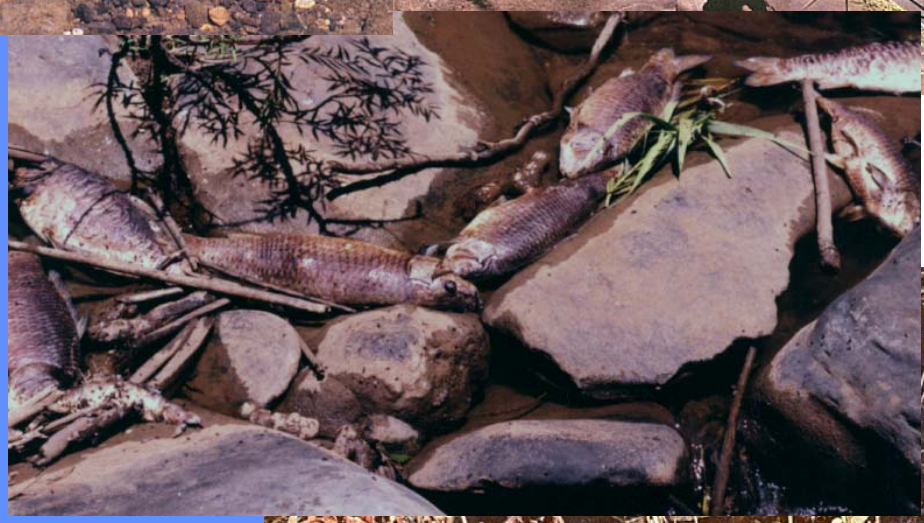
FIELD GUIDE TO FISH KILL ASSESSMENTS

B.C. Hohls and A.L. Kühn



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1. Introduction

This document is intended to provide background to the phenomenon of fish kills and to provide a methodology to be followed when encountering or investigating such a kill. The report consists of background and theory relating to fish kills and fish kill investigations, a questionnaire that will assist in the gathering of pertinent information when investigating a fish kill, and also presents a case study conducted by members of the Institute for Water Quality Studies. Furthermore, the method of calculating unionised ammonia of Dr Peter Wade is also included in an Appendix.

Fish kills take place due to both natural and man-induced causes. They may involve just a few fish or many thousands of fish. Fish kills are not restricted to inland water bodies and rivers. Estuarine and marine environments also present a backdrop for fish kills. Fish kills may occur in these environments where rapid changes in salinity can result in kills of species not adapted to the new salinity changes or the pace at which the changes take place. The significance of a fish kill is not always directly related to the size of the kill, but may be related to economic, geographical, and political factors associated with the site, as well as the ecological effects (Hale, 1996).

Fish kills are a worldwide phenomenon, with kills being reported in the local and international news media.

2. Common Causes of Fish Kills

Mortality from natural causes is the largest single cause of death of individual fish in a population (Hale, 1996). Factors that have been identified in natural fish kills include: oxygen depletion, gas supersaturation, toxic algal blooms, turnover of the water column, toxic gases, natural toxic substances, sudden or excessive temperature changes (for example, as a result of hail), salinity changes, lightning, bacterial infections, fungi, viruses, parasites, and others.

Outbreaks of bacterial disease in a fish population generally involves three factors: susceptible hosts, pathogenic organisms, and predisposing environmental conditions (Hale, 1996). These conditions can include crowding, inadequate food supply, spawning activity, storms, or seasonal changes. Kills caused by bacteria are seldom sudden, but usually there is a gradual build-up of fish losses. Death caused by viruses, fungi or parasites may require histological examination by trained biologists or veterinarians.

However, non-natural man-induced factors/practices are also responsible for fish kills. These practices include the discharging of pesticides, fertilisers and other chemicals into water bodies that may act directly on the fish, or indirectly by, for example, resulting in a sudden decrease in available dissolved oxygen.

3. Examples of Fish Kills

As a means of showing a selection of the possible causes of fish kills experienced worldwide, the following examples are given. Within the Jacksonville, Florida, Metropolitan area (Scanlan, 1997), a fish and snake kill was possibly due to a chemical discharged in to the stormwater system.

In Falls Lake, Durham, North Carolina, several thousand fish died, with some even found stuck in trees after high flows subsided (Cochran and Shiffer, 1996). There was no evidence of a sewage or chemical discharge, and swamp water and decomposed storm debris was thought to have disrupted oxygen levels so severely that fish couldn't breathe. However, downstream of Falls Lake along the Neuse River, sewage and pig waste mixed with swamp water to further exacerbate the situation.

Low water temperature was implicated in a fish kill in Lake St. Lucia in 1987 (Cyrus and McLean, 1996). A fish kill in Heart Lake, Ontario, was associated with the collapse of a massive population of dinoflagellates (Nicholls and Kennedy, 1980).

Changing pH has also resulted in fish kills. Natural acid water conditions resulted in a large fish kill in Australia (Brown, Morley, Sanderson, and Tait, 1983). Fish kills have occurred at low pH in a Norwegian river (Leivestad and Muniz, 1976), and it is also known that elevated pH and temperature results in a higher proportion of available ammonia being in the toxic un-ionised form (DWAF, 1996). Un-ionised ammonia is produced naturally by the biological degradation of nitrogenous matter and provides an essential link in the nitrogen cycle, however, this form of ammonia is very toxic to fish (the ammonium ion, NH_4^+ has little or no toxicity to aquatic biota (DWAF, 1996). Temperature and pH are modifying factors that alter the acute toxicity by altering the concentration of un-ionised ammonia in the water through changes in the ammonia-ammonium ion equilibrium, or may increase the toxicity of the unionised form to aquatic organisms (DWAF, 1996). Although temperature has a significant effect on the proportion of ammonia that is in the unionised form, pH has an even greater effect. At a constant pH, say pH 7.5, a change in water temperature from 15 °C to 25 °C results in an increase in the proportion of unionised ammonia from 0.85 to 1.7 %. In contrast, at a constant temperature, say 25 °C, increasing pH from 7.5 to 8.5 results in an increase in the proportion of unionised ammonia from 1.7 to 15 % (refer to Table 1). According to the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996), the target water quality range (TWQR) for unionised ammonia is = 7 μg N.ℓ^{-1}. The Chronic Effect Value (CEV) is 15 μg N.ℓ^{-1}, while the Acute Effect Value (AEV) is 100 μg N.ℓ^{-1} (DWAF, 1996).

Table 1. Contribution of un-ionised NH_3 to total ammonia (expressed as a percentage) as a function of pH value and water temperature (DWAF, 1996)

pH	Water Temperature (°C)							
	0	**5**	**10**	**15**	**20**	**25**	**30**	**35**
6.0	0.0083	0.012	0.019	0.027	0.039	0.056	0.079	0.11
6.5	0.026	0.039	0.059	0.086	0.12	0.18	0.25	0.35
7.0	0.083	0.12	0.18	0.27	0.39	0.56	0.79	1.1
7.5	0.26	0.39	0.58	0.85	1.2	1.7	2.4	3.4
8.0	0.82	1.2	1.8	2.6	3.8	5.3	7.3	9.9
8.5	2.6	3.8	5.5	7.9	11	15	20	26
9.0	7.6	11	16	21	28	36	44	52
9.5	21	28	37	46	55	64	71	78

Take note, if you have access to a spreadsheet computer package, or are not scared to engage yourself in some lengthy calculations, the UIA calculation method of Dr Peter Wade appears in an appendix at the end of this document. It provides a more accurate means of calculating the UIA concentration than that of Table 1 in that it does not require interpolation between the discrete water temperature and pH values.

4. Other Factors Involved in Fish Kills

Species, and ages, of fish vary in their susceptibility to toxic substances, and so a progression of selectivity among fish species and/or ages is usually evident. Juvenile fish may be more likely to be negatively effected by lower concentrations of chemical toxins than adults, whereas adults may be quicker to show signs of distress as a result of declining dissolved oxygen levels. Such an observation made on the site of a fish kill can aid in directing the investigators' attention to the possible nature of the kill. Laboratory analyses are usually

necessary to verify the presence of a toxic substance. Slow but continuous fish kills often occur even when all environmental factors appear to be normal. Such kills may be in a location adjacent to areas where chemicals are used or stored and where chronic exposure to sublethal levels of the chemical are possible. Many factors can modify the toxicity of a chemical and it is, therefore, important that physical parameters such as pH, dissolved oxygen, temperature and conductivity are made during the investigation of a fish kill. The collection of a toxicity sample and the utilisation of fish in conducting a toxicity test can guide the process of investigation and the determination of the cause of the fish kill. Indeed, adequate sampling involving the taking of a macro-, trace metal, and toxicity samples go a long way towards providing indicators as to the factors resulting in a fish kill. However, there are still times when the cause of a fish kill cannot easily be determined. This is especially true if a lengthy period of time has elapsed between the fish kill taking place and the start of the investigation.

Because of the broad range of possible causative factors in a fish kill, the investigating team should ideally come from a variety of disciplines and should gather as much information as is possible in order to attempt to determine the natural or man-induced reason for the kill.

5. Investigation and Reporting Procedure

The following section describes the procedure that should be followed when alerted to the occurrence of a fish kill.

As a member of the field staff of one of the Department of Water Affairs Regional Offices, a fish kill will either be seen by you, or it will be brought to your attention. It must be reported to the Regional Director responsible for the location in which the fish kill takes place as soon as possible. The following information must be supplied as completely as possible when reporting a fish kill:

a) The name, address, and telephone number of the person who reported the fish kill (ideally also get them to accompany you on the investigation).
b) An estimate of the number of dead or dying fish.
c) The time when the fish kill was first noticed.
d) What other types of organisms were affected by the suspected pollutant.
e) The species of fish affected.
f) The size of the fish affected.
g) The size of the area affected.
h) Possible causes, for example: a spill, oxygen depletion, sudden drop or rise in temperature, etc.
i) What possible sources of pollution are in the area, for example, agriculture, sewage works, industries, road works, accidents, *et cetera*.

[A Fish Kill Questionnaire has been included in this report to aid the investigator to record the necessary information and to assist in making useful observations.]

This information will enable the person to whom the kill was reported to decide whether to send people to investigate the incident or not (Badenhorst, 1993). If an investigation is indicated, then the incident must be investigated as soon as possible. The Regional Office must be informed about the fish kill if they are not already aware of it. Ideally, a water pollution control officer should also be present during the investigation. Communication Services should also be made aware of the kill.

The following equipment should be taken along on the investigation:

a) Dissolved oxygen meter
b) pH meter
c) EC meter
d) Sample bottles for: Macro (and preservative), Trace Metals, Organic, Toxicity, Bacteriology, and Algal identification
e) Cooler box and ice bricks
f) Kit to examine residual chlorine
g) De-ionised water and paper towels
h) Note pad, pen, pencil, marker pen, labels for bottles and masking tape
i) Aluminium foil and plastic bags
j) Plastic beaker
k) Stainless steel bucket
l) Scoop net
m) Maps (1:50 000) of the area
n) Rubber boots or waders
o) Large plastic bags and/or a plastic tray to put fish samples in
p) A camera with sufficient film or digital storage media (a video camera would also be very useful)
q) Boat (if the fish kill is away from the shoreline of a dam)
r) A GPS (Global Positioning System - an instrument with which to record the latitude and longitude of the fish kill site and the location of any other noteworthy features). This is a very important piece of equipment in order that the sample site may be registered on the Water Management System (WMS) where precise geographic co-ordinates are required.

It is important to liaise with the relevant laboratories to ensure that they are on stand-by to be able to process the various samples. In some cases (for microbiological samples) sample media must be prepared prior to the samples being delivered. The different microbiological indicator organisms have individual growth media requirements.

The extent of the kill must be determined/verified once on site, and an attempt must be made to estimate as precisely as possible the time that the incident started (Badenhorst, 1993). The investigation process requires a high level of observation and discrimination in order to look for signs that do not look natural, such as excessive turbidity, unusual water colouration, odours, algal blooms, unusual state of submerged/emergent vegetation, the absence of aquatic macroinvertebrates etc. The Fish Kill Questionnaire (attached) will guide the process of making and recording observations. Gather as much information as possible by consulting with the farmers and other people in the area. In an agricultural area, find out if crop spraying has occurred recently, and if so, what chemicals/pesticides were used (and how the containers were cleaned and maintained). Decide on where to take samples and take a complete range of sample types, ensuring to take samples in water that you believe to be contaminated as well as that which is away from the location of the fish kill. When the likely cause of the fish kill can be determined in the field, it may not be necessary to take a complete range of samples; macro-, trace metals and toxicity samples may be adequate.

In those situations where it is unclear what the cause of the kill is, in addition to water quality samples and observations made at the site, fish samples should also be collected. Where dead and dying fish are present together, collect a number of dying fish of each species and size, the New South Wales (Australia) EPA recommends that 10 of each species be collected (NSW EPA, undated). These should be chilled and transported to the appropriate laboratory as soon as possible. The choice of dying fish over dead fish is made so that putrefaction does not mask the cause of death.

It is also important to note the behaviour of the affected and dying fish (and other aquatic organisms). Observations should relate to the presence or absence of: injuries; lesions; gasping; loss of equilibrium; erratic behaviour; flared gills; attempts to leave the water; lethargy; convulsions; and other indications of distress (Hale, 1996). It is also important to note what other organisms are affected and what organisms are present in the area that do not appear to be affected (such as macroinvertebrates on/under rocks or on vegetation and fish of different ages/sizes than those affected).

It is a very good idea to take many photographs as well as video material of the affected fish and the surroundings for later study. In the case of fish and scums, etc., the photographs should be taken as close up as possible to the specimens so that they are clearly identifiable and show any features of special concern.

The completed Fish Kill Questionnaire (attached) must be copied and sent to the Director: Institute for Water Quality Studies, Department of Water Affairs and Forestry, Private Bag X313, PRETORIA, 0001. Once the results of laboratory analyses have been obtained, a fish kill report must be written and the findings of which must be provided to the news media through Communication Services.

An example of a fish kill investigation report is included as a guide to assist in writing a report on the incident. It must be borne in mind that each fish kill incident is unique and will have different difficulties and causative factors.

It should also be borne in mind that a significantly large proportion of fish kills may result without the influence of man.

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7. Acknowledgement

Mr Dana Grobler, formerly a staff member at the IWQS and now a consultant to DWAF, is hereby acknowledged for his contribution to the initial draft fish kill assessment investigation document that was prepared jointly with the primary author of this document.

FISH KILL QUESTIONNAIRE

GENERAL INFORMATION

WHO IS THE INFORMANT?

NAME:_____ TELEPHONE: (____)_____
CELLULAR PHONE: _____
ADDRESS: _____
_____
DATE AND TIME REPORTED: _____

REPORTING SOURCE

NAME OF OFFICIAL/INVESTIGATOR: _____
ORGANISATION: _____
ADDRESS: _____
TELEPHONE: (W): (____)_____ CELL: _____ (H): (_____)_____
DATE AND TIME OF INVESTIGATION: _____

SITE INFORMATION (Details of the site of the fish kill)

Type of water body: Stream ? Reservoir ? Other ?
Wetland ? Ocean ?
Name of water body: _____
Latitude: _____ S Longitude: _____ E
Has a fish kill been observed at this site before? Yes ? No ? Unsure ?
Duration of the kill (first noticed and ended) - Date and time: _____
Extent of the kill or the area covered (kilometers of river or size of pond or reservoir):
_____
Approximate total number of fish affected: 10-100 ? 100-1 000 ? 1 000-10 000 ?

Species affected (please specify)	Number	Size (length – min and max)

Behaviour of the affected fish (indicate which is applicable)
- Rate of mortality was abrupt and most fish died within 24 hours ?
- Small fish died first ?
- Large fish coming to the surface and gulping for air ?
- Small fish alive and well ?
- Jerky movements of fins ?
- Fish hyperexcited ?
- Fish swim erratically ?
- Any other behavioural observation _____

External appearance of affected fish (any abnormalities): _____
_____
Own opinion as to the cause: _____
_____
_____

Known recent activities in the surrounding area (crop spraying, weather change, etc.): _____
_____
_____

Possible sources of pollution: _____
_____
_____

Field measurements (at the same locations as samples taken):

Variable Measured	Units	Upstream	In Kill Area	Downstream
Temperature	°C			
pH	pH units			
Electrical conductivity				
Dissolved Oxygen				
Odour/Colour/Foam	-			

Collected sample information:

	Upstream	In Kill Area	Downstream
Water sample (major inorganic)			
Water sample (trace metals)			
Water sample (organic)			
Water sample (toxicity)			
Fish sample (fresh)			
Fish sample (frozen)			

General remarks:

_____
_____
_____
_____
_____

COPY FOR YOUR RECORDS AND SEND A COMPLETED QUESTIONNAIRE TO:
The Director: Institute for Water Quality Studies
Department of Water Affairs and Forestry
Private Bag X313
PRETORIA
0001

A Case Study

TITLE	**:**	*Fish Kill in the Apies River: 6th October 2000*
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Table of Contents

1. Introduction and Background

This report relates to the request by Mr L. van Niekerk, a farmer on the banks of the Apies River, to Mr B. Hohls of the Institute for Water Quality Studies. He requested that the IWQS provide assistance in determining the possible cause, or contributing factors, of a fish kill in the Apies River reported on 6th October 2000. Mr J. Daffue of the Gauteng Regional Office of DWAF was informed of the fish kill and notified of the IWQS's intention to assist with the water quality sampling. Mr B. Hohls and Mr H. van Niekerk of the IWQS conducted the investigation on 6th October 2000.

It was reported that fish, including carp and "onderbek" (possibly Silver Labeo) and other fish of varying sizes, had been dying for a number of days prior to the fish kill being reported to the IWQS. It was reported that many dead fish had already been removed from the Apies River prior to the investigation by the IWQS for aesthetic reasons. It was reported that there had not been a fish kill in the river during the preceding number of years.

Water quality samples and physical measurements described in the following sections of the report were taken at the locations indicated in Figure 1. Site 1 corresponds to Mr Van Niekerk's farm where the fish kill was initially investigated, and where the dead fish were evident on 6th October 2000. Site 2 is approximately 1 km downstream of the Rooiwal Water Care Works (WCW) and the Rooiwal power station, and 10 km upstream of Site 1. Site 3 is situated immediately downstream of the Bon Accord Dam. In between Site 2 and Site 3 there are a number of industries, including an abattoir and a plant nursery. Dead fish were only in evidence in the vicinity of Site 1 during the investigation on the 6th October 2000.

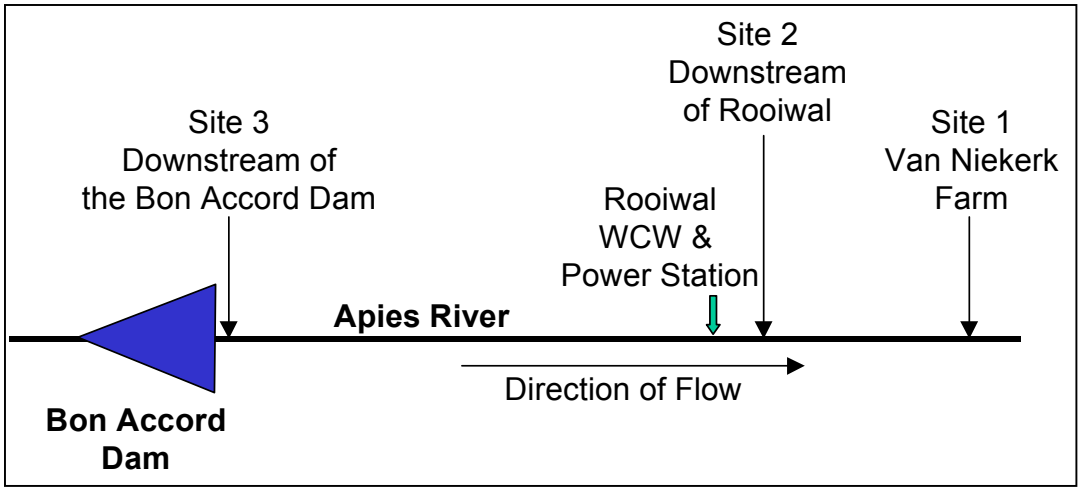

Figure 1. Diagrammatical representation of the sampling sites

Various verbal reports indicated the possibility of the spraying or discharge of a herbicide or pesticide upstream of the fish kill site (unconfirmed) and also of maintenance conducted on the cooling towers at the Rooiwal power station at the time of the fish kill (unconfirmed). It is possible that the herbicide or pesticide containers may have been rinsed in the Apies River.

The following sections outline the sampling and various analyses that were conducted at the three sites. This is followed by conclusions and recommendations made by the authors.

2. Sampling and Analysis Conducted

Various water quality samples were taken at the three sites indicated in Figure 1. These samples were used to determine the concentration of major inorganic constituents, micro inorganic constituents including trace metals, chemical oxygen demand (COD), bacteriology, the presence or absence of algal toxins, a general scan of the organic constituents, and the toxicity of water samples. Temperature, pH and dissolved oxygen readings were taken on site. A large carp that died while the investigation at the Van Niekerk Farm (Site 1) was underway was taken to the pathology laboratory at Onderstepoort for histopathological examination. A sample for organic constituent analysis was only taken at Site 1, not at Sites 2 and 3.

3. Analytical Results and Discussion

Detailed results of the various water quality samples and measurements taken at the three sites are listed in the following sections.

3.1 Physical Measurements

Physical measurements were taken of as many variables as possible with the available field instrumentation, especially of those variables such as temperature, dissolved oxygen, and pH that are prone to change prior to analysis in the laboratory. The values for these variables are listed in Table 1.

Table 1. Measurements taken at the three sites on 6th October 2000

Variable	Site 1. Van Niekerk Farm	Site 2. Downstream of Rooiwal	Site 3. Downstream of the Bon Accord Dam
Temperature (°C)	22.5	22.4	21.5
Dissolved Oxygen (%)	52.2	51.0	51.7
Dissolved Oxygen (mg.ℓ^{-1})	4.48	4.44	4.57
pH	8.7	8.3	8.6
Conductivity (mS.m^{-1})	68.0	67.2	51.0
Latitude of sampling site	25° 28’ 47.3” S	25° 32’ 14.6” S	25° 37’ 07.5” S
Longitude of sampling site	28° 15’ 29.9” E	28° 14’ 03.9” E	28° 11’ 44.1” E

Dissolved oxygen concentrations of 80 – 120 % of saturation are considered to constitute the Target Water Quality Range for aquatic ecosystems (DWAF, 1996a). The minimal allowable dissolved oxygen values according to DWAF (1996a) are not less than 60 % for sub-lethal effects and not less than 40 % for lethal effects, respectively. The sub-lethal value relates to the 7-day mean minimum, and the lethal value relates to the 1-day minimum. According to DWAF (1996a), both the 7-day minimum and the 1-day minimum should be used together. It is stated that the violation of these minimum values is likely to cause acute toxic effects on aquatic biota.

The low dissolved oxygen levels that were recorded at all three sites in the Apies River are indicative of water quality problems and the oxygen levels are low enough to be problematical to the survival of fish. It was unexpected to find such low dissolved oxygen levels, especially when taking into account the turbulent nature of the flow evident in the Apies River in the vicinity of Site 1. Site 1 was characterised by a number of areas containing

riffles. Some chemical or biological reaction must have resulted in the dissolved oxygen levels being depleted.

Assuming that the temperature and dissolved oxygen readings taken during the investigation are representative of those during the preceding days and weeks, it is expected that the fish would have been under stress and that would have made them more susceptible to additional stressors.

3.2 Major inorganic constituents

The major inorganic constituents analysed by the IWQS are listed in Table 2, together with aquatic ecosystem guidelines (DWAF, 1996a) where they are available.

Table 2. Major inorganic constituent results of samples taken on 6th October 2000

Constituent	Site 1. Van Niekerk Farm	Site 2. D/s of Rooiwal	Site 3. D/s of the Bon Accord Dam	Aquatic Ecosystem Guideline/ Category
pH	8.4	8.1	8.2	pH change should not be > 5 %
Kjeldahl nitrogen as N	1.47	1.57	1.44	Mesotrophic
Ammonium (NH_4^+) as N	0.17	0.39	0.40	
Nitrate + nitrite as N	4.03	4.05	0.72	Eutrophic at sites 1 & 2, Mesotrophic at 3
Fluoride as F	0.3	0.3	0.2	TWQR ≤ 0.75
Alkalinity as $CaCO_3$	203	199	170	
Sodium as Na	70	67	33	
Magnesium as Mg	17	17	23	
Silicon as Si	9.0	9.1	6.2	
Total phosphate as P	1.107	0.864	0.044	Hypertrophic at sites 1 & 2, Eutrophic at 3
Orthophosphate as P	1.000	0.500	0.043	Hypertrophic at sites 1 & 2, Eutrophic at 3
Sulphate as SO_4	62	60	54	
Chloride as Cl	60	64	47	
Potassium as K	11.4	11.2	6.1	
Calcium as Ca	50	49	42	
EC ($mS.m^{-1}$ at 25 °C)	75.7	74.7	58.6	
Total Dissolved Salts (TDS)	540	531	416	TDS should not change by > 15%

- Concentrations are in $mg.\ell^{-1}$ except for pH and EC

The un-ionised ammonia (UIA or NH_3) concentration was calculated according to the method of Wade (1999) using the NH_4^+ concentration, pH, temperature, and electrical conductivity (EC). The UIA (NH_3) concentration at the Van Niekerk Farm Site (Site 1), the site downstream of Rooiwal (Site 2), and at the site downstream of the Bon Accord Dam (Site 3) were 0.02006 $mg.\ell^{-1}$ NH_3, 0.024351 $mg.\ell^{-1}$ NH_3, and 0.029183 $mg.\ell^{-1}$ NH_3, respectively. The Target Water Quality Range for UIA is less than 0.007 $mg.\ell^{-1}$ NH_3, with the chronic effect value being 0.015 $mg.\ell^{-1}$, and the acute effect value being 0.100 $mg.\ell^{-1}$ NH_3. The concentrations at all three of these sites are, therefore, above the chronic effect value for an aquatic ecosystem (DWAF, 1996a). The UIA concentration was highest at the site downstream of the Bon Accord Dam (Site 3). It was, therefore, possible that the conditions

within the impoundment itself, or those upstream of the impoundment, favoured the conversion of ammonium ions to the more toxic un-ionised ammonia. The conversion process is favoured under conditions of higher water temperature and elevated pH.

If the UIA concentrations recorded on the 6th October 2000 are indicative of the situation over the long term, then un-ionised ammonia could have contributed to the fish mortalities. This could be thought of as being sufficient reason for fish dying over a protracted period of time even in the absence of any other factors (such as the low dissolved oxygen levels mentioned previously).

From Table 2 it would appear that there is a point source of nitrogen between Site 3 and Site 2. There was a dramatic increase in the nitrate-nitrite concentration on going from Site 3 to Site 2. The high concentration was also found at Site 1, albeit at a fractionally lower concentration. It is likely that the Rooiwal WCW was a large contributor to the high nitrogen levels evident at Sites 2 and 3.

A progressive increase in total phosphorus and orthophosphate was also evident from Site 3 to Site 1 (Table 2). In contrast to the situation evident with the nitrogen concentrations mentioned above, it is likely that the source of the phosphorus was diffuse.

The Total Dissolved Salt (TDS) concentrations showed a reversal of the trend evident for UIA concentrations. The lowest TDS concentration was recorded downstream of the Bon Accord Dam (Site 3), with a higher TDS at the site downstream of the Rooiwal WCW and the power station (Site 2). The highest TDS concentration was recorded at the Van Niekerk Farm site (Site 1), however, the TDS concentration was only slightly higher than that recorded at Site 2.

3.3 Acute Toxicity Tests

Samples for toxicity analysis were taken at the three sites on the Apies River. The samples taken at all of the sites resulted in 0 % mortality of 17 to 18 day-old *Oreochromis mossambicus* when exposed to 100 % sample concentration for 96 hours. The water samples at these locations could not, therefore, still be considered to be toxic at the time of sampling on 6th October 2000. The fish deaths were, therefore, most likely a result of some contaminant or event that had passed through the system by the time that the sampling was conducted.

3.4 Bacteriological Determinands

Water samples for the determination of faecal contamination were taken at all three of the sites sampled on the Apies River. The samples were analysed for faecal coliform counts, faecal streptococci, and *Escherichia coli* (*E. coli)* (reported as counts per 100 mℓ sample). The faecal coliform to faecal streptococci ratio provides an indication of whether the faecal contamination is of human or animal origin – values greater than 4 indicate contamination of human origin, while values less than 0.7 indicate contamination of animal origin. The results of the bacteriological analyses appear in Table 3.

Table 3. Results of the bacteriological analyses on the samples taken at the three sites

Microbiological Indicator (counts per 100 mℓ)	Site 1. Van Niekerk Farm	Site 2. D/s of Rooiwal	Site 3. D/s of the Bon Accord Dam
Faecal coliform (FC)	315	1620	20
Faecal streptococci (FS)	555	780	50
Escherichia coli	299	1458	20
FC:FS Ratio	0.568	2.077	0.400

Escherichia coli is not pathogenic to fish (DWAF, 1996b). There are no guidelines for bacteria in aquatic ecosystems. The microbiological levels do, however, provide an indication that human and animal waste is present in the Apies River. This is not unexpected due to the Rooiwal WCW located upstream of Site 2 and the numerous farms adjacent to the Apies River in this area.

There are tentative guidelines for bacteria in the aquaculture guidelines (DWAF, 1996b) that state that aquaculture in domestic waste water has been a practice for a long time, and that in India, for example, fish are cultured in sewage ponds without apparent detrimental effects to the fish. Therefore, it is highly unlikely that bacteria from human origin could have been responsible for the fish kill.

The full-contact recreational guidelines for bacteria (DWAF, 1996c) place the 315 faecal coliform counts per 100 mℓ water sample at the Van Niekerk Farm (Site 1) in the "risk of gastrointestinal illness" category, showing unsuitability of the site for swimming or other full contact recreation. For intermediate contact recreation, this value is within the Target Water Quality Range.

The ratios of faecal coliforms to faecal streptococci were: 0.568 at Site 1; 2.077 at Site 2; and 0.400 at Site 3. This indicates that the microbiological contamination is more likely to be of animal origin at Sites 1 and 3, but more likely of human origin immediately downstream of the Rooiwal WCW and Power Station at Site 2. This confirms what would be expected taking the landuse into account.

3.5 Trace Metal Analyses

Samples for trace metal analyses were taken at each of the three sites in order to determine whether trace metal concentrations could have been at levels that would have resulted in, or contributed to, the fish kill. The results of the analyses appear in Table 4.

Table 4. Trace metal concentrations recorded at the three sites

Constituent (mg.ℓ^{-1})	Detection limit	Site 1. Van Niekerk Farm	Site 2. D/s of Rooiwal	Site 3. D/s of the Bon Accord Dam
B – dissolved	0.014	0.081	< 0.014	0.027
Al – dissolved	0.059	< 0.059	< 0.059	< 0.059
V – dissolved	0.005	0.010	< 0.005	< 0.005
Cr – dissolved	0.006	< 0.006	< 0.006	< 0.006
Mn – dissolved	0.003	< 0.003	< 0.003	< 0.003
Fe – dissolved	0.006	< 0.006	0.050	< 0.006
Ni – dissolved	0.009	0.031	< 0.009	< 0.009
Cu – dissolved	0.019	< 0.019	< 0.019	< 0.019
Zn – dissolved	0.005	< 0.005	< 0.005	< 0.005
As – dissolved	0.100	< 0.100	< 0.100	< 0.100
Sr – dissolved	0.003	0.150	0.043	0.139
Mo – dissolved	0.012	< 0.012	< 0.012	< 0.023
Cd – dissolved	0.005	< 0.005	< 0.005	< 0.005
Ba – dissolved	0.003	< 0.003	< 0.003	0.033
Pb – dissolved	0.054	< 0.054	< 0.054	< 0.054

Mr P. Botes (Botes, 2000) indicated that the trace metal concentrations were low and are not likely to have been linked to the fish kill.

3.6 Chemical Oxygen Demand

Chemical Oxygen Demand (COD) provides a measure of the oxygen requirement of organic material present in the water (DWAF, 1996d). A high COD would imply that there is a large amount of organic material present in the water sample. The results of the COD analysis are reflected in Table 5.

Table 5. Chemical Oxygen Demand (COD) concentrations recorded in the samples taken at the three sites

Constituent	Detection limit	Site 1. Van Niekerk Farm	Site 2. D/s of Rooiwal	Site 3. D/s of the Bon Accord Dam
COD (mg.ℓ^{-1})	10	36	30	31

Analytical results indicated (Botes, 2000) that the COD concentrations were low and are not likely to have been linked to the fish kill.

3.7 Organic Constituent Analyses

Only one sample was taken for organic constituent analysis, that being at Site 1 where the dead fish were seen during the investigation. One part of the sample was analysed for the presence of algal toxins, Microcystin-LR, Microcystin-RR, Microcystin-YR, and Nodularin using the Enzyme-Linked ImmunoSorbent Assay (ELISA) Microcystin Tube Kit. The limit of detection (LOD) of the ELISA Microcystin kit is 0.5 ppb (µg.ℓ^{-1}). The second part of the sample was extracted with dichloromethane and analysed with a gas chromatograph with a

mass selective detector (GC-MS). The compounds were identified using a library search routine and a spectral library containing typical spectra of the compounds. The organic scan indicated the presence of Endosulphan in the sample. An Endosulphan standard was, therefore, used to determine the concentration of the pesticide in the sample.

The results of the various analyses are listed as follows:

1.) The estimated algal toxin concentration was less than 0.5 ppb ($\mu g.\ell^{-1}$).

2.) A 2 $\mu g.m\ell^{-1}$ Endosulphan standard was also injected. The concentration of Endosulphan in the sample taken at Site 1 can be estimated at less than 1 $\mu g.m\ell^{-1}$.

The concentration recorded may not reflect the actual peak concentration since it was already breaking down in the sample when the analysis was conducted. Note that the pesticide Endosulphan is highly toxic to fish. In addition to the Endosulphan, Atrazine, a herbicide, and Phthalates were also isolated in the sample. Phthalates were also present in the blank and would, therefore, most likely be of other origin (such as in the water supply or in the material of the containers used).

3.8 *Postmortem* Examination

Dr E. Du Plessis, of the Pathology Laboratory of the Onderstepoort Veterinary Institute, conducted the histopathological examination on the carp delivered to their laboratories by the IWQS. The results are listed below under the headings used by Dr Du Plessis (Du Plessis, 2000).

Macroscopical Pathological Changes

Severe, generalised congestion, intestinal contents scanty and catarrhal; few gills flukes present on a gill smear.

Histopathological Changes and Morphological Diagnosis

Kidney – Pigmentation, tunica media of medium arteries, moderate.
Intestine – Congestion, lamina propria, multifocal, moderate.

Bacteriological Examinations

No pathogenic bacteria could be cultured.

Discussion

No specific lesions were present to indicate a cause for this fish die-off, including gill lesions indicative of soluble oxygen deficiency. Bacterial presence can be excluded as a cause of death. Further toxicological examinations will hopefully give a better indication of a cause for these mortalities.

At the same time as the above examination, a fish specimen was delivered to the Toxicology Laboratory at Onderstepoort by someone not associated with the IWQS. The herbicide Atrazine was found in the fish tissue of this specimen (Joubert and Basson, 2000).

3.9 Other Possible Factors

Mr J. Daffue (Daffue, 2000) reported that there are pollution problems (including eutrophication problems) upstream of the Bon Accord Dam. This would begin to explain why dissolved oxygen levels are so low in the Apies River, even downstream of the Bon Accord Dam. This may also explain the high UIA concentrations recorded, especially that the UIA levels were highest immediately downstream of the Bon Accord Dam and then progressively improved in the direction of Site 1 (where the UIA concentrations were the lowest of the three sites, albeit still at chronic levels).

Mr Daffue further reported that farmers in the area of the fish kill were complaining of negative growth effects to crops that had been irrigated with water from the Apies River. This is consistent with the presence of Atrazine (a herbicide) in the water sampled.

4. Conclusions and Recommendations

The most obvious cause of the fish kill would appear to be the detectable levels of the pesticide Endosulphan and the herbicide Atrazine in the water. Endosulphan is especially toxic to fish. The source of these toxins is not known. The toxins were already beginning to break down at the time of analysis and it can, therefore, be assumed that their levels had been higher than those recorded.

Un-ionised ammonia (UIA) levels were high enough to result in chronic (long-term) effects on fish. The UIA concentration was surprisingly highest at the site downstream of the Bon Accord Dam (Site 3), with a decreasing gradient to Mr Van Niekerk's Farm (Site 1). It would appear that the conditions within the impoundment itself, or those upstream of the impoundment, favoured the conversion of ammonium ions to the more toxic un-ionised ammonia. These UIA levels, together with the low dissolved oxygen levels recorded, would have made the fish much more susceptible to the pesticides and herbicides than if their levels were more favourable to the well-being of the fish.

It would appear that there is a point source of nitrogen between Site 3 and Site 2 since there was a dramatic increase in the nitrate-nitrite concentration on going from Site 3 to Site 2. The high concentration was also found at Site 1, albeit at a fractionally lower concentration. It is likely that the Rooiwal WCW was a large contributor to the high nitrogen levels evident at Sites 2 and 3.

A progressive increase in total phosphorus and orthophosphate was also evident from Site 3 to Site 1. In contrast to the situation evident with the nitrogen concentrations mentioned above, it is likely that the source of the phosphorus was diffuse.

The effects of the developments between the Bon Accord Dam and the Van Niekerk Farm site, including the effects of the Rooiwal WCW and the Rooiwal power station, can be seen in the increasing salt concentrations that were recorded in the Apies River. There was a significant increase in the TDS concentration at the site downstream of the Rooiwal WCW and the power station (Site 2). There was a further slight increase at the Van Niekerk Farm site (Site 1).

Toxicity tests conducted by the IWQS resulted in 0 % mortality of 17 to 18 day-old *Oreochromis mossambicus*. This indicates that at the time of sampling, the toxin was no longer present at a sufficient concentration to result in mortalities. The conditions in the laboratory would also have resulted in a higher dissolved oxygen level than that evidenced on-site.

Results of the *post-mortem* investigation conducted on the carp delivered to the Pathology Laboratory at Onderstepoort, showed that no specific lesions were present to indicate a cause for the fish die-off. It was indicated that bacterial presence could be excluded as a cause of death (Du Plessis, 2000). It was further reported that a fish sample had been delivered to the Toxicology Laboratory at Onderstepoort at the same time by someone not involved with the IWQS investigation. It was found that Atrazine was present in the fish tissue of that specimen (Joubert and Basson, 2000).

In order to address the fish kill adequately, the cause of the elevated UIA concentrations and low dissolved oxygen levels should also be determined. If these factors are improved, then the fish will be in a better position to resist toxins or other factors that threaten their survival.

It is suggested that the Gauteng Regional Office of DWAF, together with the appropriate experts, undertake an information-sharing session with the various land users in the area to inform them of the dangers of insecticides and herbicides to the aquatic ecosystem, and also to their crops. If this information is disseminated to all people managing and using such chemicals, the chance of a similar incident occurring will be decreased.

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Appendix A: The Calculation of Un-Ionised Ammonia in River Water Samples

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Method

Un-ionised ammonia can be calculated in river water samples from the ammonium concentration using temperature, electrical conductivity and pH.

$$\text{Un-ionised ammonia } (NH_3)\ (mg/l) = NH_4 / (1 + 10^{logK-pH})$$

where NH_4 is the total analysed ammonia concentration in mg/ℓ, pH is the pH of the sample and logK is calculated as follows:

$$\begin{aligned}logK = {} & 0.00035130T^2 + -0.044736T + 10.119 \\ & + [-0.00055400T^2 + 0.029236T + -0.59920]*[sqrt(I)/(1+sqrt(I))] \\ & + [-0.00021131T^2 + -0.0010510T + 0.46909]*I\end{aligned}$$

where T is the water temperature in degrees Celsius (°C) and I is the ionic strength in mol/ℓ which can be calculated from the electrical conductivity as:

$$I = 0.00013(EC)$$

where EC is the electrical conductivity in mS/m. This ionic strength: EC relationship is valid for river waters and soil extracts with an electrical conductivity less than 3200 mS/m.

Example

A river water sample has the following characteristics:

Total Ammonia concentration (NH_4)	0.010 mg/ℓ
Water temperature	20 °C
Electrical concentration	40 mS/m
pH	8.0

a. Calculate the ionic strength, I (mol/ℓ):

$I = 0.00013(EC) = 0.00013 \times 40 =$ **0.0052** mol/ℓ

b. Calculate logK

$$\begin{aligned}logK = {} & 0.00035130T^2 + -0.044736T + 10.119 \\ & + [-0.00055400T^2 + 0.029236T + -0.59920]*[sqrt(I)/(1+sqrt(I))] \\ & + [-0.00021131T^2 + -0.0010510T + 0.46909]*I \\ = {} & 0.00035130 \times 20^2 + -0.044736 \times 20 + 10.119 \\ & + [-0.000554 \times 20^2 + 0.029236 \times 20 + -0.59920] \times [sqrt(0.0052)/(1+sqrt(0.0052))] \\ & + [-0.00021131 \times 20^2 + -0.001051 \times 20 + 0.46909] \times 0.0052 \\ = {} & \mathbf{9.3508}\end{aligned}$$

c. Calculate the un-ionised ammonia concentration as :

$$NH_3 = NH_4 / (1 + 10^{logK-pH}) = 0.010 / (1 + 10^{9.3508 - 8.0})$$
$$= \mathbf{0.00043}\ mg/ℓ$$