NSW DEPARTMENT OF
PRIMARY INDUSTRIES

FISH KILLS IN NSW

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Introduction

A fish kill is defined as "any sudden and unexpected mass mortality of wild or cultured fish".

Fish kills are often very "visible" events which cause considerable interest and concern to the public and the media because they are often perceived to be the result of pollution or contamination of waters. The reality is that there are many and varied causes of fish kills, and a large proportion are due to natural events.

This paper outlines the general situation with regard to fish kills in NSW, examines and discusses some of the more important causes and details the observations which might be made during a field investigation of a fish kill which could help to isolate a cause.

It is divided into four parts - Part 1 summarizes the fish kill situation in NSW. Part 2 examines various potential causes. Part 3 provides advice on what should be done in the event of a fish kill being reported so that it may be investigated and a cause determined. Part 4 is the Fish Kill Investigation and Reporting Protocol developed jointly by NSW Fisheries and the Environment Protection Authority including the reporting forms and contacts.

1. Fish kills in NSW

NSW Fisheries maintains a database of fish kills in NSW. It contains some 750 records dating back to the early 1970's. The following information is drawn from that database.

Since the mid 1980's there has been around 20 - 50 kills reported to NSW Fisheries each year whereas prior to 1987 less than 20 kills per year were reported (Figure 1). The change in reporting rate is probably explained by improved awareness and better reporting arrangements rather than an increase in the number of kills that have been occurring. Since many smaller kills probably go unnoticed and others remain unreported, the real number of kills possibly exceeds 60-80 per year.

The species most frequently affected include mullet, eels, Common Carp and trout although a total of nearly 60 species including molluscs and crustaceans have been affected overall (Figure 2). It may seem surprising that species such as carp and eels which are generally thought to be "tough" should be commonly killed. The explanation is probably that they are tolerant of poor conditions and therefore are the only species to occupy marginal habitats which are periodically subject to influences which exceed the tolerance limits of these species.

Figure 1 Total Number of Fish Kills recorded for each calendar year from 1970 to 1996

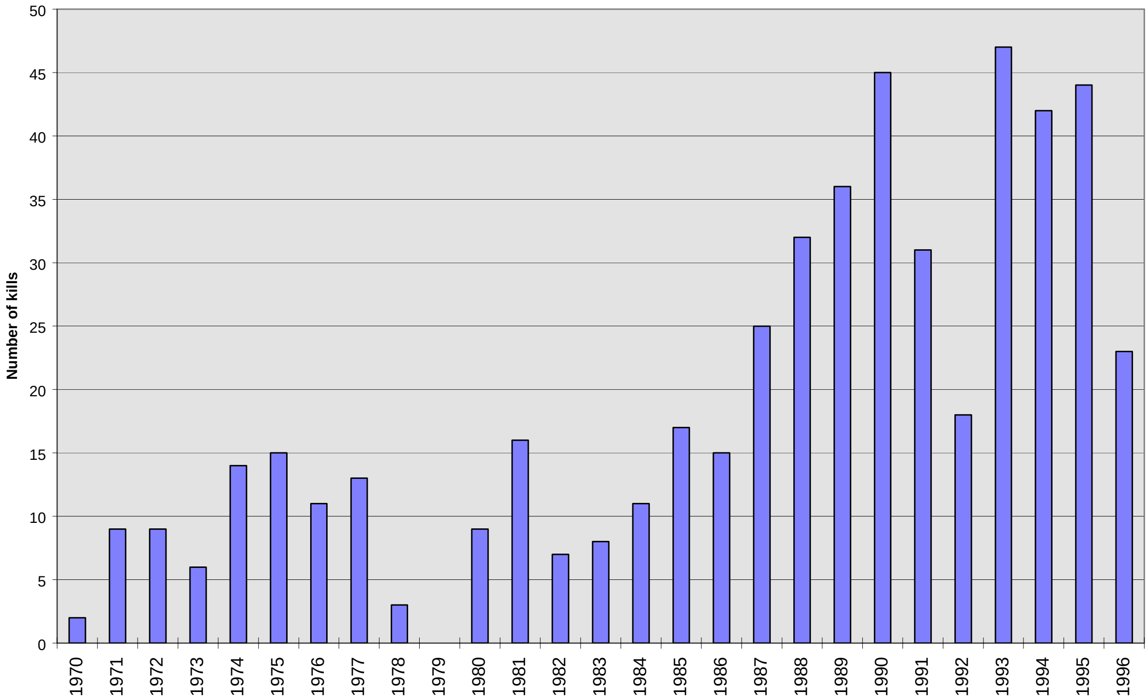
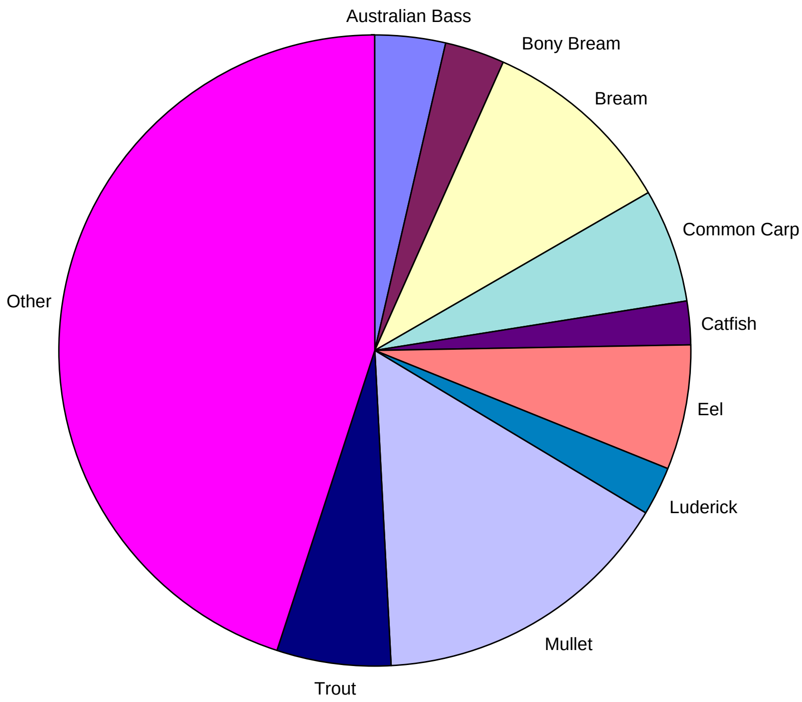


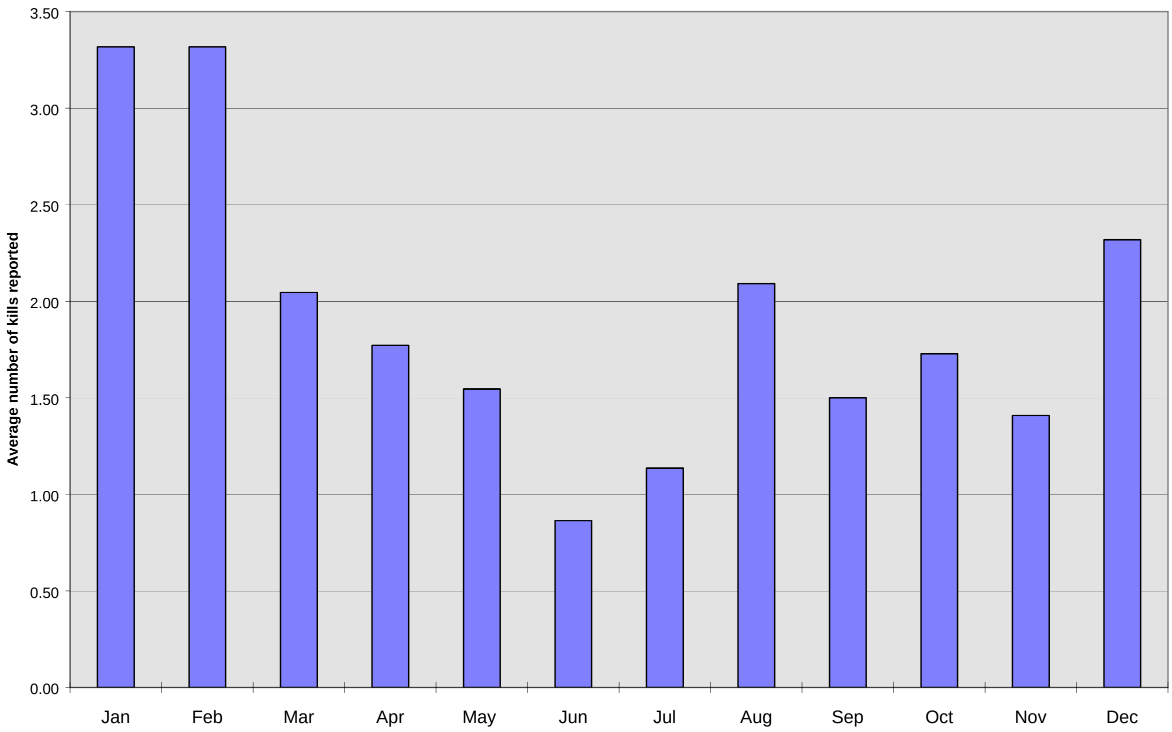
Figure 2 Species affected



Both freshwater and estuarine areas are equally affected (each contributing approximately 45% of all kills respectively) with fewer kills reported from oceanic waters (approximately 10%). However, kills in oceanic waters when they do occur, can be very large and spectacular (eg pilchards in 1995).

Relatively more kills occur in summer (January/February) although they are likely to happen at any time of the year (see Figure 3). The main contributing factor appears to be higher water temperatures (and consequently lower dissolved oxygen levels) and lower water levels in freshwater systems.

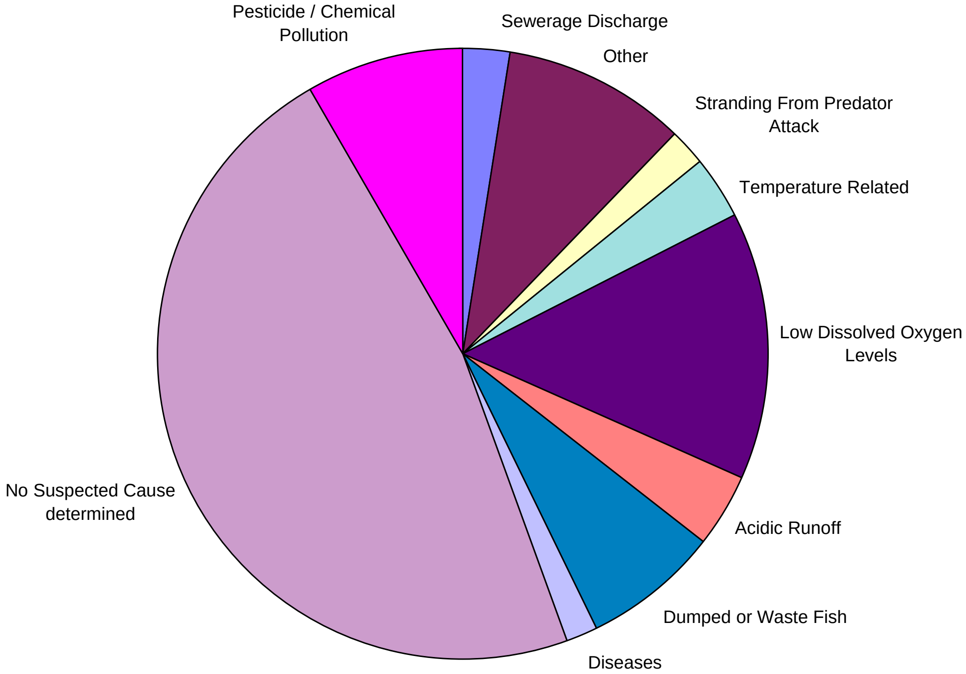
Figure 3 Reporting Rate - average number of kills per month for the years 1970 to 1996



Over the whole state, particularly problematic areas have been the Murrumbidgee River, Lake Macquarie, the Hawkesbury and other rivers and estuarine areas in the Sydney metropolitan area . There are only five river basins for which no kills have been recorded - Paroo, Warrego, Condamine-Culgoa, Castlereagh and Moonie.

No cause was determined for 47% of the fish kills reported in NSW over the last twenty years (see Figure 4). Of those where a diagnosis was made, the main causes were low oxygen levels (14%), pesticide/chemical pollution (8%), acidic runoff (4%), high or low temperatures (3%) and sewerage pollution (3%). There were also a significant number of incidents attributed to dumping of waste fish or bycatch (7%). In many cases the cause was based on circumstantial evidence and should not be considered definitive.

Figure 4 Suspected causes of fish kills

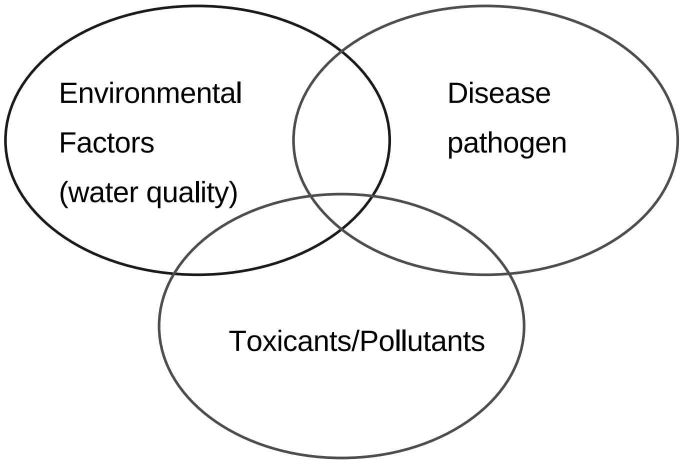


2. Potential causes of fish kills

Clearly many fish kills are a natural phenomenon, often related to natural events and normal environmental cycles. There is therefore, little that can be done to prevent them and, in most cases, no serious cause for concern as in many cases the number of fish affected is insignificant in comparison to the total population size. However, some kills are **not** natural events, being related to pollution of waterways, introduced diseases, or fishing and other human related activities.

A large percentage of kills are caused by a multiplicity of factors acting together - eg. poor water quality due to industrial or urban pollution predisposing fish to disease or temperature shock. Three main factors play a role - toxicants/pollutants, environmental factors (eg salinity, temperature, acidity levels, dissolved oxygen levels), and disease pathogens. These can interact in a number of ways as indicated in Figure 5 (see following page). It is possible for a kill to be due to a single factor acting alone, or two or three factors acting together.

Figure 5. Interaction between factors involved in fish kills



As far as possible, fish will avoid areas with adverse environmental conditions such as low dissolved oxygen concentrations. Provided the area of water affected is not too great, the fish will be able to swim away and no kill results. This is mostly the case in oceanic waters. However, if the entire or a large proportion of the waterbody is affected, or the adverse conditions appear very rapidly, then avoidance reactions are of little use. Small dams, ponds, residual pools and other enclosed waters present an obvious problem since the fish cannot readily move elsewhere.

Lack of oxygen or deoxygenation

Oxygen dissolved in water comes from both the atmosphere (by exchange across the air-water interface, especially in association with wind rippling or agitation during flow over riffle sections of streams) and from aquatic plants (by photosynthesis). The normal range for well oxygenated water is around 8 to 10 milligrams per litre (mg/L).

Most aquatic animals, including fish, extract the oxygen they need from the water through their gills. A low level of dissolved oxygen (it is not necessary for the water to become completely deoxygenated) will cause suffocation and death of aquatic animals. The critical minimum level varies with different species and different physical conditions, but as a general guide, few species will tolerate prolonged exposure to dissolved oxygen (DO) levels below 3 mg/L. As oxygen levels decrease, the more susceptible species (eg, trout, mullet) will die first, and the less susceptible (eg, eels, carp, catfish) later. The larger fish and more active species also tend to die first due to their greater oxygen requirements. A fish kill that effects only the more oxygen-sensitive species is likely to be due to partial deoxygenation of the water. A total kill of all species could be due to severe deoxygenation, but other causes are also possible.

Cool water can contain a greater concentration of DO than warm water with the result that deoxygenation induced deaths are often associated with a rise in temperature.

The main response by fish to lack of oxygen is gasping at the surface. This is done in an effort to force air across the gills, and some species (eg Common Carp) can survive in this way for hours. Others, however, will die quickly under low oxygen conditions, and the gasping does not substantially prolong their life. However, gasping at the surface may also be due to gill damage caused by pesticides or parasites.

Fish killed by oxygen depletion often exhibit 3 symptoms - a wide open mouth, flared gills and a bent back head.

Depletion of DO in water is a common cause of fish kills. It is however a **secondary** cause in that the oxygen levels have been depleted by some other factor (eg. excessive plant or algal growth, inputs of organic matter following rain etc). It is almost always due to the use of the dissolved oxygen in the water column by living organisms other than fish. An exception is in aquaculture where fish are maintained in crowded conditions.

Factors that may lead to deoxygenation include:

- **Excessive plant growth.** Plants produce oxygen by photosynthesis under conditions of strong light, but also continually consume oxygen by a process known as respiration. This applies to all plants, whether phytoplankton (microscopic algae suspended in the water) or vascular plants (such as reeds). At night or on dull days, consumption of oxygen exceeds production and a progressive removal of dissolved oxygen from the water column results. DO levels will generally reach a minimum just before dawn. This mainly occurs during the warmer months, when dense growths or “blooms” of phytoplankton (“green water”) are more prevalent, and when the oxygen saturation value (the amount of oxygen that can be dissolved in the water) is lower. If calm weather prevails and re-oxygenation from wind rippling does not occur, DO levels can fall to dangerously low levels. Paradoxically, the water can become supersaturated with oxygen in the middle of the day due to photosynthetic activity.
- **Rain.** Heavy or prolonged rain often results in extensive surface runoff, which can carry large loads of biologically active matter such as animal manure, fresh or decomposing plant material or organically rich soil into a waterway, where rapid bacterial decomposition occurs. The decomposition process creates a high Biochemical Oxygen Demand (BOD). Fish kills from this cause most often occur in enclosed bodies of water such as farm dams, small lakes or billabongs.

- **Flooding.** Decomposition of biologically active material can also occur when floods inundate areas that are heavily vegetated (eg swamps) or those with high levels of other biodegradable material (eg animal manure or green immature crops) (eg McKinnon and Shepheard 1995). Kills will generally occur on the flood recession as deoxygenated water on the flood plain drains back into the main stream where the majority of fish are living.

  Fish deaths have also occurred in rivers where a section of “black water”, so named because of the colour resulting from the high level of dissolved tannins and lignin from decomposing organic matter, moves downstream at the beginning of a flood. (See Gehrke *et al*. 1993 for information on the fish impacts of black water.)

- **Sediment disturbance.** Still, quiet waters (eg lakes and billabongs) that contain large quantities of aquatic vegetation or have excessive nutrient loads (from sewage, farm or industrial wastes) will accumulate thick, biologically active bottom sediments. If undisturbed, these sediments will remain in a "reduced" (ie highly reactive) state as insufficient oxygen is available for their full decomposition. This condition is evident if there is a strong smell of hydrogen sulphide ('rotten egg gas') when the sediments are disturbed. Normally, the overlaying water remains well oxygenated and aquatic life is not adversely affected. However, if these sediments are mixed through the water, then rapid deoxygenation will occur (an effect termed benthic biochemical oxygen demand). Turbulence following heavy rain or the influx of flood waters can mix sediments in lagoons or billabongs.

Pollution

Contrary to popular belief, pollution with artificial chemicals or substances is a relatively uncommon cause of fish kills with only 8% of kills in NSW attributable to this cause although it can be the most significant cause in some areas such as urban Sydney. However the sublethal effects of pollutants are cause for concern. Exposure to toxic substances may not result in immediate fish kills, but may affect fish populations by decreasing fecundity (number of eggs produced), reducing the viability of sperm, eggs and larvae, decreasing life expectancy, increasing the incidence of abnormalities and increasing natural mortality.

From its effects on aquatic life, pollution can be conveniently divided into three types: physical, industrial chemicals and agricultural chemicals.

- **Physical pollution.** This involves pollution by suspended solids such as coal washery waste, asbestos, paper or wood fibres. The gills of the fish can be blanketed by layers of the material preventing oxygen uptake. Mechanical damage due to sharp, angular particles can also seriously impair gill function. Fish kills from physical pollution are rare in New South Wales.

- **Industrial chemicals.** Most kills caused by these agents result from accidental or deliberate short-term releases of chemicals, often into stormwater drains. Some very large kills have resulted from this form of pollution. Fish are usually killed by direct poisoning, although acid or alkali releases can change the pH (the acidity of the water) so greatly that this may be the cause. The release of chemicals is often difficult to observe directly and since the source is often difficult to trace, prompt observation and reporting of the kill is necessary if industrial pollution is suspected. Discharge of chemicals (accidental or deliberate) is a violation of the Protection of the Environment Operations Act, and the Environment Protection Authority (EPA) should be notified immediately.
- **Agricultural pesticides.** Herbicides and insecticides used in agriculture have been responsible for a number of fish kills. Endosulphans used in the cotton growing areas have been particularly problematic over the last decade (Napier et al. 1998). Pesticides are often highly toxic to fish and may persist for long periods in dry soil, although many are degraded fairly rapidly (48 hours to 2 weeks) in water. They can enter waterways by overspray, wind-drift from aerial and ground spraying, washing of spray equipment in water bodies, deliberate dumping of out-of-date or empty drums into streams, release of irrigation tail waters and by runoff from recently sprayed land. There is little or no physical evidence of death resulting from pesticide toxicity and it is often difficult to prove pesticide contamination has resulted in fish deaths without water quality and/or fish tissue sampling.

Physical and chemical water quality changes

- **Acid Water.** Acid Sulfate Soils underlay many wetlands and floodplain soils adjacent to estuaries along the east coast. These soils contain high levels of iron pyrite which oxidizes to form sulfuric acid when it is exposed to the oxygen in the air. This most commonly occurs where wetland or floodplain soils are drained or excavated. Water trapped in drains can frequently be close to the strength of battery acid following prolonged dry spells. Rainfall can mobilize the sulfuric acid and transport it into adjacent waterways lowering the acidity of the waterway to lethal levels. High levels of aluminium dissolved from clay minerals in the soil by the acidic water and released into the waterway often result in a characteristic turquoise-bluish appearance at the same time. A pH level less than 4 will kill most species of fish, while levels between 4 and 6 will be stressful and may predispose individuals to diseases such as "Red Spot" or Epizootic Ulcerative Syndrome (EUS) . Further detail on Acid Sulfate Soils and their relationship to fish kills and disease can be found in Sammut and Lines-Kelly (1996), Creagh (1991), Green (1993) and Sammut *et al.* (1996) and references therein. Similar effects can be found in acid runoff generated from abandoned or derelict mine sites and poorly managed operating mines.

- **Water temperature.** Changes in water temperature, both rapid fluctuations and slow seasonal variations, can result in fish deaths. Generally, these only occur at the limits of a species distribution (both geographical and altitudinal) where individuals are likely to be exposed to temperatures above or below their tolerance levels. Kills caused by rapid temperature changes are rare and generally only occur in small waterbodies that heat and cool rapidly. A sudden cold snap can occasionally kill native warm water species (for example, Golden Perch and Eel-tailed Catfish) in farm dams or small streams. Rapid temperature changes due to human activities in localised areas of larger water bodies (for example, discharge of cooling water from electric power generators) have been responsible for fish deaths in some areas.

  Seasonal temperature changes can also result in fish kills. Warm water (greater than 27^0C) will kill trout, although it is rare to see large numbers of dead fish, and cold water will kill warm water species. An example of the latter is the large number of fish (mainly Bony Bream) that die each winter in the lower Murray River. The affected fish, weak from thermal stress, are susceptible to bacterial and fungal infections that eventually kill them.

- **Salinity.** Rapid salinity changes (for example, where a major storm causes a rapid inflow of freshwater into an estuary) can lead to fish deaths. Similarly, breaching of a sand barrier allowing saltwater to rapidly fill a lagoon can affect fish that have slowly become acclimatised to a relatively low salinity. Salinity increases as a result of evaporation of water in coastal and inland lakes, can also cause fish deaths.

Diseases and Parasites

Disease is usually a result of secondary infection. The primary precursor to disease is stress from physical or chemical factors such as lowered water temperature, rapid changes in salinity, chronic pollution or crowded conditions. Under these circumstances, resistance to disease is reduced and the fish become susceptible to infection. The much publicised 'red spot' disease in northern estuaries is often associated with floods that reduce salinity and cause acidic runoff (Callinan undated).

Australia is relatively free of many of the major fish diseases present overseas. One that is present and frequently causes deaths of juvenile Redfin and sometimes juvenile Rainbow Trout is Epizootic Haematopoietic Necrosis (EHN) virus (Langdon 1989). The much publicised death of millions of pilchards in coastal waters of southern Australia in May 1995 is thought to be due to a newly introduced viral disease (Whittington 1996).

Diseases rarely kill fish instantaneously, rarely affect the whole population and rarely affect all species to the same degree. Characteristically a kill due to disease will only affect one or two closely related species, occur over a period of days or weeks, and there will generally be some individuals that are only mildly affected or not affected at all.

Even healthy fish will carry a low level of parasite infection in the gut, gills and flesh. Fish that are under stress due to other factors may have reduced resistance to parasite infection and carry a much higher load. Examination of fish from a kill may indicate large numbers of parasites, but it would be wrong to assume that this was the primary cause of death. It may simply indicate that the fish was living under marginal environmental conditions while some other factor caused its death.

Detail of the impacts of fish diseases on native and introduced freshwater fish is given by Rowland and Ingram (1991) and Langdon (1989).

Dumping of Waste Fish or Bycatch

Although not a fish kill by the strict definition of the term, dumping of waste fish or bycatch is a commonly encountered problem which can give the impression of being due to other causes. Observations that might suggest that dumping is the cause include:

- fish species that are uncharacteristic of that location (eg deep sea species in inshore waters),
- predominantly non-commercial species,
- commercial species which are mostly less than the legal size limits,
- external damage such as net mesh marks or hook marks,

- fish of commercial species and size which are deformed or have obvious external disease lesions or sores which render them unmarketable.

3. Fish kill reporting and investigation

Government agency and local council staff are often required to investigate fish kills, provide advice on the cause of kills, and to collect specimens of fish and water for later analysis. There are good reasons why fish kills should be investigated and a cause determined including:

- Understanding the cause of a kill may help prevent a recurrence. This is especially relevant in an aquaculture situation or where endangered species are involved.
- There may be public health risks associated with a kill, especially where a disease is present within the fish population, or there has been contamination with some form of pollutant.
- A kill may be a sign of a fundamental problem with the habitat which needs to be addressed.
- There may be an opportunity for legal action against individuals or organisations for polluting a waterway.
- The kill may be due to a new disease organism and there is a need to implement quarantine measures or other treatments.

Initial reports of fish kills will often come as a telephone message from members of the public or those involved in the fishing industry. The report may come to a range of organisations including NSW Fisheries, Department of Land and Water Conservation (DLWC), Environment Protection Authority (EPA) and local government councils. Details sufficient to enable completion of **Part A** of the **Fish Kill Notification and Investigation Report** should be taken from the informant.

Fish kills have the potential to generate a large amount of community concern and media publicity. It is often important to manage this aspect of the kill. A decision needs to be made relatively early as to whether the kill is likely to generate large amounts of publicity. This would be the case where:

- large numbers of fish are involved,
- the site is highly visible to the public,
- pollution or contamination of the waterway is involved,

- rare or threatened species have been killed.

If the kill is likely to generate high levels of media publicity, then notification of Regional Managers, head office staff and media units should be undertaken.

As a matter of course other government agencies and local councils should be notified since:

- they may have already sent someone to investigate or they have someone nearby who can respond quickly,
- they may be aware of other circumstances (eg chemical spill) which is related,
- it may be appropriate for them to assist in any subsequent investigation, or they have access to equipment (eg for water quality sampling) which can be used in a subsequent investigation,
- they are then in a position to notify other people who ring in that they were aware of the report and can advise them whether the matter is/is not being investigated.

In all cases where chemical or physical pollution or contamination is strongly suspected the EPA should be informed.

Site inspection

The causes of a fish kill are almost invariably transitory. It is therefore important to act quickly. Deoxygenation or `slugs' of chemical only occur for a few hours and the effects are rapidly reduced by natural factors such as dilution or reoxygenation. It is therefore of little benefit (in many cases) to visit the site to take direct measurements of water quality, collect specimens, or collect water samples, if the fish have been dead for more than a day or so.

A site inspection should be undertaken if:

- the kill has involved rare, threatened or protected species,
- the fish kill involves a large number of fish (eg more than 40);
- there is a possibility that pollution is the cause and is still occurring;
- polluting agents are readily identifiable with legal action possible;
- mortalities are still occurring and there is a good possibility of isolating a cause.

When conducting site inspections the following readily available equipment should be taken -

- measuring tape for recording the size of fish,

- camera to record the habitat and the extent of the kill,
- rubber gloves for handling dead fish,
- new plastic bags (freezer bags) and wire ties for storing fish samples,
- labels, pencils and marker pens for labelling samples,
- Esky and ice or cold bricks (frozen) for transporting samples,

If it is available, water testing apparatus should also be taken. This would include meters or instruments for measuring pH, dissolved oxygen, turbidity, salinity and temperature, and sample bottles for collecting samples for later algal or chemical analysis.

During the site inspection, details sufficient to enable the completion of **Part B** of **the Fish Kill Notification and Investigation Report** should be recorded.

Determining the cause of a fish kill

There are a number of simple measurements and observations that can be made to assist in the presumptive diagnosis of kills. However, the causes of many fish kills result in no external symptoms or signs, and the causative agents are often transitory. Normal conditions may re-establish within hours. Diagnosis is therefore often by inference or by piecing together a number of shreds of evidence. Determining a cause may well involve a process of elimination resulting in one or two potential causes remaining as high probabilities.

Table 1 lists observations that might be made in the field, what these observations might indicate concerning the cause of the kill, and what further action should be taken to verify or disprove the preliminary indication.

OBSERVATION	MAY INDICATE	ACTION REQUIRED
• Only 1 or 2 species of fish are affected when many more species are known to be present in the waterway.	• Partial deoxygenation of the water column is killing susceptible species. • A disease specific to those species is responsible for kill.	• Measure dissolved oxygen levels. • Check fish for signs of disease. Collect samples for expert analysis.
• Fish die over a reasonably long period of time.	• A disease is responsible for deaths.	• Check fish for signs of disease. Collect samples for expert analysis.
• Sick or unhealthy fish are also present with the dead.	• A disease or toxin is responsible.	• Collect samples for expert analysis.
• Dead fish with mouths agape, gills flared and heads bent back. Gills are deep bluish - red in colour.	• Deoxygenation.	• Measure dissolved oxygen levels.
• Fish apparently died overnight and/or live fish are swimming at surface and gulping air, especially around margins of waterway and in the early morning.	• Deoxygenation due to respiration by abundant aquatic plants (may be microscopic algae or submerged macrophytes).	• Measure dissolved oxygen levels. • Check for abundance of aquatic plants.
• Kill occurred during or after period of extreme cold or frost, **&** only cold intolerant species or those at the southern geographic or upper altitudinal limit of their distribution are affected, **&** kill occurred in a small water body (eg dam, pond, creek pool)	• Lethal low temperatures may be responsible.	• Measure water temperatures and compare result with known temperature tolerance limits for affected species.
• Kill occurred during/after period of extreme heat, **&** only heat intolerant species (eg trout) or those at the northern geographic or lower altitudinal limit of their distribution are affected, **&** kill occurred in a small water body (eg dam, pond, creek pool).	• Lethal high temperatures may be responsible.	• Measure water temperatures and compare result with known temperature tolerance limit for affected species.

• Kill occurred in proximity to cooling water outlet.	• Lethal high temperatures may be responsible or some toxic substance has been released with the cooling water.	• Measure water temperatures (near and away from influence of outlet) and compare results with known temperature tolerance limit for affected species.
• Kill occurred after heavy rain or flooding.	• Rapid change in water quality parameters (eg. pH, turbidity, salinity) may be responsible.	• Test water for pH, salinity and dissolved oxygen levels.
• Kill occurred in estuary after heavy rain or flooding and smell of "rotten egg" gas is apparent.	• Release of hydrogen sulphide gas from sediments following lagoon breakout.	

OBSERVATION	**MAY INDICATE**	**ACTION REQUIRED**
• Gas bubbles (often very small) visible on head or in fins (when held up to light) **&** kill occurred in vicinity of dam/weir outlet, or aeration device (eg in aquaculture ponds).	• Gas bubble disease.	• Collect samples for expert analysis.
• Dead fish have burst swimbladders.	• Explosives have been used in the water.	• Investigate legality of use of explosives
• Fish have obvious lesions on body.	• Disease (may be related to poor water quality).	• Collect samples for expert analysis and disease identification.

• Dead fish infected with grey/white fungal growth.	• Fish have been dead for a number of days and have suffered from a secondary infection (*Saprolegnia*).	•
• Only small fish (less than legal length) of commercial species are affected or mainly non-commercial species are present. • Dead fish are not characteristic of that waterbody. • Dead fish are of commercial species and size but exhibit obvious physical damage.	• Discarded waste or bycatch from fishing operations.	• Contact local fishers to verify.
• All species and size classes expected to occur in the waterway are dead.	• Poison, pollutant or contaminant has entered waterway, **or** • Severe deoxygenation.	• Collect water and fish samples. • Measure dissolved oxygen levels.
• The kill is confined to a relatively small section of waterway.	• Poison, pollutant or contaminant has entered waterway at one point and then dispersed or been diluted, **or** • Deliberate poisoning.	• Collect water and fish samples.
• Kill occurred in an estuary flanked by land that has flood mitigation works, **&** followed a reasonably major rainfall event which caused runoff from the land **&** estuarine waters now have a clear turquoise-bluish appearance.	• High aluminium and acidic (low pH) conditions associated with acid sulfate drainage.	• Collect water samples and/or test pH levels.
• Deaths occurred in water that has reddish appearance.	• Dinoflagellate (red-tide).	• Collect water samples for analysis.

OBSERVATION	MAY INDICATE	ACTION REQUIRED
• Deaths occurred in water that has a black coloration.	• High lignin or tannin content from rotting vegetation, especially bark and leaves from native species.	
• Kill occurred during or following flood recession.	• Receding floodwaters have carried large quantities of organic material into the waterway which has decomposed causing deoxygenation **or** • Deoxygenated water stored on the floodplain has re-entered main water body.	• Measure dissolved oxygen levels.
• Fish are washed up along the shoreline.	• Kill occurred some time previously or elsewhere and fish have been blown on shore, **or** • Fish have stranded themselves following predator attack, **or** • Fish have been discarded into shallow water.	

• Fish all of one species (especially "prey" species such as anchovies, pilchards, yellowtail, frigate mackerel) and relatively uniform size.	• Stranding from predator attack.	
• Kill occurred in water which has a greenish coloration with or without green scum.	• Toxins associated with a Blue-green algae bloom are responsible. • High levels of algae in water have contributed to deoxygenation	• Collect water samples. • Notify DLWC for potential notification of toxic algae bloom • Measure dissolved oxygen levels.
• Kill occurred in industrial area.	• Possible pollutants from deliberate discharge or accidental spill.	• Contact EPA and/or local Council for joint investigation.
• Kill occurred in an urban area.	• Pollutants or sewage overflow has caused or contributed to kill.	• Contact EPA and/or local Council for joint investigation.
• Kill occurred in agricultural/horticultural area where crops are grown.	• Possible pollutants or pesticides.	• Contact EPA and Department of Agriculture for joint investigation.

Collections of samples from a fish kill

Collection of samples (both fish and water) is particularly important when the cause of the kill is suspected to be related to a pollutant, contaminant or disease. In many cases, samples can be tested weeks or even months later to help determine or eliminate potential causes although some contaminants such as chlorine will evaporate quickly and need to be tested on site. If, for example, evidence comes to light 2 or 3 days after an investigation, that a pesticide or other contaminant or disease is to blame, it is preferable to have samples on hand properly preserved and available for analysis, than to have nothing. This is not to say however, that samples should always be collected. If the cause is quite **clearly** due to some environmental problem or mishap, collecting samples probably serves little useful purpose unless the matter is likely to be subject to legal prosecution and samples are required as evidence.

Fish

Fresh specimens (preferably still showing signs of life) should be collected. Specimens are required of all the common species (i.e. those representing more than 30 per cent of the kill). Eight specimens of each are required - two for physical/pathological analysis (**which should be refrigerated or preserved in formalin but not frozen**) and six for chemical analysis (which can be frozen). Individual fish should be placed into individual new plastic bags (freezer bags or zip seal bags), sealed and labelled (collector's name, date, locality, species).

To preserve fish in formalin

1. Obtain 100% formalin (equivalent to 38% formaldehyde),
2. Dilute this to 10% - ie 1 part formalin and 9 parts water,
3. Open body cavity of fish by cutting from vent to gills,
4. Immerse fish in formalin mixture for 48 hours,
5. Remove, drain and wrap in formalin moistened paper and seal in a plastic bag.

When using an 'Esky' avoid having the fish slopping around in the bottom with half-melted ice. Use 'cold bricks' or ice in plastic bags to avoid contamination from water. As soon as possible, forward fresh specimens to the relevant laboratory. A cold, well insulated fish will remain fresh for several hours and, provided air freight is used, there should be no difficulty in ensuring that the specimens arrive in a condition suitable for analysis. **Notify the receiving laboratory of the date, flight number and arrival time of the specimens.** There is less urgency with frozen and formalin preserved specimens and firm arrangements should be made with the analytical laboratory before shipment.

Water

Collecting water samples for quality testing can be a specialised field requiring standardised procedures and specific equipment. However many fish kill investigations can be adequately carried out without collecting water samples - for example in those instances where field instruments are used to measure physical parameters such as temperature, DO, pH and turbidity on site.

Where pollution of the waterway by some toxic substance is suspected it will be necessary to collect water samples to have them analysed in a laboratory at a later date. It is important to avoid the possibility of contamination and maintain "chain of custody". Given that it may be necessary to respond to a kill report at short notice, the correct materials and equipment needs to be on hand. In general, specialist assistance from an agency that routinely undertakes water quality sampling and testing such as EPA, local government, or DLWC is advisable.

The best guide is "**Water Sampling Protocols - A Training Manual for NSW Fisheries in the Collection of Water Samples for Fish Kill Investigations**" prepared by the Science Management and Support Section, Environmental Science Branch of the EPA.

Algae

In some fish kills there is a thick scum of algae (green, red or brown) or the water is a bright green colour (occasionally a red-brown colour, which is obviously not silt). Collect a small amount of the scum or about 0.5 L of the green water. Label the specimen as 'algal sample' and include the information regarding the site from where it was collected and the date. It may be necessary to "fix" the sample if it is to be stored for more than a few hours - refer to the Department of Land and Water Conservation for advice.

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