< 10 fish) supplied by non-department staff were also obtained from Middle Island (east of Esperance), Augusta and Dongara. Samples of fish collected from normal fishing operations in the time preceding and subsequent to the kills were also

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examined from each of the areas. Unfortunately no information is available for the Esperance samples as these were misplaced.

All fish collected were measured (fork length), weighed and most had their gonads weighed, sex determined and otoliths removed. Only the large samples are reported here.

6.2.2 Results

The range in lengths for pilchards killed in WA was from 130 to 190 mm fork length (22-68 g). The range in otolith weights was from 0.96 to 2.75 mg, which equates to a range in ages between 2 and 9 (Fletcher, 1995). None of the individuals examined was virgin (gonad stage 1); all had some level of gonad development to indicate that they had achieved sexual maturity. The gonad stages were, however, very different between individuals and between sites (Figure 6.5).

A summary of the biological information that was able to be collected is located in Table 6.2. This indicates that the mean values for the affected fish were not significantly different from the fish caught immediately prior to the deaths occurring and were generally not different from those caught in the ensuing months. The GSI values, whilst different between sites, were also identical between classes of fish, indicating that the affected fish were not in some different spawning phase from the surrounding unaffected fish.

The only clear difference between the affected and unaffected fish was in the sex ratio. All samples collected from the commercial fishery of live fish were heavily biased towards females, whereas the numbers of males and females obtained in the mortality samples were approximately equal.

6.2.2.1 Lengths

Bremer Bay

The lengths of dead fish found at Bremer Bay were relatively large with a distribution from 145 to 190 mm (Figure 6.6) and a mean of 161 mm (Table 6.2). This was almost identical to the distribution of the fish that had already been caught by the fishery during April (145-185 mm, with a mean of 159 mm) (Table 6.2). In the months subsequent to the mortalities, the distributions of lengths became progressively smaller with mean values of 156 and 151 mm respectively (Table 6.2) suggesting some impact from the deaths. By August, however, the values were again larger at 158 mm.

Albany

The spread of lengths in Albany was smaller than at other locations which was evidenced by the smaller standard deviations (Table 6.2). The mean fork lengths before, during and after the kills were all in the vicinity of 156-160 mm with the total distribution being from 145 to 180 mm (Figure 6.7).

West coast

There were differences in the lengths of the dead individuals collected from different locations on the west coast. Those collected off Fremantle had a distribution very similar to those caught by the fishery in the previous months (April-May) being composed mainly of large individuals (Figure 6.8). Whilst the mean length of the dead fish collected at Bunbury was similar to Fremantle, the distribution of lengths were disparate, having a much larger spread with individuals. Thus individuals from 130 to 185 mm were collected, with a large component of small individuals not seen on the south coast. These small individuals, whilst not caught at Fremantle before the kills occurred, were caught by this fishery in the August period, following the kills (Figure 6.8).

6.2.2.2 Otolith weight (age)

Bremer Bay

There was a similar distribution of otolith weights for fish collected during the mortalities compared to the month before (April) and after (May) (Figure 6.9). The main peaks in otolith weight were at very similar locations in each of the independent samples and these correspond to where a number of the age classes have previously been determined to occur. Thus the peak at 155 corresponds to age 4; 170 being age 5 with the peak at 190 being age 6.

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Albany
The otolith weight distributions for fish killed during early May were almost identical to those from live fish collected in April and late May (Figure 6.10). The spread of otolith weights in all these samples was relatively small: most were in the 1.60-2.00 mg range which equates to 5 and 6 year olds. These two age classes made up the bulk of the commercial catch during the entire year (Fletcher, unpublished data).

West coast
The dead pilchards found in the Fremantle region were mostly large old individuals with otolith weights greater than 1.60 mg (Figure 6.11). These were similar to those collected before the kills occurred in April-May. The younger 2-3 year olds (otolith weights 0.96-1.40 mg) found dead off Bunbury were similar to those caught in subsequent months off Fremantle (Figure 6.11). Thus, even though there was a larger distribution of ages killed in this region, it merely reflects those available.

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Table 6.1 Results of the electron microscope examinations of pilchard gills.

Geelong AAHL SAN	Date	Perth AHL Sample	From	Type	When	Structure	EM Visual	Method	grams (see note)	ml
950278		1595	California	Frozen	before	fish	negative			
		cal	California	Frozen	?	gills	negative	NCEM	28.56	112
		1676 (2)	Esperance	Frozen	before	gills	negative	NCEM	21	84
	early 94	1703	Bremer Bay	Frozen	before	gills	negative	NCEM	13.8	54.8
	23/04/95	1670 (6)	Bremer Bay	Frozen	before	gills	negative	NCEM	19.3	77
	01/05/95	1676	Fremantle	Frozen	before	gills	negative	NCEM	22.94	92
	01/05/95	1300	Bremer Bay	dead	kill	gills	positive	NCEM		
	02/05/95	1300 (1)	Bremer Bay	dead	kill	gills	positive	EM	bits off 2 fish	
	02/05/95	1300	Bremer Bay	dead	kill	gills	positive	NCEM	not recording	
	07/05/95	1670 (2)	Bremer Bay	Frozen	after	gills	negative	NCEM	25.3	100
	07/05/95	1373	Torbay	live	during	gills	positive	NCEM		
950251	18/05/95	1485	Albany	Frozen	after	KLSG	negative			
950251	18/05/95	1485	Dunsborough	Frozen	healthy	KLSG	negative			
950290	25/05/95		Rockingham	Frozen	kill	fish	positive			
950267	25/05/95	1576	Bunbury	Frozen	kill	fish	positive			
950266	25/05/95	1576	Bunbury	Frozen	kill	fish	positive			
950277	29/05/95		Rottnest	Frozen	kill	fish	negative			
	29/05/95	1610	Rottnest	live	kill	gills	positive	NCEM	12.5	49.7
	29/05/95	1610	Rottnest	live	kill	gills	positive	EM	bits off 3 fish	
	22/06/95	1873	Fremantle	Frozen	after	gills	negative	NCEM	13.2	52

For NCEM, gills of approx. 20 fish examined, except California = 15 fish

Table 6.2 Summary of the biological characteristics of affected and unaffected pilchards.

	Length	STD (L)	Weight	STD (Wt)	Oto Wt	STD (OtoWt)	No. Fem	No. Male	GSI Fem.	STD	GSI Male	STD GM
Albany												
Kills (May)	157	6.5	36.5	4.7	172	15	42	50	1.23	0.5	1.14	0.4
April	160.1	6.1	42.6	4.8	174	20	184	65	1.47	1.1	1.54	0.52
May	161.2	6.5	42.4	5.4	176	21	248	123	1.3	0.67	1.48	0.52
June	156	6.3	35.3	4	169	21	95	55	0.8	0.45	0.97	0.42
Bremer Bay												
Kills (Apr–May)	161	9.9	42	7.3	176	24	64	74	1.27	0.65	1.34	0.53
April	159	7.8	42.9	6.2	182	24	112	110	1.26	0.58	1.41	0.43
May	156	9.4	41.1	9	170	22	170	77	1.31	0.7	1.47	0.6
June	151	10	32.7	7	158	23	165	93	0.74	0.4	0.95	0.48
Fremantle												
Kills (May)	159	10	37	7			6	11	0.24	0.1	0.49	0.2
April–May	163	6.7	46	7	186	23	75	54	0.83	0.5	0.89	0.42
August	159.9	10	44.6	8	163	48	57	43	3.92	3	1.91	0.9
Bunbury												
Kills (May)	158.8	14	37	11			19	28	0.55	0.2	0.99	0.56

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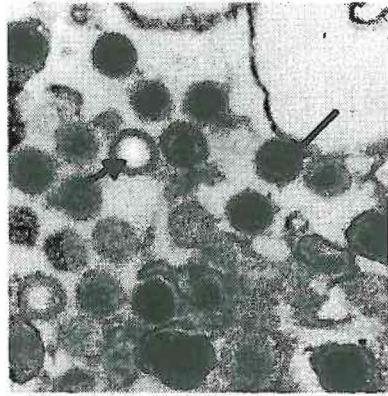
Figure 6.1   Autolytic secondary gill epithelial cells of pilchard (*Sardinops sagax*) showing virus particles (capsids and nucleocapsids) (arrow) within cell.
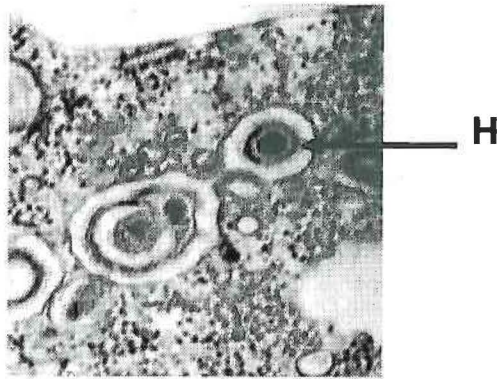
Figure 6.2   Pilchard (*Sardinops sagax*). Herpesvirus particle (H) within the cytoplasm of autolytic secondary gill epithelial cells.
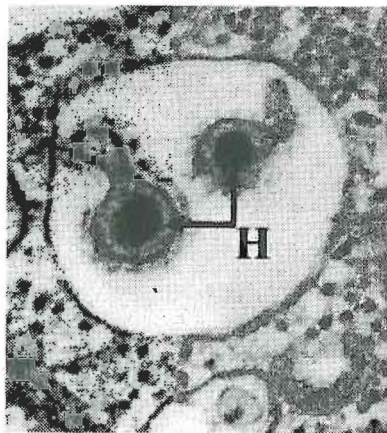
Figure 6.3   Pilchard (*Sardinops sagax*). Enveloped virions (H) within vacuoles in cytoplasm of autolytic secondary gill epithelial cells.

Figure 6.4(a) Healthy Gill

Figure 6.4(b) Focal lesions

Figure 6.4(c)
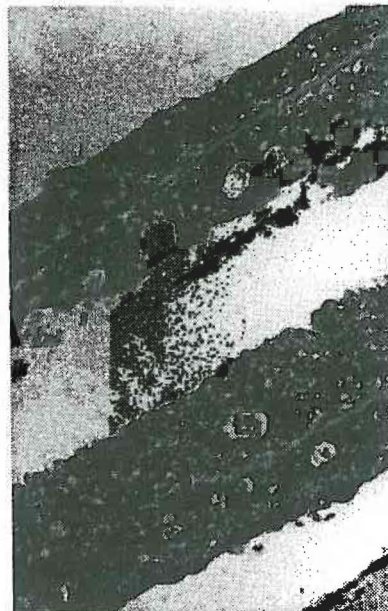
Figure 6.4(d)

Figure 6.4 Pilchard (*Sardinops sagax*) (a) Normal gill lamellae. Note the thin squamous layer of epithelial cells; (b) Focal infection of gills. Note hyperplasia of the epthelial cells of the secondary gill lamellae; (c) gill lamellae with extensive hyperplasia of the secondary lamellae, some sloughing of the epithelial cells, but secondary lamellae still recognisable; (d) secondary lamellae become fused and unrecognisable with the presence of crypts containing detached cells. Death by asphyxia occurs within a short time. Scale bar = 0.04mm.

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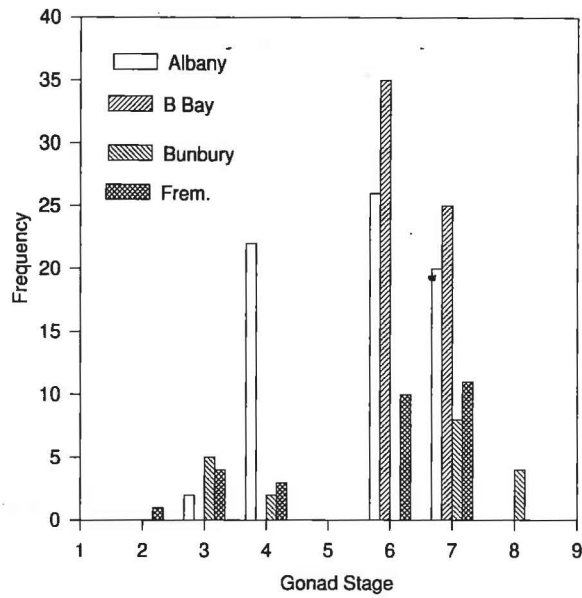
Figure 6.5   Gonad stages of the affected fish from all sites.
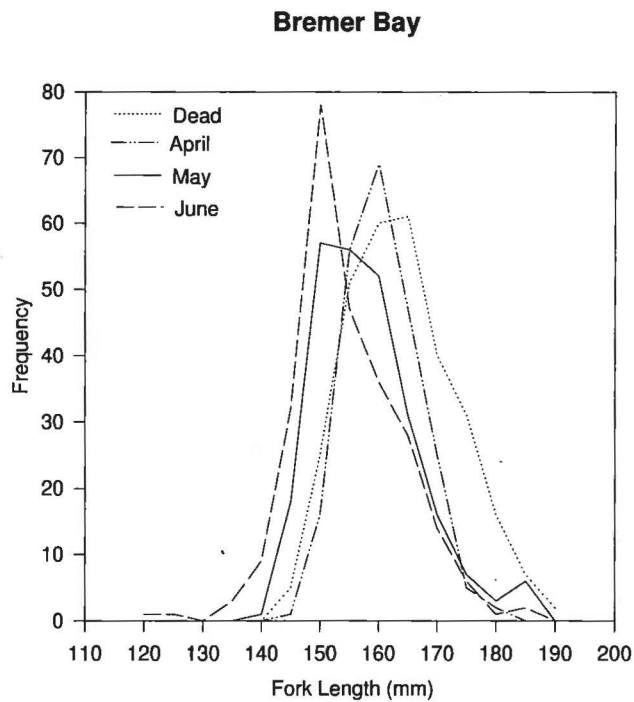
Bremer Bay

Figure 6.6   Lengths of the affected fish in comparison to unaffected fish caught in the months preceding, during and after the deaths occurred at Bremer Bay.

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Albany Region

Figure 6.7   Lengths of the affected fish in comparison to unaffected fish caught in the month preceding, during and after the deaths occurred at Albany.

West Coast Region
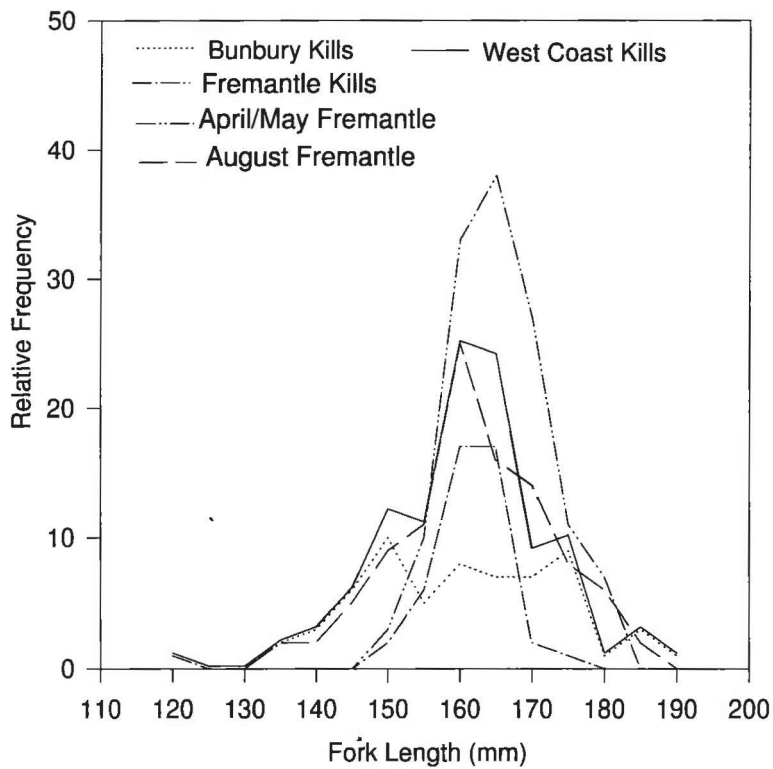
Figure 6.8   Lengths of the affected fish in comparison to unaffected fish caught in the month preceding, during and after the deaths occurred along the west coast."

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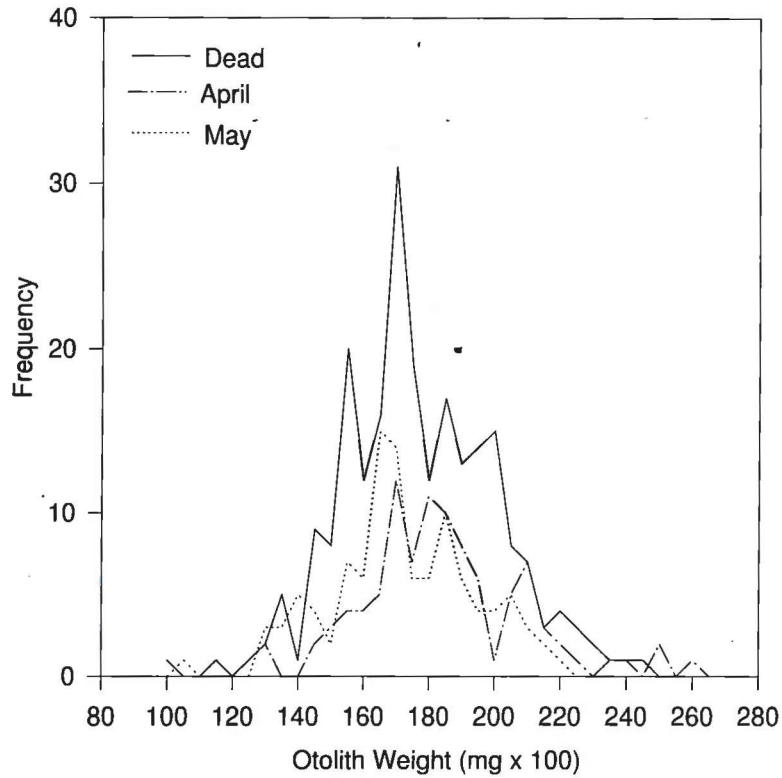
Bremer Bay

Figure 6.9   Otolith weights of the affected fish in comparison to unaffected fish caught in the months preceding, during and after the deaths occurred at Bremer Bay.

Albany

Figure 6.10   Otolith weights of the affected fish in comparison to unaffected fish caught in the months preceding, during and after the deaths occurred at Albany.

West Coast

Figure 6.11 Otolith weights of the affected fish in comparison to unaffected fish caught in the months preceding, during and after the deaths occurred along the west coast.

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Section 7   Impact on the Stock

7.1   Introduction

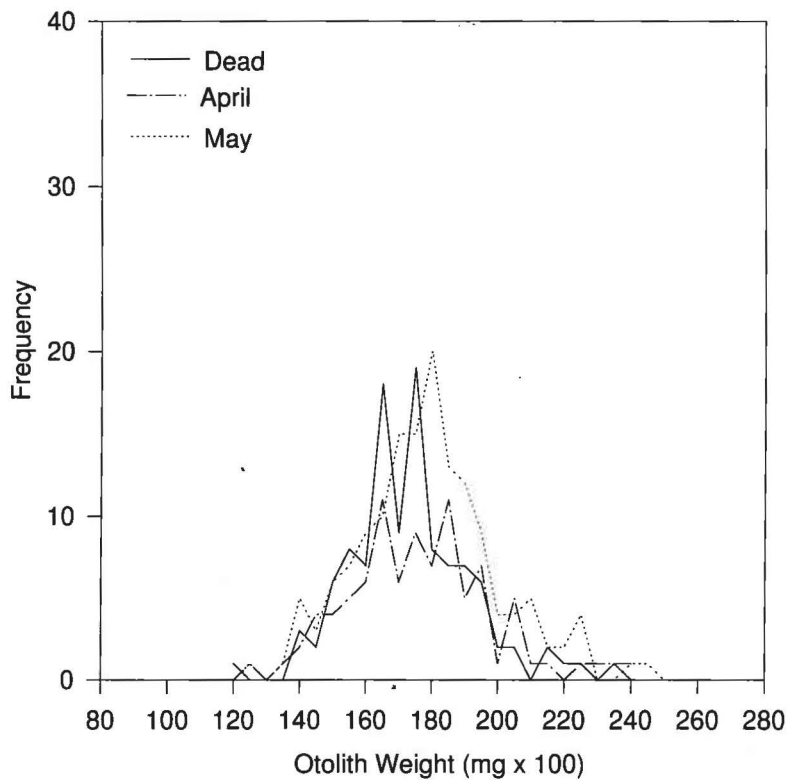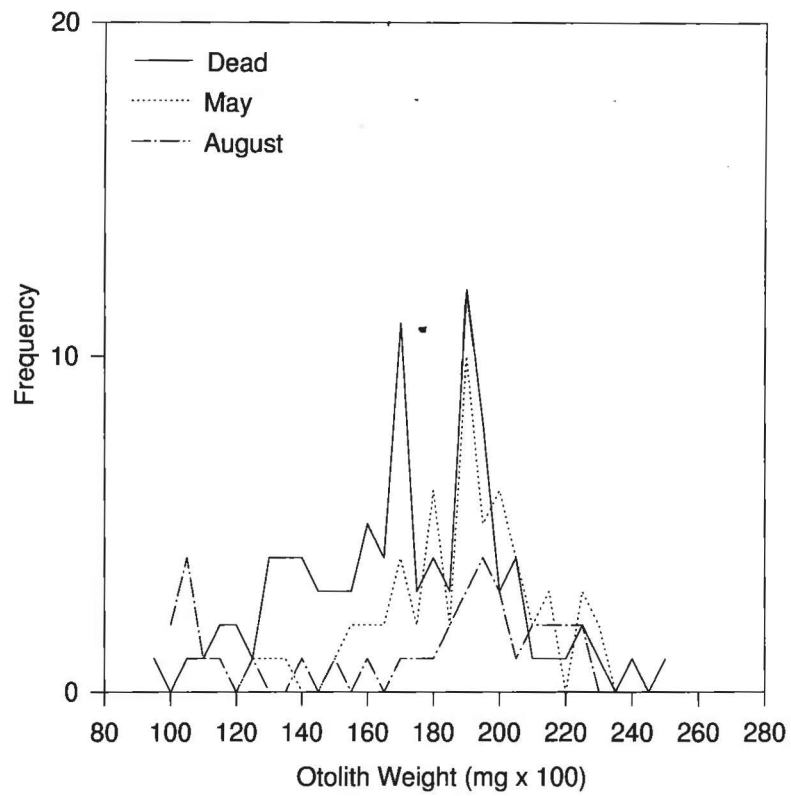Scenes with immense numbers of dead pilchards washed up on beaches and floating in offshore rafts ignited fears that the extent of the mortalities was large enough to have had a significant impact on the size of pilchard stocks in WA. There were even petitions by conservation groups for a moratorium on pilchard fishing because of concerns for the welfare of the pilchard stock and that of other species. Pilchards play a key role in the marine ecosystem of southern Australia, constituting a major dietary item for fish (e.g. Cappo, 1987), sharks (e.g. Compagno, 1984; Simpfendorfer, pers. comm.), sea mammals (e.g. Gales and Pemberton, 1990) and sea birds (e.g. Klomp and Wooller, 1988; Wooller *et al.*, 1991). It was imperative, therefore, to obtain objective estimates of the extent of the kills. This involved assessing not only the numbers of pilchards that were affected but, more importantly, what percentage of the original stock remained. If the reduction was found to be substantial (> 20%), then reductions in fishing (possibly up to a complete closure) may have been required.

In this section, we describe the surveys of dead fish from which the numbers of fish affected in WA were estimated. Plankton tows completed both during and after the kills enabled indirect assessments of the stock that remained using the daily egg production method (see Lasker, 1985).

Analyses of the collected information were made more effective because previous estimates of the stock size of pilchards at a number of the south coast regions were available (see Section 2) which enabled informed comparisons. Furthermore, the patterns for the normal distribution and abundance of pilchard eggs in WA with which to compare the results of the plankton tows completed following the kills are well known (Section 2). Finally, with pilchard fisheries located at a number of locations around the coast, examination of their catch rates during 1995 was possible in relation to the historical variations in both seasonal and yearly catch rates (e.g. Fletcher, 1991a).

7.2   Methods
7.2.1   Surface counts
Counts of dead fish encountered on the surface (Figure 7.5a) were made whilst travelling between stations. A standard search area of 4 m width on one side of the vessel was scanned with the numbers counted in this transect strip recorded at the arrival at the next station. The distances between stations were either 1 n mile (1.8 km) or 5 n miles (9 km), making a sampled area of either 7400 or 37 000 m^2. Each of these counts was subsequently converted into an estimate of the density of dead fish (no./m^2) for the area. Within some of the regions (Albany and west coast), zones were delineated (on a *post hoc* basis) based upon the variations in density observed, with counts used as a replicate within each of these zones. The total numbers of dead fish were calculated for each zone using the average density of dead fish, with the estimated tonnages determined using the average weight of dead individuals for that region (see Section 7).

7.2.2   Bottom counts
An underwater video camera was used to assess the numbers of pilchards that had sunk to the bottom. The camera was attached to a frame and towed along the bottom with the images recorded to videotape for later analysis. The distance travelled·on each transect was determined using GPS fixes at the beginning and end of each tow. To keep the camera from straying from the bottom too frequently and ensure that the images were sufficiently clear, the speed of the vessel during towing was kept to less than 0.5 kt. The field of view of the camera, at a distance of approximately 1 m in front of the lens, was determined to be approximately 1 m using the average size of pilchards seen in the videos as a scale. Individuals were only included in the count if they passed within this field of view. Using the width and length of the transects, the area sampled and consequently the density of dead pilchards (no./m^2) could be calculated.

The Albany region was divided into four sectors: inshore, inner shelf, mid shelf and outer shelf. Each tow was assigned to one of these sectors with a mean for each sector calculated. These values were scaled

up by the area they represented to obtain an estimate of the total number of dead fish for the entire region.

7.2.3 Beach counts

Counts of dead pilchards were made at a number of beaches in various locations around the WA coast. In most cases, the density of dead fish was calculated by counting the numbers along a known distance, from which numbers per linear metre were calculated.

7.2.4 Plankton sampling

Surveys utilising vertical plankton tows of the CalCOFI style CalVET net ($2 \times 0.05m^2$ opening; 300 μm mesh; see Smith *et al.*, 1985 for details) were completed in the south coast region (Esperance and Albany) during April–May 1995 which was during and just after the passage of the front in this region. Samples were also collected in the Albany region during July 1995 which was two months after the passage of the front in this region.

Sampling involved lowering the nets to within a few metres of the bottom (cod-ends first) and hauled immediately to the surface (i.e. the nets only sample on the return journey). The contents were immediately preserved in a 10% buffered formalin solution and subsequently sorted in the laboratory with all pilchard eggs and larvae counted and staged according to White and Fletcher (1997).

7.3 Results

7.3.1 Surface counts of dead fish

7.3.1.1 General

All surface counts of the dead pilchards made in WA are located in Figure 7.1. On the west coast, the recently dead pilchards were located offshore closer to the edge of the shelf with few found inshore. On the south coast, near Albany, the majority of dead and dying fish were seen floating on the surface inshore, close to the main areas where fishing occurs in Torbay and King George Sound. Further west, in the Esperance region, the dead fish were located over a much wider area, from inshore locations out to the edge of the relatively broad shelf.

7.3.1.2 Esperance

Distribution
Counts of dead fish in the Esperance area were made over a week-long period with a total of 105 sectors recorded (Figure 7.2). Counts began on 26 April. The boat travelled all day through patches of dead and dying fish (e.g. Figure 7.5a) which began only a few kilometres from the coast and were observed out to the offshore limit of the survey, 80 km from the coast. Up to 300 fish were seen floating on the surface in each of the 5 n mile transects.

The following day (27 April) the boat returned in an easterly direction, covering the same region as sampled the day before, but much lower counts were obtained (< 20 fish per transect). Furthermore, on the same day an additional transect was made 30–50 km east of Esperance in which no dead fish were seen.

Further transects were made during the next few days directly offshore and up to 200 km east of Esperance in which no further sightings of dead fish were made.

Estimates of mortality
In the region sampled on 26 April, the estimated density was 0.0027 dead fish/m^2 floating on the surface within an area of 6440 km^2. This equates to a total of 745 tonnes (Table 7.1). This was the only day that the front of dying fish was observed in the Esperance area, hence these data were assumed to be the most representative densities for the calculation of total mortality within this area.

A similar density of dead fish was assumed to have occurred over the entire Esperance region (22 000 km^2). This was assumed given the counts of dead fish on the beaches to the east of Esperance on the previous days, indicating that deaths had occurred in this region, and from our knowledge of the distribution of adults from plankton tows completed during this and previous surveys. The total

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numbers of dead fish in the Esperance region, using these criteria, was in the order of 60 million individuals which equated to 2500 tonnes (Table 7.1).

7.3.1.3 Albany

Distribution

The P.V. *Baudin* travelled from Fremantle to Albany on 5 May and during this time no deaths were observed, at least for the periods when observations were possible (i.e. daylight). Extensive surveys were completed over the next few days in the Albany region with transects covering the area out to and beyond the shelf break with 169 sectors scanned (Figure 7.3). On 6 May, the transects to the east of Albany found concentrations of dead fish inshore from Bald Head across to Bald Island. The boat travelled offshore to the edge of the shelf passing through regions where only a few dead fish were sighted. At the shelf break, however, larger numbers of dead fish were once again recorded (Figure 7.3).

On 7 May, very large concentrations of dead and dying pilchards were observed on the water surface near Torbay Head (50 km west of Albany) with some counts over the 1.8 km sector in excess of 1000. No dead fish were seen west of this region but the vessel encountered a strong, warm, south-easterly flowing current in this region. This was probably the Leeuwin Current. Whilst completing the offshore transect, few dead pilchards were detected in the mid-shelf region; many dead (but no dying) pilchards were on the surface near the shelf break, but not at densities as high as those encountered inshore (< 200 per sector). It is possible that these dead pilchards had been transported offshore by the Leeuwin Current.

Estimates of mortality

The region between Torbay and Bald Island out to a distance of 20 n miles (beyond the shelf break) was divided into six strata for the calculation of the numbers of dead pilchards. There were two narrow coastal areas, two large mid-shelf regions and two large shelf break regions (one for each survey day; Figure 7.3). When combined, a total of 39 million pilchards were calculated to have died, which is equivalent to 1400 tonnes (Table 7.1).

Given our knowledge of the distribution of this stock, the relative density of pilchards in the region surveyed (Torbay – Bald Island) is usually the largest (see Fletcher *et al.*, 1994). Consequently, the remainder of the stock which is located between Bald Island and Cape Riche (see Section 2), whilst having approximately the same total area as the region surveyed, was assumed to only have approximately half the density of adults and therefore only half the number of casualties. Consequently, the estimate of mortality for the entire Albany stock from surface counts was calculated as being 2150 tonnes (Table 7.1).

7.3.1.4 West coast

Distribution

Off the west coast, counts of dead fish were made offshore of Bunbury (8 sectors) and in the Fremantle-Mandurah region (80 sectors; Figure 7.4). These counts were made on 29 and 30 May, shortly after the pilchards had died. The majority of the dead fish were found well offshore (> 25 km from the coast); subsequent strong onshore winds resulted in many washing up on metropolitan beaches some three to four days later (2-4 June).

The dead fish appeared to be in 'wind rows' with densities in some areas of up to 1000 fish per 1.8 km sector. The counts of fish made off Fremantle-Mandurah found totals of 8.5 million fish in an area of 1722 km^2 which equates to 309 tonnes (Table 7.1). For Bunbury, the area sampled was only 27 km^2 in which 8500 fish were counted (Table 7.1).

To calculate an estimate for the entire lower west coast, the distance from Cape Naturaliste to Fremantle (80 n miles) was used. Because the width of the kills found off both Bunbury and Fremantle was approximately 25 n miles, this value was used for the width for the entire region. This results in an area of kills of 6900 km^2. The mean density of dead fish within this region (0.005) was calculated using a weighted average from the Bunbury and Fremantle counts based on relative size of each area sampled. These two calculations were combined to obtain an estimate of the total number of fish that died on the

west coast of 34 million fish, which equates to 1240 tonnes (Table 7.1). This does not include dead fish located north of Fremantle nor south of Cape Naturaliste, and is therefore a minimum estimate.

7.3.2 Bottom (underwater video) counts

A total of eight underwater transects were completed in the Albany region on 10-11 May in which the numbers of dead pilchards seen on the sea bed were counted (Figure 7.5b). It should be noted that by this time the numbers of dead fish still floating on the surface were greatly reduced.

The areas sampled were located from inside King George Sound out to 20 km from the coast. A total of four hours of videotape was recorded; only pilchards were observed to be lying on the bottom, confirming that other species were not affected.

The density of pilchards found on the bottom varied widely from one area to another (Table 7.2). The counts indicated that in some inshore areas, for example off Limestone Head, the density was as great as 0.33 fish/m^2. The counts were lower in the transects completed further offshore with none found in the outermost transect, 20 km offshore. The estimates of density were classified into strata according to distance from shore. There were a number of estimates for the inshore region but only one estimate per region out to the mid-shelf region. Unfortunately no counts were made in the outer shelf region near the shelf break due to a storm that was passing through the area at the time of sampling which restricted the vessel's range. Consequently, we were unable to determine if any dead fish had sunk to the bottom in the offshore region where significant numbers had been seen on the surface a few days before.

To obtain estimates for the Albany stock, we used the average bottom density for each sector combined with the values for surface counts made at the same time (i.e. independent from those cited above). These densities were scaled up by the total area each sector represents and summed to provide an estimate for the entire Albany region. The estimates using this sampling method and assumptions suggest that at least 1600 tonnes of pilchards were killed in the Albany region (Table 7.3). It must be emphasised that the lack of offshore sampling makes this estimate a minimum.

7.3.3 Beach counts

Counts of the dead pilchards made on the beaches are located in Table 7.4. These varied greatly between sites, and between days at the same site. The highest counts were obtained at Thistle Cove near Esperance (25/m) and at Cape Riche near Albany (23/m; Figure 7.6). At these densities there would be a tonne of fish for every 1.5 km. Most beaches surveyed, however, had relatively low densities (< 5/m) with merely a scattering near the high tide line (Figure 7.6).

Given the great variation in densities between adjacent beaches (e.g. Cape Le Grande – Thistle Cove on 25 April), the difficulty in assessing beach distances, and the knowledge that the majority of fish took a number of days to arrive on the beaches and many would not have done so at all, no estimates of total deaths were attempted using this data.

7.3.4 Plankton tows

7.3.4.1 April–May (during the kills – Esperance and Albany)

Plankton tows completed in the Esperance region between 26 and 30 April collected large numbers of pilchard eggs whereas those completed in early May at Albany found very few eggs (Figure 7.7). This discrepancy in egg density is almost certainly due to the difference in timing of spawning by pilchards between the two regions as suggested by the variations in the pattern of GSI readings (see Section 2).

For the tows completed in Esperance, positive tows (those with newly spawned pilchard eggs) were widespread (Figure 7.8). Thus, there appeared to be little difference in the numbers of these day one eggs on transects completed in the areas in which the fish had just died, in comparison to those just behind the 'front' or some days following the passage of the front. As with previous surveys in this region, the distribution of eggs was widespread with no specific area of the shelf having greater concentrations than others.

The production of eggs in this survey was relatively high with an estimate of 5.1 eggs/0.05m^2 (Table 7.5). Given the area over which eggs were found (9 700 km^2; Table 7.5) this suggests that a large amount of pilchard stock remained following the mortalities. Unfortunately no 'live' adult samples were collected

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during this survey to determine spawning frequency and fecundity estimates. Nonetheless, a range of biomass estimates can be obtained by using known fecundity values and a range of spawning frequencies (15-21%) all of which puts the remaining stock size at above 20 000 tonnes (Table 7.5).

7.3.4.2　July 1995 (post kills – Albany)

Spawning activity in the Albany area was intense during the July period. Of the 120 stations, there were a large number of positive tows with most, as with previous surveys, located inshore, particularly in the region close to King George Sound (Figure 7.9).

The small CalVET nets ($0.05m^2$ net openings) which were used in this survey still caught up to 270 eggs in a single vertical haul. The calculated egg production was large at 10.5/0.05 m^2 with the area of spawning being 2930 km^2 (Table 7.5).

Combining this information with the batch fecundity data (Table 7.5) and the spawning frequency of females collected during the survey (Table 7.5), we were able to calculate an estimate of spawning biomass for this region at this time of approximately 17 100 tonnes (CV = 0.42).

7.3.5　Aerial surveys

A number of aerial surveys were made in WA. The first was completed on Tuesday 8 May at Albany; this covered the region to the west over where the concentrations had been the day previously. This flight found no dead pilchards further west of Torbay.

Flights were also made in the Cape Naturaliste and Bunbury areas on 18 and 21 May respectively, but no dead pilchards were sighted on either of these.

7.3.6　Fishery catch rates

Information on the possible impact on the stock was generated from the catch rates by the various purse seine fleets in the months prior to and subsequent to the mortality events. The catch figures were analysed by years, and within 1995, by month (Figs 7.10 and 7.11). In none of the four areas where fishing occurs for pilchards in WA was there an observable reduction in the catch rates following the mortalities.

At Albany, the yearly catch rates were good in comparison to previous years (Figure 7.10) despite the prediction made before the mortalities had occurred that this would not be a good year (based upon variations in recruitment; see Figure 2.8). When examined on a month-by-month basis, the pattern of catch rates was very similar to previous years with no decline in the period just after the deaths. At Bremer Bay and Fremantle, the catch rates for 1995 were actually higher than for the previous year. The monthly values again mirrored the general pattern seen during the past five years (Figure 7.11).

7.4　Discussion

The number and spatial coverage of the surface counts and plankton tows were large with over 400 counts and 200 tows which covered an area of more than 25 000 km^2. Whilst the techniques used to obtain data on the numbers of dead and dying pilchards were in some respects relatively simple, the similarity in the estimates of the kills between the regions and the use of a number of independent techniques substantially increases the confidence that can be placed in them.

7.4.1　Distribution

The distribution of the dead fish, particularly the location of large concentrations, was remarkably similar to the pattern of distribution usually found for pilchard eggs (see Section 2). This agreement between the usual location of adult pilchards and where the dead pilchards were observed suggests that the numbers of deaths in an area was probably directly proportional to the numbers of pilchards present. Thus, concentrations or lack of dead fish in an area were most likely to be due to differences in density of adults, not to any variations in the influence of the causative agent.

7.4.2　Counts

The confidence that can be placed in the estimate of deaths in each zone is enhanced due to the similarity between the surface and bottom counts at Albany, and also the similarity between the totals

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calculated for the three sampled locations. The information we have on the biomass in each of these areas is that there are similar tonnages, therefore similar quantities of fish should have been affected.

The general decline in the counts of dead pilchards in the same region on consecutive days probably reflects the limited time that they floated following death. Whilst tests completed by fishermen found that some of the dead fish were capable of remaining afloat for some two to three days under controlled conditions, it is likely that this is the maximum rather than the average. The numbers found on the sea bed at Albany, two days after large counts were made on the surface, supports the hypothesis that most had sunk after a day or two. What is unknown, however, is the numbers that did not float at all but sunk immediately to the bottom. Whilst videos shot in Wellington harbour by NIWA indicate that this can be substantial, observations off Albany suggest that this may not always be the case.

What is also not included in any of the estimates is some evaluation of the quantity eaten by predators. There were numerous reports of fish and sharks having gut contents filled with pilchards during this period (Simpfendorfer, pers. comm.). Thus, the estimates cited here ignored the individuals consumed prior to the counts having been made and should, therefore, be viewed as minimum estimates. The consumption of the dead pilchards may have hastened the decline in numbers observed on successive days and may have contributed to the lower estimate of mortality made from the bottom counts because they were made some three days after the majority of the fish had died.

7.4.3 Impact on the stocks
The estimates of total numbers affected in each of the zones were relatively similar at 1500-2500 tonnes with the total deaths for WA being approximately 8000 - 10 000 tonnes. With what is known about the stocks in these locations (see Section 2), this equates to a decline of about 10-15% of the spawning biomass within each region. This estimate of only a relatively minor impact on the stocks was supported by the plankton studies which both indicated that reasonable stocks of pilchards remained along the south coast subsequent to the passage of the kills. Similarly, the good catch rates of pilchards obtained by all fisheries in WA after May or June also suggests that there were reasonable stocks remaining. Finally, other indirect evidence that there has not been a major decline in pilchard biomass comes from the lack of any reported wide-scale deaths of seabirds since this time, especially of penguins, which are highly susceptible to variations in food abundance (e.g. Klomp and Wooller, 1988). Variations in pilchard biomass of 10-15% occur regularly from normal variations in recruitment levels (Caputi et al., 1996). Consequently, if this situation does not recur, then no long-term effects on the pilchard stocks are anticipated.

Table 7.1 Estimates of the tonnages of pilchards that died made from the surface counts of dead fish completed in WA. Note: values in italics were not measured but were assumed values given constraints discussed in the text.

Zone	Mean Density (per m^2)	Area of Zone (km^2)	Estimated Number of Fish (millions)	Mean Fish Weight (g)	Estimated Tonnage of Fish
Esperance					
Esperance (west)	.0027	6441	17.7	42	745
Esperance Stock	*.0027*	22046	59	42	2500
Albany					
inshore (1)	0.0660	86	5.6	36	204
midshore (1)	0.0010	1722	1.7	36	62
offshore (1)	0.0118	1033	12.2	36	440
inshore (2)	0.0769	86	6.6	36	238
midshore(2)	0.0031	1722	5.2	36	194
offshore (2)	0.0079	1033	8.1	36	296
total			39.4		1437
Albany Stock	*.0052*	11360	59.8	36	2156
West Coast					
Fremantle (1)	.018	1722	31	36	
Fremantle (2)	.0009	1722	0.31	36	
Average	.0049	1722	8.5	36	309
Bunbury	.03	0.3	.008	36	0.3
West Coast Stock	*.005*	6867	34	36	1236

Table 7.2 Underwater video counts of dead pilchards in the Albany region. Note: width of the transect was 1 m.

Site	Distance (m)	Bottom Count	Bottom Density no./m^2	Surface Count	Surface Density no./m^2
King George Sound	200	0	0	0	0
Breaksea Island	240	28	0.116	1000	1.0
Limestone Head	1200	401	0.334	21	0.004
Bald Island	1100	47	0.04	30	0.0055
Torbay	900	112	0.133	3	0.0009
3 n miles offshore of Torbay	700	129	0.19	0	0
6 n miles offshore	2800	49	0.0175	0	0
10 n miles offshore	1800	0	0	0	0

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Table 7.3 Estimates of total deaths in the Albany region based on underwater video transects. The distance used for calculating the total area for this region was between Torbay and Cape Riche – 190 km. Given the missing shelf break data these estimates should be treated as minimum values.

Site	Offshore Extent (km)	Area (mill. m^2)	Residual Surface Density (no./m^2)	Bottom Density (no./m^2) Measured off Albany	Bottom Density (no./m^2) Assumed for Region	Estimated Number of Deaths (millions)	Tonnes
Inshore	10	190	0.0104	0.2	0.15	30.5	1097
Nearshore	6	114	0	0.19	0.14	15	540
Inner Shelf	5	95	0	0.017 .	0.012	1.1	41
Mid Shelf	10	190	0	0	0	0	0
Outer Shelf	10	190	?	?	?	?	?
Total							1678*

Table 7.4 Counts of dead pilchards made on beaches in WA by Fisheries staff.

Region	Location	Date	Length of transect (m)	Number	Density per linear m
Esperance	Cape Le Grand	25/4	2000	50	0.025
	Lucky Bay	25/4	70	100	1.3
	Thistle Cove	25/4	100	2500	25
	Thistle Cove	26/4	100	750	7.5
	Duke of Orleans	30/4	100	500	5
	Qualup Beach	1/5	1000	0	0
	Duke of Orleans	2/5	1000	0	0
Bremer Bay	Bremer Beach	2/5			2
	Trigalow	2/5			0.7
	Bremer Bay	3/5			12
	Peppermint Beach	3/5			1.5
Albany	Cape Riche	3/5	100	23	.23
	Bluff Creek	3/5	100	46	.46
	Cape Riche	4/5	100	2300	23
	Cheynes Beach	4/5	100	830	8.3
	Middleton Beach	5/5	present, no counts		
	Whaling Station	5/5	present, no counts		
Metropolitan	South Beach	2/6	200	0	0.0
	Coogee Beach	2/6	400	0	0.0
	Kwinana Beach	2/6	200	0	0.0
	Rockingham	2/6	200	0	0.0
	Point Peron	2/6	30	19	0.63
	Port Beach	2/6	30	56	1.87
	Leighton	2/6	present, no counts		
	Cottesloe	2/6	100	7	0.07
	North Cottesloe	2/6	200	5	0.025
	Swanbourne	2/6	200	2	0.01

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Table 7.5 Preliminary DEPM estimates for the sampling completed in the Esperance area during April 1995 (during and immediately after the kills) and the Albany region during July 1995 (two months after the kills).

Parameter	Esperance	Albany
(A) Area of day 1 eggs (km^2)	9700	2930
(P) Egg production (eggs/0.05m^2/day)	5.1	10.5
(W) Average weight (g)	42[#]	34.6
(F) Average batch fecundity	13000[#]	11167
® Sex ratio	0.6[#]	0.6[##]
(S) Spawning fraction	(0.15 - 0.21) [#]	0.187
(B) Biomass (t)	21000-32000	17100±7000

[#] The parameter values for average weight, sex ratio and batch fecundity for Esperance were assumed from previous samples. The spawning fraction used for Esperance was a range of likely values.

[##] The actual sex ratio obtained during the survey was 0.72 which we know from seven years of regular sampling is too high, therefore the more common value of 0.6 was assumed.

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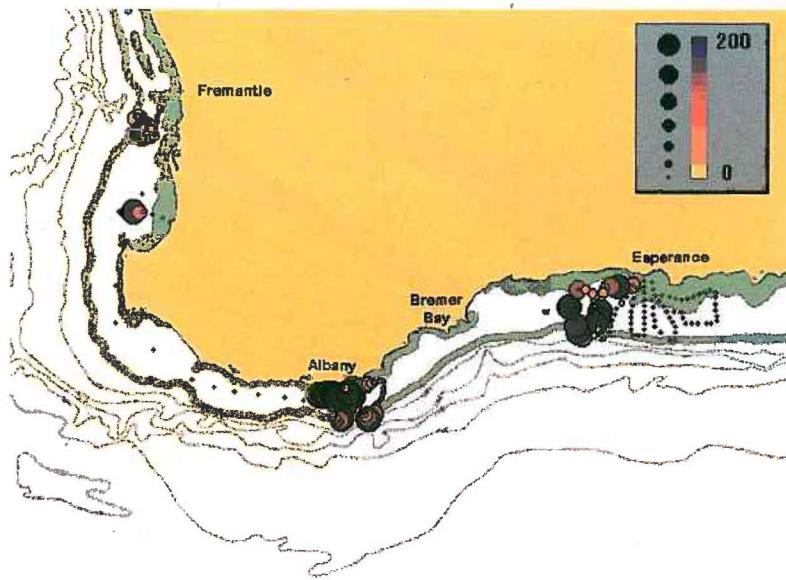
Figure 7.1   The number of dead pilchards counted on transects over the entire WA region.
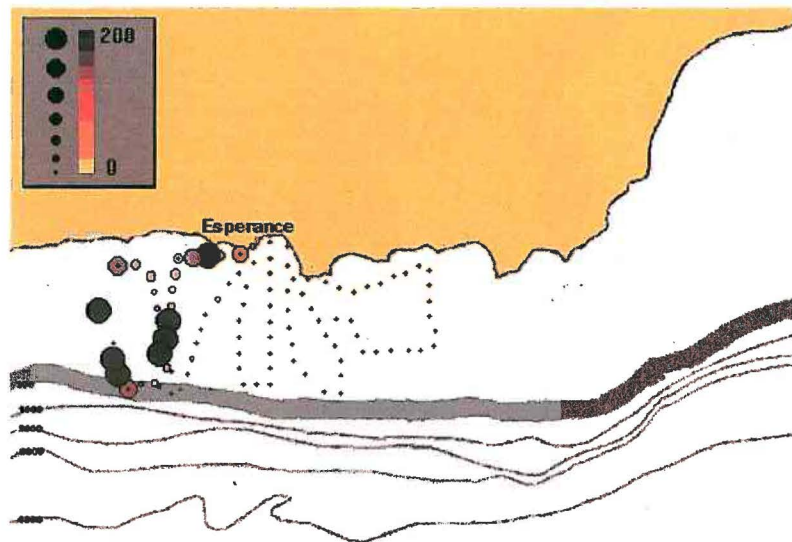
Figure 7.2 The numbers of dead pilchards counted on transect of 5 nm length in the Esperance area during the period 26th April - 3rd May, 1995.

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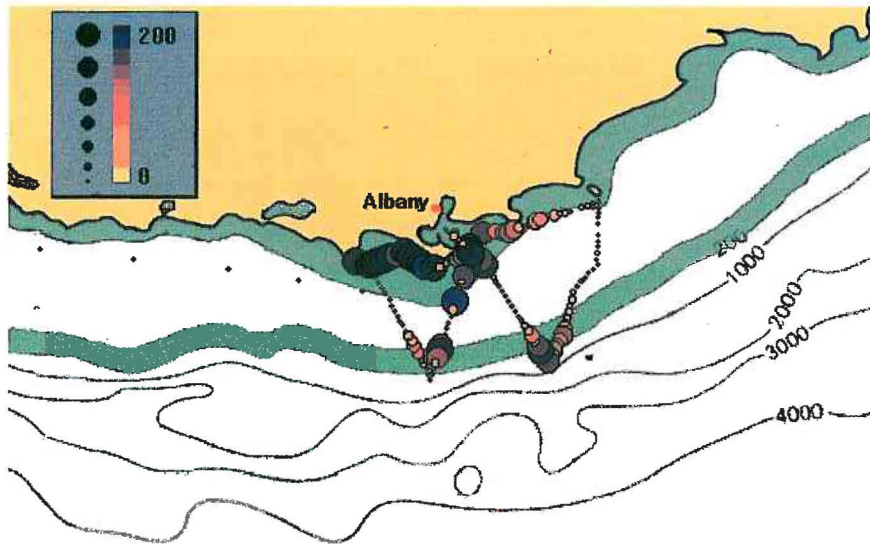
Figure 7.3 The number of dead pilchards counted on transect of 1.8 km length (1 nm) in the Albany area over the period 5 - 7 May 1995.
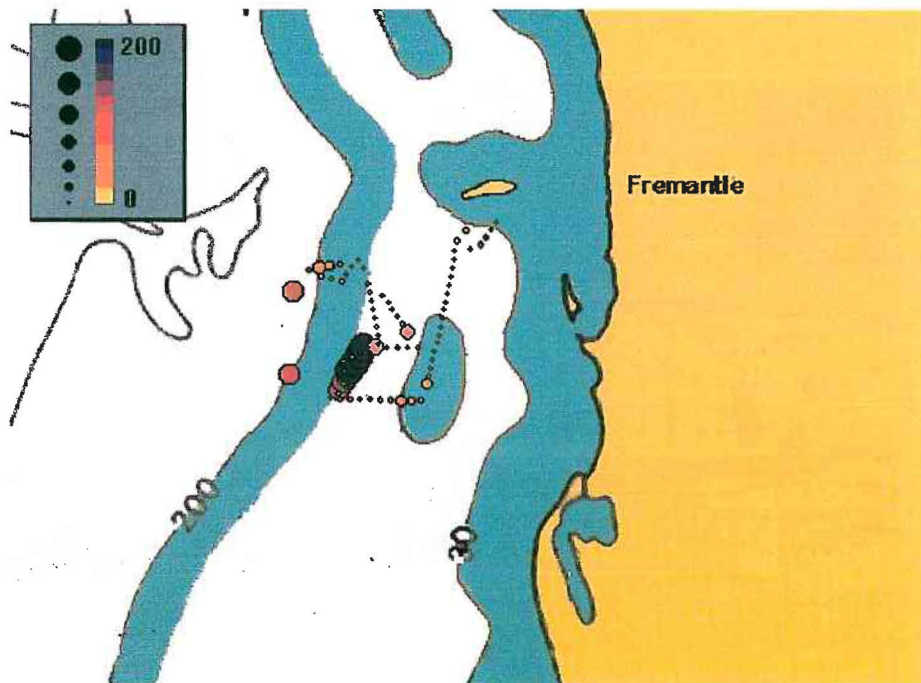
Figure 7.4 The number of dead pilchards counted on transect of 1.8 km length in the Fremantle/Mandurah area during the period 29 - 30 May.

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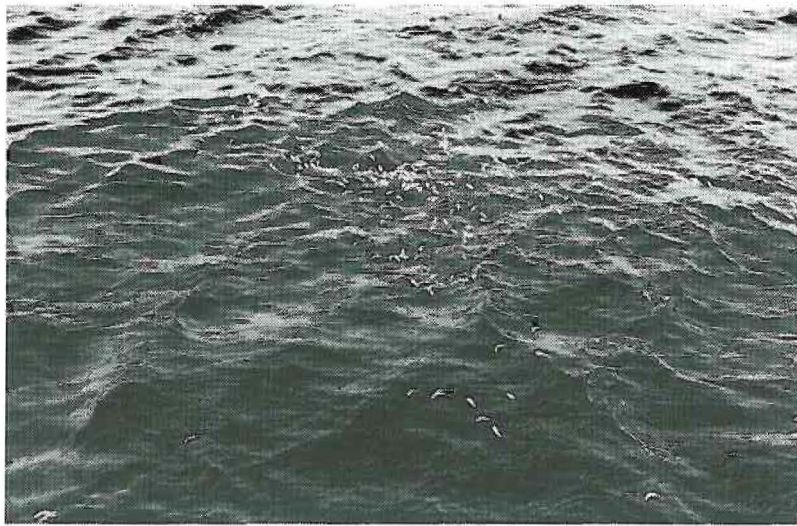
Figure 7.5(a)   Photograph of dead pilchards floating on the surface off Albany.
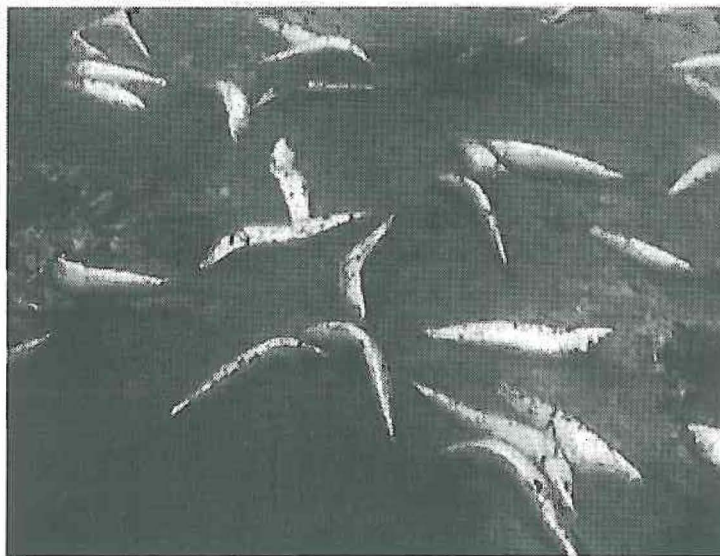
Figure 7.5(b)   Video image taken of the seabed off Albany showing the dead pilchards.

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Figure 7.6   Photographs of an area of beach with dead pilchards

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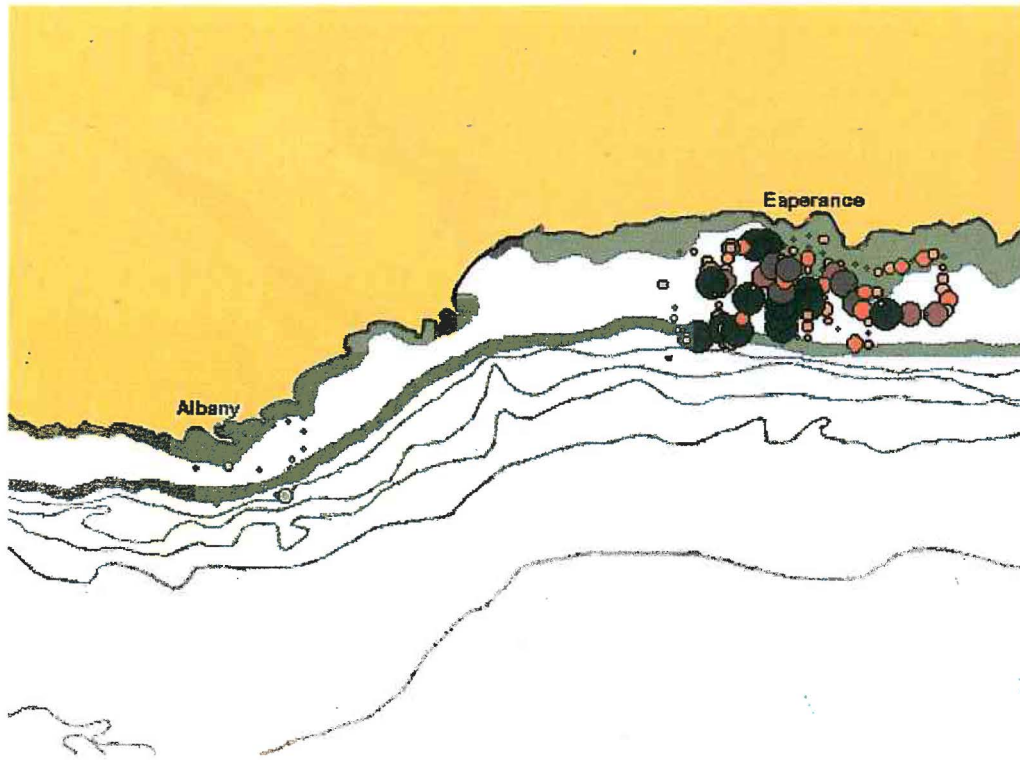
Figure 7.7 Relative density of all pilchard eggs taken in plankton tows completed in the April/May period of 1995 off Esperance and Albany.
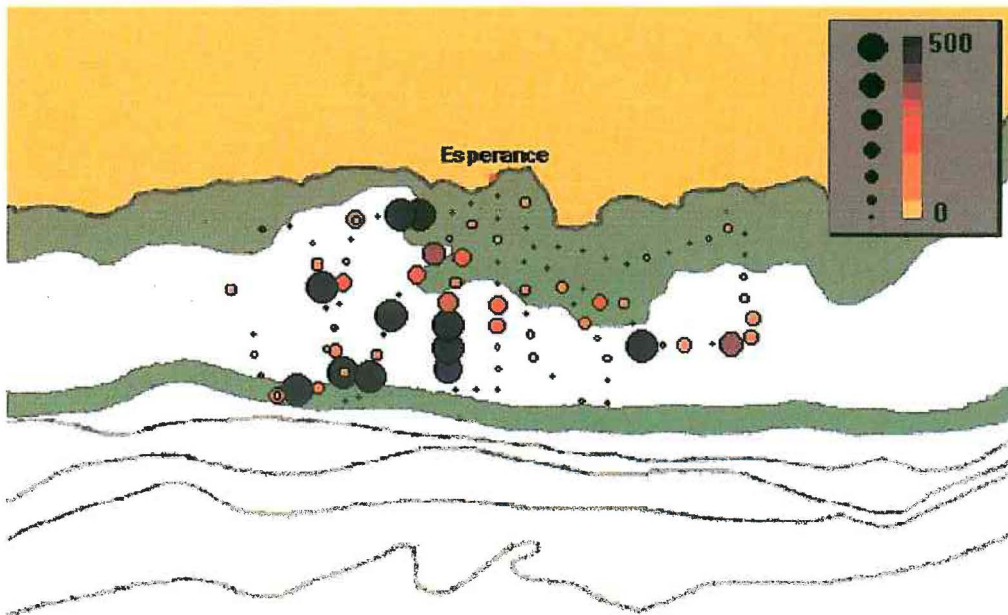
Figure 7.8 Relative density of day 1 pilchard eggs collected off Esperance in April/May 1995 using the 0.05 m² CALCOFI net.

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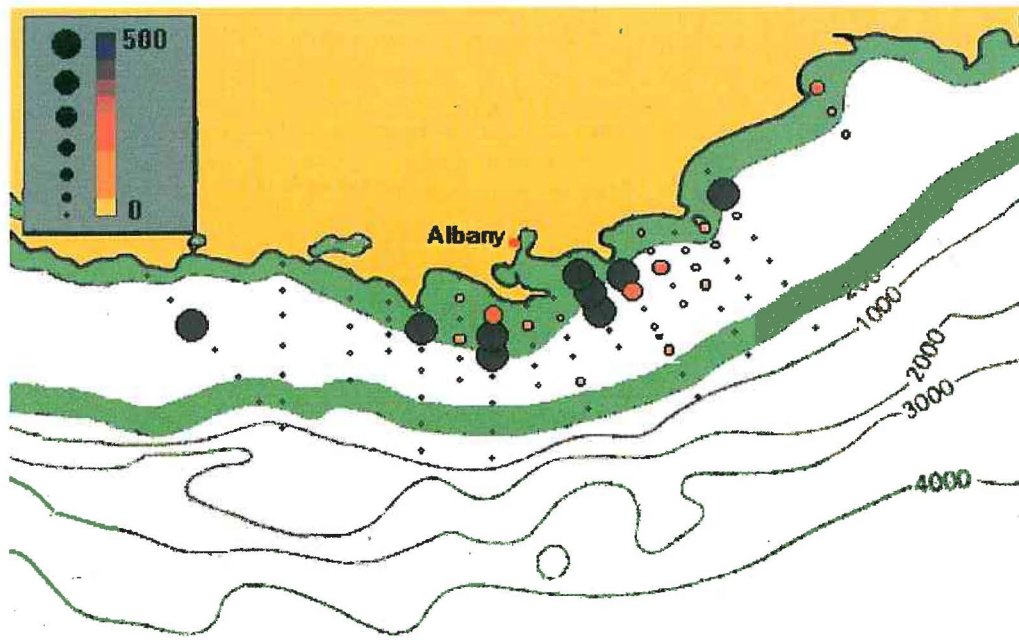
Figure 7.9   Relative density of day 1 pilchard eggs collected off Albany in July 1995 using the 0.05 m² CALCOFI net.

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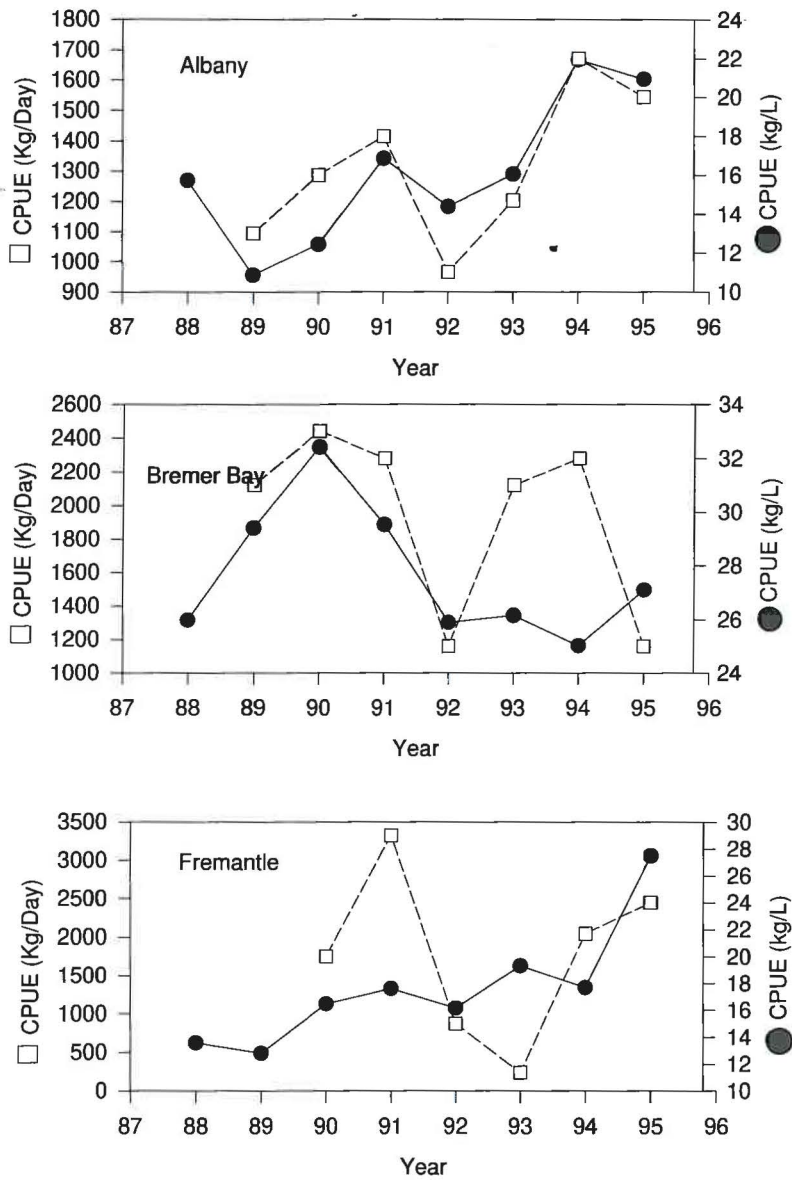
Figure 7.10 Annual mean CPUE values (kg/day and Kg/litre of fuel) for each of the pilchard fisheries in WA during the period 1988 - 95

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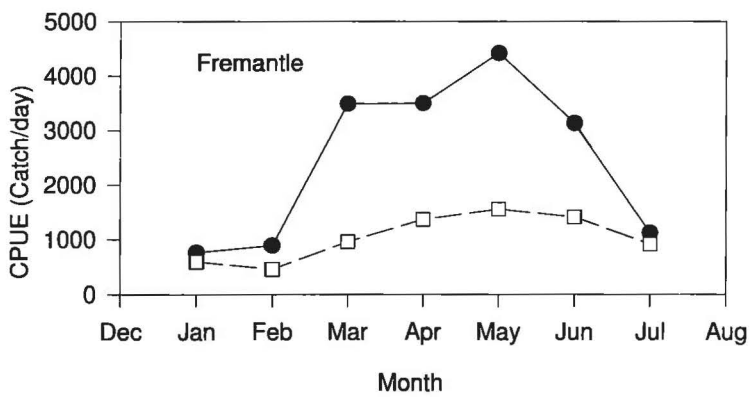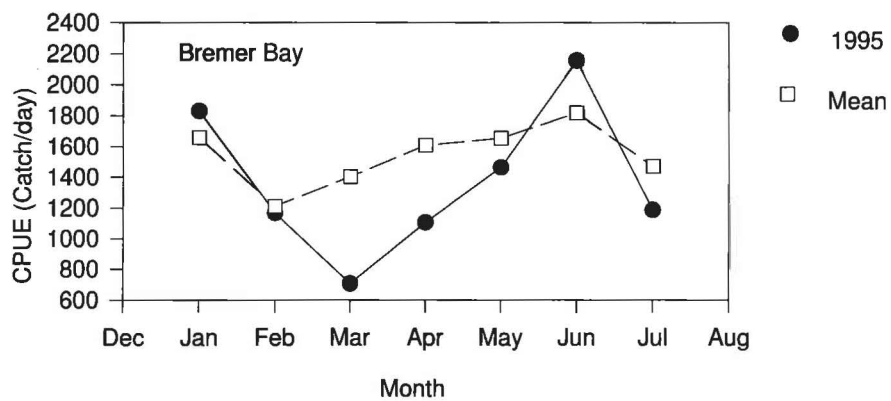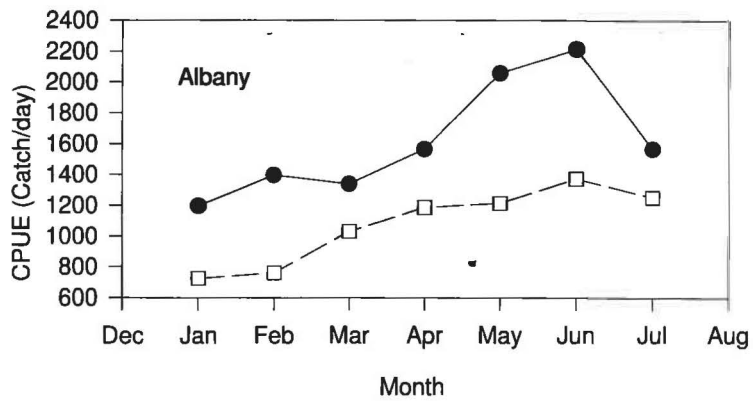
Figure 7.11 Monthly mean CPUE values for 1995 for each of the pilchard fisheries in WA compared to the monthly average during the period 1988 - 94.

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Section 8   Conclusions

8.1   Introduction

A wealth of information was collected during the pilchard mortality incident. In this section we summarise these data by comparing the findings to the hypotheses generated in Section 1 and the possible scenarios and preliminary conclusions cited in the interim report of the task force (Anon., 1995). Finally, we report the most likely cause for the mortalities.

The summary of the initial hypotheses (from Section 1) is that the kills could have been associated with:

(a) phytoplankton;
(b) environmental changes/upwellings;
(c) pathogens;
(d) some combination of these.

Tables 8.1, 8.2 and 8.3 contain summaries of the comparisons between the observations expected and observed during this incident examining the first three scenarios.

8.2   Comparison with Task Force Report

Given the wide circulation and use of the task force report in other publications (e.g. Douglas, 1995) and political debates, it is useful to comment upon each of the scenarios and conclusions contained and point out their inconsistencies and errors. It must be reiterated that it was known within the first few weeks (from the similar aetiology of the deaths and gill histopathology of affected individuals) that we were dealing with a single problem. Therefore, unless the conditions in all areas where the kills occurred met the criteria, then as scientific hypotheses they were not supported and should have been rejected.

The possible scenarios reported in the task force report in June 1995 were:

> (a) activation by environmental factors of latent infections of virus or amoebae already present in the pilchard population, indicating that there was not a point source for the current event.

It has been clearly shown in this report (Section 3) and elsewhere (Griffin et al., 1997) that over most of the area where the kills occurred there were no unusual environmental conditions, either on an interannual scale or during the period when the mortalities were occurring, that could have caused or even contributed to the deaths of the pilchards. Consequently the extensive data collected do not support the first half of this scenario. It should be noted that much of this information was known at the time the task force report was published.

There are several possible sources for the Herpesvirus epizootic implicated in this event. The virus may have been latent in the population and have been triggered by an unidentified event; the virus may have transferred to pilchards from another species already present in Australia; the virus may have mutated to become more virulent or the virus may have been introduced into Australia.

Latency in Herpesviridae causing recurrent infections is well documented (Roberts, 1989). However, 'latency' requires that the infected animal survive the initial infection and that the pathogen recreate the disease in the host once 'triggered', usually by temperature or hormonal changes. In the case of Australian pilchards, if the virus was latent, then the initial infection must have occurred sometime within the life of those affected by the 1995 mortality event. No previous wide-scale mortalities of pilchards have been reported. The one previous report of pilchard mortalities in Australian waters was by Copas (1982) and was restricted to a few thousand pilchards washed ashore in Tasmania with indications 'consistent with crushing' which, therefore, is inconsistent with the present epizootic (Section 4). There was no evidence of pilchards recovering from the gill damage associated with the Herpesvirus, which would be necessary for the development of 'latency'. The indications were that the disease was rapid and progressive, and the outcome was death. This is consistent with fish exposed to a

novel pathogen for which they have no previous exposure or resistance. Furthermore, the lack of any widespread environmental anomaly or cause of stress found during this study precludes the present event, which covered the entire range of the pilchard population, from being due to a latent virus. The possibility of a latent virus was also dismissed by Hyatt *et al.* (1996).

Herpesvirus is known to be relatively species-specific, and the mortalities involved only pilchards; no other species was found to be affected. It remains possible that the virus has developed as a virulent strain. However, there was no indication that any of the affected fish were only lightly infected or had recovered from the infection. Such recovery could be expected if the population had some previous resistance to this Herpesvirus.

The lack of any previous history of large-scale mortalities, and the rapid and dramatic spread of the disease in a 'front' from a point source, are strongly indicative of a novel virus infecting a susceptible population. Similar patterns have been found for propagative epidemics in a number of groups (eg Heide-Jørgensen *et al.*, 1992; Lafferty and Kuris, 1993). Once the infection had passed through an area reinfection did not occur, suggesting that the surviving fish were resistant to infection.

The phrase relating to a supposed consequent lack of a point source if the environment was responsible is incorrect on two counts. First, even if this situation was environmentally driven there could have still been a point source. Furthermore, the epidemiology clearly shows the advance of the deaths in both directions away from a 'point source' in South Australia. Only the size of the point source was ever in question.

> (b) *damage by phytoplankton. There are reports of phytoplankton (some potentially damaging) associated with kills in some regions, but there is insufficient information to link them to the kills in all areas (i.e. it is not known if they were present at all kill sites). It is known that they were not present in all kill sites in WA, and the histopathology is not consistent with phytoplankton-induced mortalities.*

The last sentence covering the data collected in WA on phytoplankton clearly contradicts the previous statement concerning the 'unknown' relationship between the kills and phytoplankton. It was known; there was no relationship. To summarise, phytoplankton were scarce at most locations where the kills occurred, and the composition of species present varied greatly between areas (Section 5). Furthermore, other clupeoid species and juvenile pilchards (and other filter-feeding organisms such as mussels) which can ingest phytoplankton should also have been affected if this had been the cause. Finally, as stated in the last line of the interim report scenario, the histopathology was not consistent with phytoplankton being the cause of death, nor even a contributing factor.

> (c) *the introduction of a pathogenic organism in imported pilchards used as feed in tuna farming, for bait (by tuna longliners, recreational fishers and the rock lobster fishery), or in ships ballast water. It should be noted that if there is a point source for this event then it is difficult to explain the spread of the original sightings, and the rapidity and occasional discontinuity of spread unless non-susceptible carriers are involved. There is no evidence to support or reject the involvement of such carriers.*

The only consistent pattern in the data collected from all of the deaths was the gill histopathology and the presence of a Herpesvirus in affected fish (Section 6). The virus was not seen in fish collected ahead of the front or in the survivors behind the front in WA (Section 6) or New South Wales and New Zealand (Hyatt *et al.*, 1996; Whittington *et al.*, 1997).

The gill epithelial layer covering the secondary gill lamellae constitutes 90% of the surface area of a fish. Hyperplasia and subsequent loss of this layer will lead to a reduction in oxygen and carbon dioxide transfer resulting in asphyxia. The loss of epithelial cells would also result in severe osmotic imbalance, which would also be lethal. While the involvement of the Herpesvirus in the mortalities has not been conclusively demonstrated by infection trials (these were attempted but failed due to the deaths of pilchards from bacterial infection prior to the tests beginning), the association of the virus with the gill damage which killed the fish, and the epidemiology consistent with a novel pathogen infecting a naive population, provide strong support for the Herpesvirus as the causative agent.

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Herpesviruses are usually associated with epithelial proliferation in fish (Wolf, 1988; Hedrick and Sano, 1989). Some are associated with epizootic mortality (McAllister and Herman, 1989; Iida *et al.*, 1989) and with hyperplasia of the branchial epithelium (Watson *et al.*, 1995). Of the three possible routes by which the Herpesvirus may have been introduced suggested by the pilchard task force (which are not the only possibilities), the first was through the use of imported pilchards to feed captive tuna in South Australian waters. The tuna industry imported about 10 000 tonnes of pilchards in 1995 which were released into the cages in and around Port Lincoln. The second suggested route was through the use of frozen bait by, for example, Japanese longline vessels. The Japanese practice is to set the 3000 hooks along a 150 km line, with the vessels set in parallel lines 4 miles apart. This means that they are placing about five baitfish in each square kilometre of ocean, and some of those will be eaten by the catch. The third route was through ballast water, but in this case the dilution factor would be immense, meaning that some other carrier of the virus had to be involved. There is no direct evidence to support any of these possibilities.

More thorough examination of reports for where and when the deaths occurred indicates that there was a single point source near the eastern end of the Great Australian Bight (CSIRO Web site, 1995). A reliance on other non-susceptible carriers is not required to account for the rate of passage of the front. An average rate of movement of 30 km/day is within the possible range of pilchards (Section 2). This does not, however, preclude other carriers being involved. A number of possibilities were suggested including birds (e.g. muttonbirds) and other species of fish (e.g. Australian salmon). However, neither of these can fully explain the smooth transmission both east and west at the same time. Furthermore, if birds were involved it is likely that the progression of the mortalities would have been significantly more patchy in its course than was recorded. Nonetheless, the involvement of other carriers in this incident remains a distinct possibility.

The lack of sightings of deaths in some areas can be explained simply by variations in the local density of pilchards. The results of extensive plankton studies have also found regions where pilchard density appears to be much lower than in other areas (Section 2). If the density is low, the deaths are unlikely to be observed because scavengers would remove the smaller number of dead individuals before they build up to noticeable aggregations.

The task force report preliminary conclusions
Underlying causes of the mortalities are complex and related to a number of factors. A likely hypothesis is that the initial factors involved stress associated with environmental changes coupled with agents such as a herpes-like viral infection and/or amoebic infestations.

As stated above the 'environmental change/stress' hypothesis has no scientific credibility and should be removed from any list of possibilities.

While it cannot be discounted, as yet, there is no evidence for or against the implication of an exotic pathogen in the pilchard mortalities.

This is a *non sequitur*. There was ample evidence that a pathogen, in the form of a previously unreported Herpesvirus, was involved in the mortalities. The bushfire-like pattern of kills, and the fact that they extended throughout the entire distribution of the pilchard population, suggests that this population had never been exposed to this virus previously.

In relation to media reports that the mortalities spread by infections from a single point source in SA, the balance of probability is that this is not the case.

Again, this statement defied the evidence. Whilst there was some confusion on the dates and locations of the initial sightings at the time of publication, there was still no doubt that the deaths occurred in fronts both east and west from the mid-South Australian region. The area where deaths were first sighted has subsequently been found to be significantly smaller in size than originally reported, clarifying the location of the point source as in the eastern Great Australian Bight region.

8.3 Final Conclusions

From the results of the investigations conducted in WA we have shown that:

1. Pilchards were affected over their entire range.
2. Approximately 10-15% of the stocks were killed.
3. There was no involvement of phytoplankton either directly or indirectly in the deaths of the pilchards.
4. There were no large or small-scale environmental anomalies which could have affected the pilchards over any of their range in WA.
5. The only consistent factor in the deaths at all locations was the presence of a previously undescribed Herpesvirus in the gills of the affected pilchards.
6. The pattern and severity of the impact suggest that the Herpesvirus was not a latent infection.

Thus:

CONCLUSION

The most likely cause of the massive mortalities of pilchards in Australia during early 1995 was from a novel Herpesvirus to which the Australian pilchard population was naive and whose origin was, therefore, most likely to be exotic.

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Table 8.1   Pilchard deaths: comparison of two hypotheses with the observed situation.

Algal bloom	Pathogenic organism	Observed situation
Associated with measurable oceanographic features.	Range is less than or equal to that of host, not necessarily associated with oceanographic feature.	Not associated with identifiable oceanic conditions. Extends to limit of pilchard population.
Dispersed by storm events.	Not affected by water turbulence.	Not stopped by severe gales off Bremer Bay, Albany, Augusta, Bunbury and Fremantle.
Does not cross current boundaries.	Moves with host.	Crosses current boundaries.
Is passive, moving with currents.	Moves with host.	Appears to move upcurrent.
Not species specific - kills a wide range of organisms in water containing algae.	Specific to its host(s).	Specific only to adult pilchards.
Does not spread out from a geographic centre.	May spread out from a central area.	Has spread out to east and west from South Australian waters.
Has never before been observed to move as a front through the water column. The mechanism whereby this could be achieved has not been explained.	'Bushfire' pattern epidemics are common.	A moving 'front' of deaths has been observed.
Kill is ongoing while bloom persists and if toxic, while toxin remains, then stops.	Susceptible population killed, resistant animals remain.	Pilchards behind 'front' are apparently unaffected. Most of the water samples containing 'suspect algae' have been recovered from behind the front where deaths have stopped occurring.
Gill histopathology ranges from no lesions to non-focal lesions. Algae such as *Thalassiosira* kill by clogging gills, not by toxin and should be present in the gills of dead fish. They have not been seen in affected gills in WA (or NSW, NZ).	Gill parasites cause focal lesions, lesions develop progressively over time until mortality occurs.	Focal lesions on gills in WA. Histopathology not consistent with any reported toxic algae but consistent with amoebic gill disease.
If toxic, often affects predators feeding on carcases of killed fish.	Does not normally kill predators feeding on carcass	No predatory animals appear to be affected other than by overeating.

Table 8.2   Causes of death from algal blooms (Jones and Rhodes, 1994)

1   Mechanical damage
2   Asphyxiation caused by oxygen depletion
3   Gas bubble trauma due to supersaturation
4   Chemical toxicity caused by ichthyotoxins
5   Increased seawater viscosity due to secretion of mucilages

Each of these has a unique gill histopathology which is not consistent with that seen in pilchards collected during this incident.

Table 8.3   Causes of fish deaths from environmental causes obtained from the literature

Oceanographic event	Mechanism	Observed situation
Upwellings	Oxygen deficit.	No oxygen deficit reported.
	Gas.bubble disease.	Inconsistent histopathology.
	Temperature shock.	Kills deep-sea fish or fish trapped in estuaries, and affects all fish in the area to some extent.
	Kills temperate phytoplankton.	Phytoplankton are replaced by deepwater species not typical of the surface layers. This has not been reported.
	Affects all species.	No other species affected.
Extreme water temperature incursions		
a) Cold shock	Temperatures typically drop to less than 5°C.	Most areas had normal water temperatures. Those that observed temperature drops were still within normal range encountered by pilchards.
	Phytoplankton are killed and replaced by cold-tolerant species.	Not reported.
	Affects all species in area.	No other species acting abnormally or killed.
b) Warm shock	Usually associated with power station effluent.	
	Phytoplankton are killed and replaced by warm-tolerant species.	Not reported.
	Affects all species in area.	No other species acting abnormally or killed.

Section 9   Outcomes

9.1   Introduction
The mass mortality of pilchards influenced a wide variety of people and resulted in both short and longer-term effects. Those affected included the fishers in the pilchard fishery, people in other fisheries, those that rely on pilchards as bait, other industries that utilise locally caught pilchards and also industries that rely upon the importation of fish and fish products. Another group that was also severely affected was the scientists who were embroiled in the investigations and consequent hysteria that surrounded this event. In this section we document these impacts and concerns and attempt to suggest rational and <u>achievable</u> goals for future situations.

9.2   Impact on the Pilchard Fishery
The pilchard fishers in WA were affected immediately by the kills, but to varying degrees. The fishermen at Bremer Bay and Esperance were only affected to the extent that the dead fish in the area interrupted their fishing operations. At Bremer Bay, in particular, a storm hit at the time when the peak numbers of fish were dying in this region (29 April 1995) which stopped fishing anyway. By the time the weather cleared there were few dead fish left in the area. At this point, the nature of the deaths was unknown and no action had been taken by the Fisheries Department of WA. When the kills began occurring in Albany a few days later (6 May 1995), it was becoming clear that this was probably not a local, environmentally driven event as had previously been assumed, but one that could be due to the impact of some pathogen. Consequently, following talks with industry, a voluntary moratorium on the capture of pilchards was imposed for the Albany area on Friday 12 May 1995, to be enforced until the nature of the problem was ascertained or at least until the danger to health from the affected fish was known. Furthermore, because it was possibly a virus and it was unclear how it could be communicated amongst fish, or over what time-frame the infections would have occurred, a self-imposed restriction was introduced on transport out of the areas already affected of pilchards caught during and after the kills. Thus, sales to the west coast and the east coast were halted. A containerload of pilchards caught during the kill event which had been consigned to New Zealand was stopped on the wharf. The last load shipped to New Zealand left Bremer Bay on 21 April, with the fish caught during the two days previous to this. This is about ten days before the first deaths had occurred at Bremer Bay. The container arrived in New Zealand a month before the documented deaths began in that country, for use mainly as IQF angling bait.

The ban on fishing in the Albany area continued for approximately a week, after which catches resumed but the moratorium on the transport continued. Subsequently, when deaths began on the west coast, the Bunbury and Fremantle fisheries were also closed until the kills had passed through these regions (27 May – 5 June). By this stage (26 May 1995), however, the transport bans had been lifted because it was decided during the phone conferences that matters relating to the importation of fish from interstate were the direct responsibility of the receiving state. Furthermore, by this time there were few places in Australia where the kills had not occurred. The only question that remained was whether other related tropical species (e.g. *Sardinella, Amblygaster*) would become affected. At this point the kills in New Zealand began and the New Zealand authorities imposed a ban on the import of further shipments of Australian pilchards (2 June 1995). This ban remained in place until the end of July 1995.

The pilchard industry members in WA should be highly commended for their responsible and professional attitude during this crisis. Whilst obviously concerned about their livelihoods, they responded favourably to suggestions concerning the appropriate action to take. In the longer term, the loss of income due to the cessation of catching was probably minimal, but this was not to be known at the time. As stated above (Section 7), the catch rates following the kills were not significantly lower than expected before the kills had occurred so their impact on this sector of the industry was probably minimal in the longer term. For Albany fishermen, especially, the long-term impact was probably minimal because it is a quota-managed fishery and most of the 1995/96 quota was subsequently caught. Nonetheless, those individuals that required a constant cash flow may have suffered during the enforced

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break in fishing activities and this event also affected the possibility of reinstating their quota which had been reduced a month before the kills had occurred.

The processing sector, and especially those involved in the human consumption market, were dramatically affected. Sales of pilchards for human consumption to supermarkets etc. stopped almost overnight, particularly with media reports highlighting the possibility that the fish may have posed a health risk. Even after the scientific reports returned findings that there was no public health risk, the sight of millions of pilchards washed up on beaches was a public relations nightmare. This was exacerbated some months later by the media reporting that a Herpesvirus was involved in the kills. Many ill-informed reports suggested that you may catch 'herpes' by eating pilchards, which severely retarded the improvement in sales that had only just begun. Consequently, it took almost a year to regain the confidence of the public.

- The longer-term effects on the pilchard stock cannot be fully determined at this stage. Sindermann (1990) noted the effects from 1954-56 fungus infection of herring included:
- Herring landings during post-epizootic years were half previous level.
- Mean ages of herring in landings decreased with fewer age groups in the fishery.
- Herring growth rate increased.
- Growth rate of cod (which fed on dead herrings) exceeded previous records.
- Cod landings increased due to increased weight of individual fish.
- Growth rate of lobster (which fed on dead herrings) increased.

Despite the favourable signs it may take some years before we can be completely assured that there were no real impacts on the pilchard stocks.

9.3   Other Fisheries

A number of other fisheries were also affected during this event. Thus, a number of rock lobster fishers in southern parts of the state reported low catch rates in the week or two after the kills. This was likely to be due to changes in the catchability of the lobsters as a result of the enormous amounts of pilchards that were accessible without the need to get into pots. This type of effect has also been seen elsewhere (Sindermann, 1990). By contrast, no effect was seen for the west coast lobster fishery (Chubb, pers. comm.).

Line fishermen on the south coast also found catching fish difficult and those that were caught had stomachs that were full of pilchards. This was also the case for sharks and other fish caught in nets (Simpfendorfer, pers. comm.).

There was a general reduction in the amounts of fish of all species sold during this event, particularly in the early stages when there were fears that the pilchards were somehow contaminated and that this would be passed on to their predators. The Fisheries Department fielded numerous requests for information and reassurance that there would be no ill-effects from eating fish that had consumed the dead pilchards. Obviously, it took a few weeks before we had the scientific evidence with which to answer these queries with certainty.

9.4   Other Industries

The cessation of fishing and moratorium on transport affected other industries such as bait wholesalers and pet food companies. Supplies of pilchards during this period for both these groups declined appreciably. The pet food industry also stopped purchasing pilchards for some time until the results of the toxicity of the affected fish were ascertained.

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9.5   Importation of Fish

The speculation that occurred during this incident raised awareness of the current quarantine practices associated with the importation of fish into Australia. On this subject, a report into these practices by Humphrey (1995) had concluded that the importation of bait and feedfish 'constitutes a high risk of introducing exotic pathogens' with the risk escalating when importing, and using in an untreated state, hosts (i.e. species) that are also present in Australia.

Prior to any simplistic courses of action being suggested, it should be remembered that a number of industries rely on the importation of fish to operate. These include the rock lobster industry which uses the fish for bait, and the aquaculture industry, particularly that in South Australia which requires thousands of tonnes to feed the caged southern bluefin tuna. The whole question of what protocol should be used to control the importation of bait and other fish products is now being examined by a separate National Task Force on Imported Fish and Fish Products. A similar review has been undertaken by New Zealand.

9.6   Impact on Research Organisations

Media interest in this event was enormous. During this period in excess of 15 television, 40 radio and 50 print interviews were given by staff of the Fisheries Department of WA. These covered local, regional, national and international agencies. This is in addition to the hundreds of public enquiries that poured in. Generally only one spokesperson was used in order to minimise confusion, which was especially necessary given that, at the time, a number of scenarios were being proposed for the cause of the deaths by other agencies.

The ability to quickly switch resources into this program was appreciated. The budgeting arrangements and the availability of sufficient contingency funding for unexpected events meant that no time was lost waiting for funding approval before work could commence. Acknowledgement is also made of the cooperation of other agencies in WA in providing assistance at short notice, enabling the best possible response to be made.

The pressure to find a cause was intense. There was also, however, an obligation to ensure that, before any major unsolicited statement was made, the actual cause (and not merely a possibility) was known. A number of 'answers' to the mystery were disseminated on television and in newspapers using limited or even incorrect supporting evidence. This caused a large amount of frustration to fisheries managers in WA, particularly when trying to maintain the cooperation of the fishermen in abiding by the suggested fishing and transport restrictions. Thus, by late May there was already sufficient information available to reject both the phytoplankton and environmental stress theories as tenable scientific hypotheses. Despite this, these two theories kept on being promoted to the extent that they were made the most likely causes in the interim task force report (Anon., 1995) and have continued to be advocated in more recent publications (e.g. Douglas, 1995).

9.7   The Pilchard Mortality Task Force

The rationale for creating the task force to help coordinate investigations of the pilchard mortalities was sensible. The first two teleconferences were particularly useful in receiving information on where and when the kills had occurred and were still occurring, and what people were doing to tackle the problem from a wide variety of locations all at the one time. The methods used to finish the interim report, however, appeared to require the appeasement of all participants, even though there was an extreme level of disagreement. Instead of highlighting the discrepancies in theories and objectively listing the data to support either case, a method of constructing convoluted 'non-conclusions' was adopted. This severely weakened the value of this document and consequently the advice coming through the process.

9.8   What is Needed for Future Situations

The events described in this report are unique in Australia's recorded history. Mass mortalities, especially on the scale witnessed for pilchards during this event, are rare and generally unexpected. Whilst there may 'never' be a repeat of this event for any species in Australia, useful lessons have been learned about how to appropriately manage fish kill events of all scales.

The interim report (Anon., 1995) identified that 'an effective framework' was required for future situations whereby a national coordination network should be in place. In addition, a well developed and documented response plan should be produced with clear guidelines concerning the types of samples and the information that need to be collected. This should be distributed widely so that it is known by many individuals within each fisheries agency (i.e. not just the pathologists).

Another area which could be improved is the collection of baseline environmental data. Access to long-term databases collected by the CSIRO and the Navy should also be available at relatively quick notice (e.g. by lodging them on the Internet). The naval records we obtained, which were invaluable to dismissing some theories, probably would have remained unknown except for the knowledge of one staff member. There may be a wealth of similar information that is never accessed by the general scientific community.

As stated in the interim report, each state should have sufficient equipment to conduct environmental surveys in a timely fashion. Thus, a conductivity, temperature and depth (CTD) profiler and appropriate water sampling equipment should be available along with phytoplankton, zooplankton nets etc. to enable specific locations to be sampled easily. The provision of 'fish kill' kits in each district office, along with appropriate instructions on their use, should facilitate the rapid acquisition of material in a useful condition for pathological examination. This was a major problem during the present mortality event.

Finally, a national register of scientific expertise is required, one that is updated regularly so appropriate expert knowledge can be obtained quickly. Obviously the spatial scale and species involved will determine who is most appropriate to coordinate any actions.

Reports should be produced in such a way that there is a clear separation between scientific analysis and political ramifications, allowing conflicts in interpretation to be highlighted, not hidden. Any additional information which should be obtained to clarify the situation could also be listed.

9.9   Finale

The mass mortality of pilchards was a major disease management crisis which for the first time was located in the marine environment, covered half the continent and involved the input of a large number of agencies, institutions and individuals. Many will have learned a great deal through this experience. Whether it was handled effectively, efficiently and appropriately will probably become clearer with the passage of time.

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Section 11 Appendices

Table 11.1 Dates and functions of Task Force teleconferences

Date	Purpose
10 May	General information — where and when events occurred
16 May	General - update on deaths and what people should do
19 May	Working Group meeting and phone link-up
26 May	General - what had been found and implications
8 June	General discussion — focused on Interim Report
14 July	Final discussion - what happens now?

Table 11.2 List of acronyms

AAHL	Australian Animal Health Laboratory
ADCP	Acoustic Doppler Current Profiler
AHL	Animal Health Laboratory
AQIS	Australian Quarantine and Inspection Service
ASP	Amnesiac shellfish poisoning
AVHRR	Advanced Very High Resolution Radiometery
CalCOFI	Californian Co operative Fishery Investigation (USA)
CalVET	Vertical plankton net
CCEAD	Consultative Committee for Exotic Animal Disease
CPE	Cytopathic Effect
CPUE	Catch per unit effort
CSIRO	Commonwealth Scientific Industrial Research Organisation (Divisions of Oceanography and Fisheries)
CV	Coefficient of variation
CTD	Conductivity, temperature and depth probe
DAL	Days at liberty
DEPM	Daily egg production method
DPIE	Department of Primary Industry and Energy
ENSO	El Nino — Southern Oscillation index
FRDC	Fisheries Research and Development Council
FIRTA	Fishing Industry Research Trust Account
GAB	Great Australian Bight
GPS	Global positioning system
GSI	Gonadosomatic index
HPLC	High pressure liquid chromotography
ICES	International Council for Exploration of the Sea
IQF	Individually quick frozen
KGS	King George Sound
LCF	Length to Caudal Fork
MSA	Marine agar
NIWA	National Institute for Water and Atmospheric Research (NZ)
NOAA	National Oceanic and Atmosphere Administration (USA)
NZ	New Zealand
NSW	New South Wales
PSP	Paralytic shellfish poisoning
SA	South Australia
SARDI	South Australian Research and Development Institute
SEM	Scanning electron microscope
SST	Sea surface temperature
TAC	Total allowable catch
TL	Total length
USA	United States of America
VIC	Victoria
WA	Western Australia
XBT	eXpendable BathyThermograph

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Table 11.3   Relevant notes made during the period of pilchard mortality.
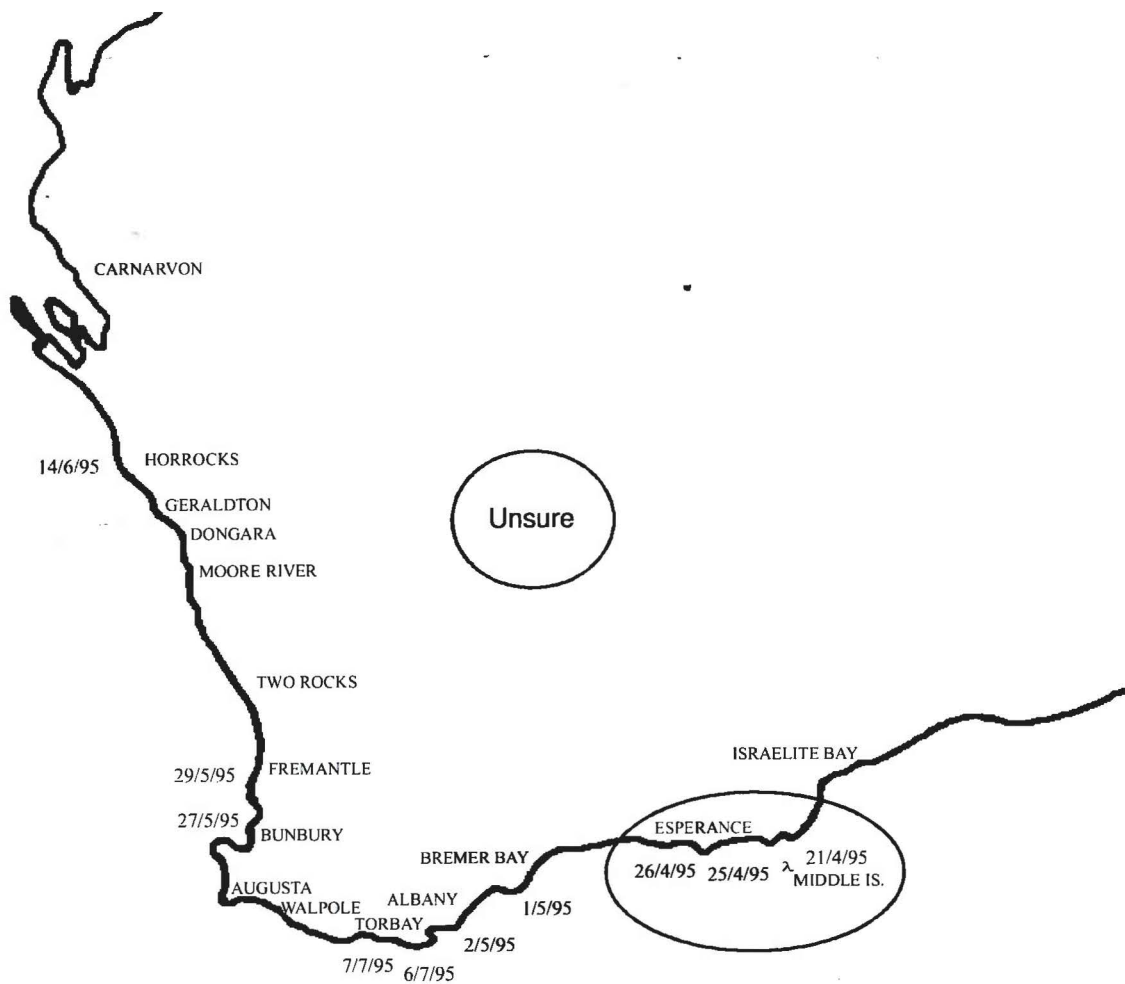
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20/04/95	First report
21/04/95	Davey Island from fisher
24/04/95	Arrived Esperance first plankton tows
25/05/95	First sighting on beaches in Esperance
26/04/95	More sightings on beaches
27/04/95	First report in Bremer Bay
1/05/95	mouse test Lots in Bremer Bay (largest)
3/05/95	mouse test Slimy fish at Bremer some dead at Cape Riche
4/05/95	Lots at Cape Riche and Bluff Creek
5/05/95	Rottnest sample fish dumped from p/seiner? Almost over at Bremer, fish less slimy. Front at Albany
7/05/95	Front at Torbay, some dead fish at edge of Shelf
8/05/95	25 km west of Torbay there were a handful of dead fish
9/05/95	BIG STORM (greater than 40 knots)
13/05/95	Front in Walpole region
14/05/95	Front in Walpole region
15/05/95	Front in Augusta region
16/05/95	More dead fish in Augusta (and dirty water)
26/05/95	Deaths offshore Bunbury
29/05/95	Off Warnbro Sound dying fish
30/05/95	Near Rottnest scattered
31/05/95	Near Rottnest scattered
1/06/95	Large numbers of dead Garden Island
2/06/95	Strong westerly winds
3/06/95	Lots of dead fish on beaches in metro region
4/06/95	Lots on beaches
5/06/95	All gone from metro beaches
6/06/95	Two Rocks – Moore River area reports of dead fish
7/06/95	STORMS
11/06/95	No deaths Dongara
12/06/95	30 miles offshore Dongara dead fish
13/06/95	Still dead fish Dongara
1406/95	Reports from Horrocks of dead fish
28/06/95	End of June – reports of dead pilchards at islands near Carnarvon

CARNARVON

END OF JUNE

HORROCKS
14/6/95

GERALDTON
12/6/95   DONGARA

MOORE RIVER
STORM

6/6/95

TWO ROCKS

STORM

29/5/95   FREMANTLE

25/5/95   BUNBURY

BREMER BAY

ISRAELITE BAY

18/4/95

ESPERANCE

λ MIDDLE IS.
21/4/95

28/4/95

STORM

STORM   AUGUSTA   ALBANY   1/5/95
16/5/95   WALPOLE
        TORBAY
15/5/95   STORM   7/5/95   5/5/95

Figure 11.1   Epidemiology: known position of the front of <u>dying</u> fish in WA.

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Figure 11.2 Dates and locations of where samples of dead fish were obtained for analysis of lengths, weights, condition and ages.

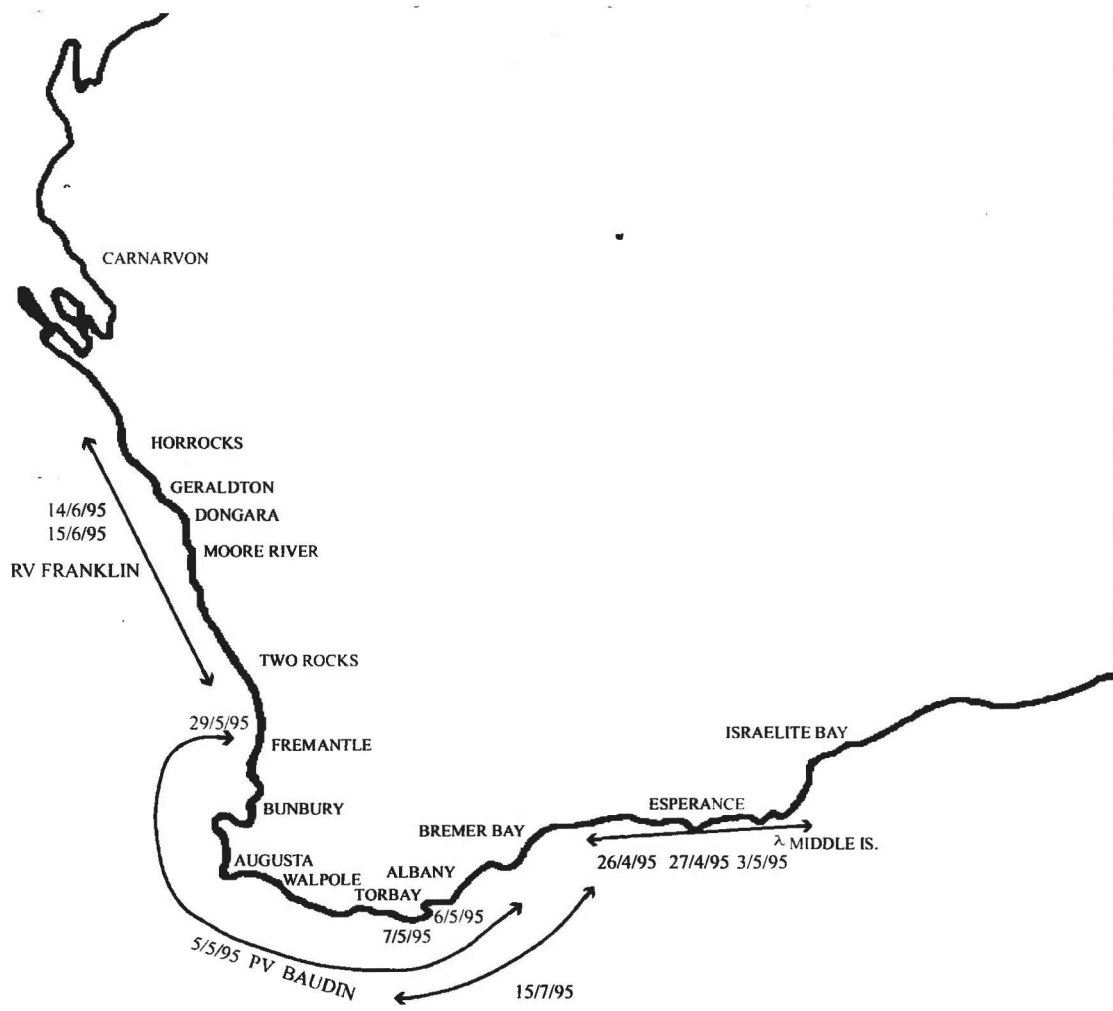Fish. Res. Rep. Fish. Dept. West. Aust.
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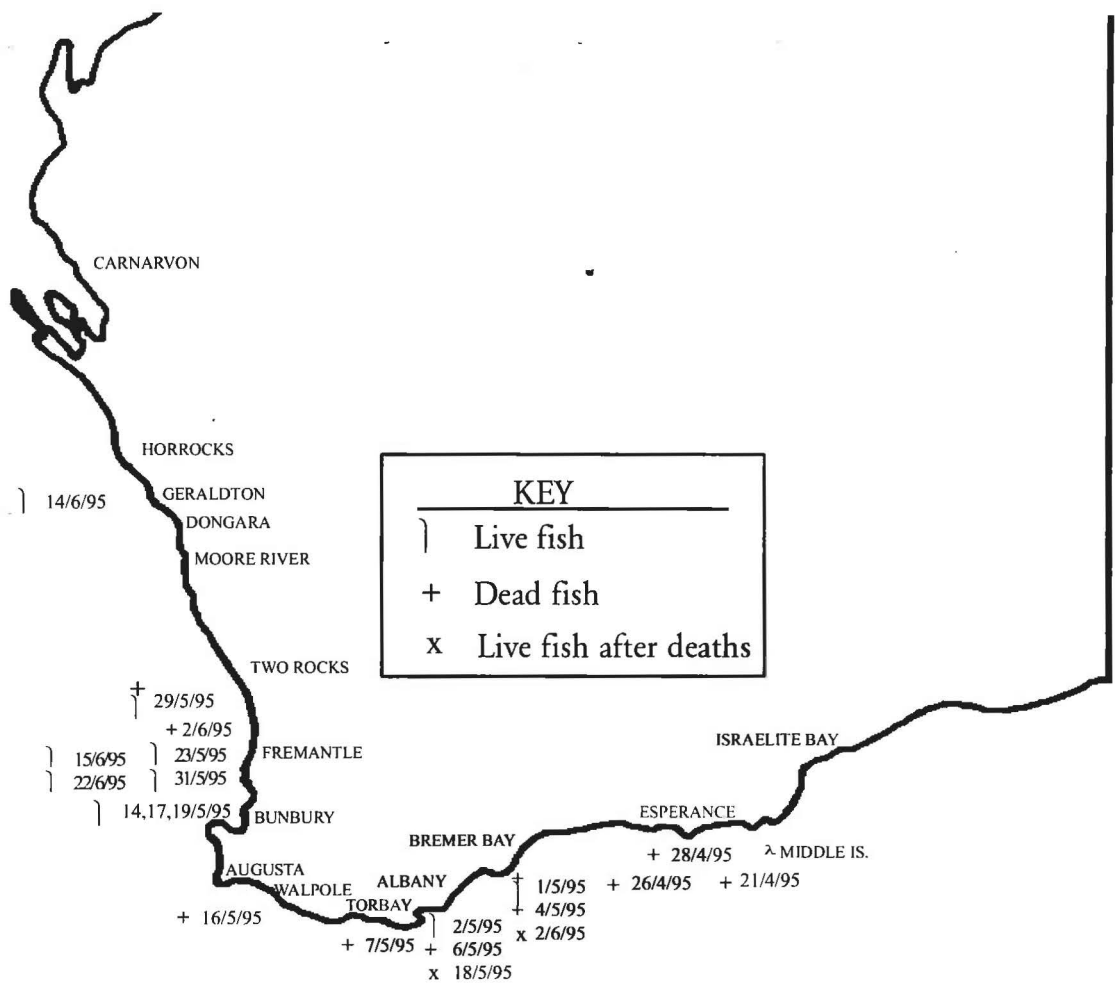
Figure 11.3 Dates and locations of dead and live pilchards collected for histological analysis.

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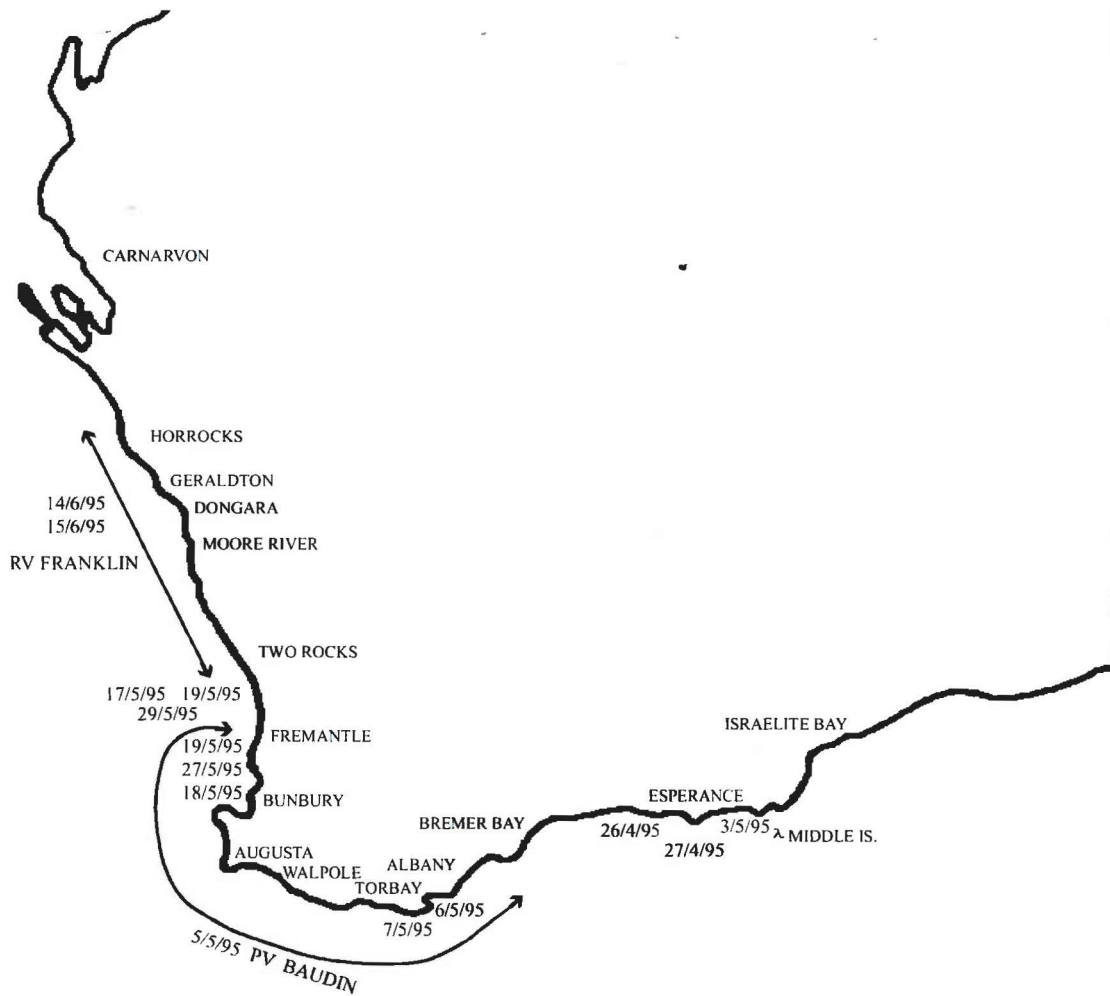
Figure 11.4 Dates and locations of phytoplankton samples taken before, during and after the pilchard kills occurred.

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Figure 11.5 Dates and locations where zooplankton samples, including those with pilchard eggs, were taken.

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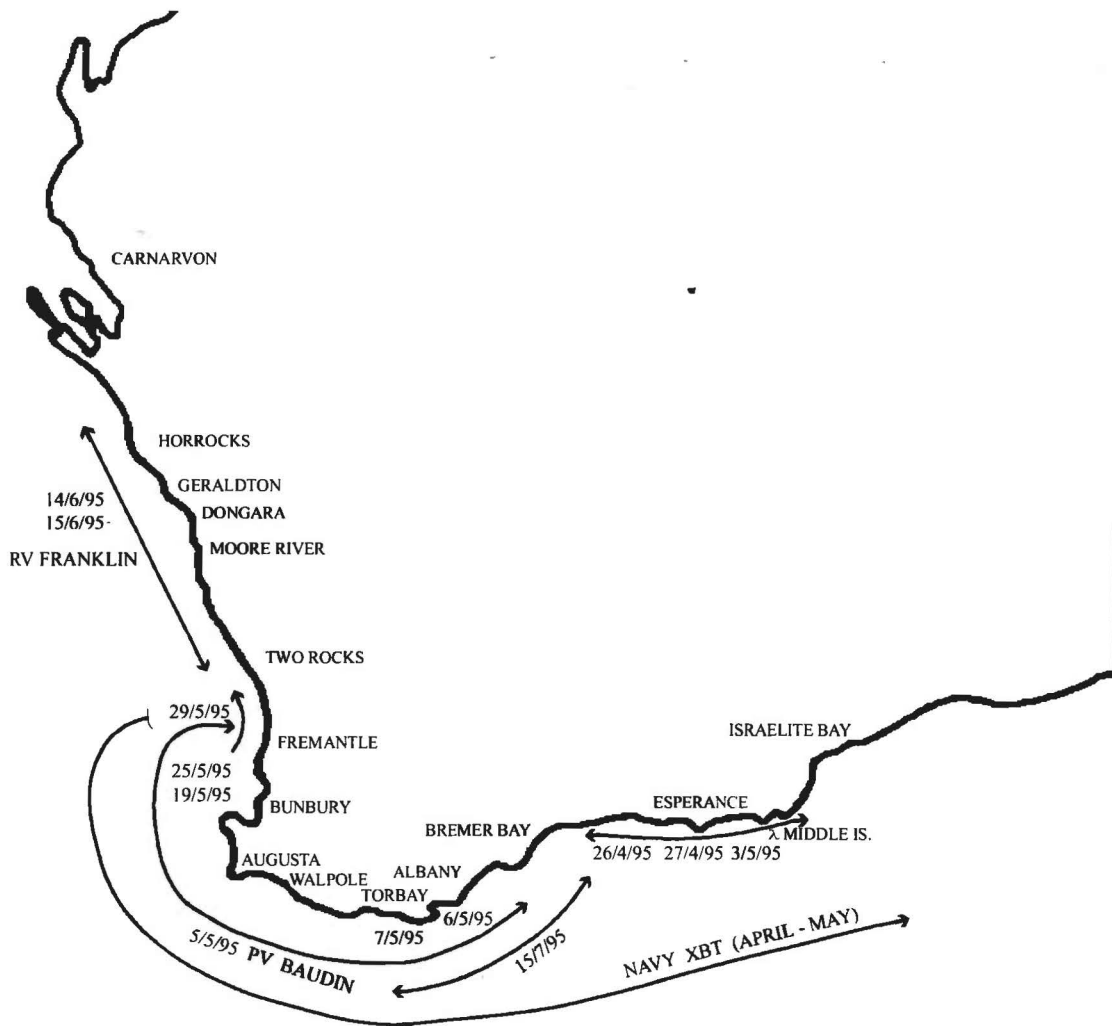
Figure 11.6 Dates and locations where environmental measurements were made, both direct sampling, plus automatic loggers and remote sources (SSTs).

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Figure 11.7 Location of generally unsubstantiated, and in some cases incorrect, anecdotal information obtained during the incident.