Tasmanian Investigation
2005 – 2008

The following report contains a summary of laboratory test results collected for the purpose of shedding light on biological anomalies in oysters and possibly people in the Break O Day area. The author (Marcus Scammell) has drafted the document to explain the results to an educated non-science audience.

Summary

Following an extensive oyster mortality event in 2004 a number of water samples were collected for chemical measurement and tested for manmade chemicals. No manmade chemicals were identified despite ongoing disease in the oysters.

In January 2005 chemical testing was abandoned in favour of toxicity testing. Two methods of collecting water samples were used; one method concentrates surface foam while the other collects a representative sample from the water column (called a grab sample). On January 17th 2005 two grab samples were collected from Pyengana and the North George River. Both were toxic to Sea Urchins and one was also toxic to oysters. During a storm event from the 2nd to 3rd of February 2005 two grab samples were taken, one of which was toxic from Moulting Bay. On the 14th of February 2005 five grab samples were taken and one was positive (Crystal Creek, although there was no clear documentation associated with this sample which was taken by DPIWE staff).

Thus, in the first two months of 2005 nine grab samples were taken four of which were toxic (although Crystal Creek method of collection is not known). All surface foam samples were toxic.

Chemistry associated with these samples was not helpful in identifying a range of organic compounds, none of which were identified as manmade and the origins of these chemicals were unknown. The detection limits of these tests were also relatively high and could well have missed some manmade chemicals.

As indicated above the Government was aware of the January results leading to a combined sampling effort on the 14th of February 2005. Despite the oyster deaths and now finding toxic surface water as well as some toxic grab samples the Government decided it was natural and therefore not an issue and to the best of the authors knowledge stopped sampling for toxicity.

In the absence of any useful knowledge to allow management of this situation a Toxicity Identification and Evaluation (TIE) was commissioned (by the author, a local doctor and the oyster farmers). Due to funding constraints and scientific complexity, this section of the study took a considerable time being completed early 2008.

The TIE commenced in full in March 2005. The important findings are as follows.

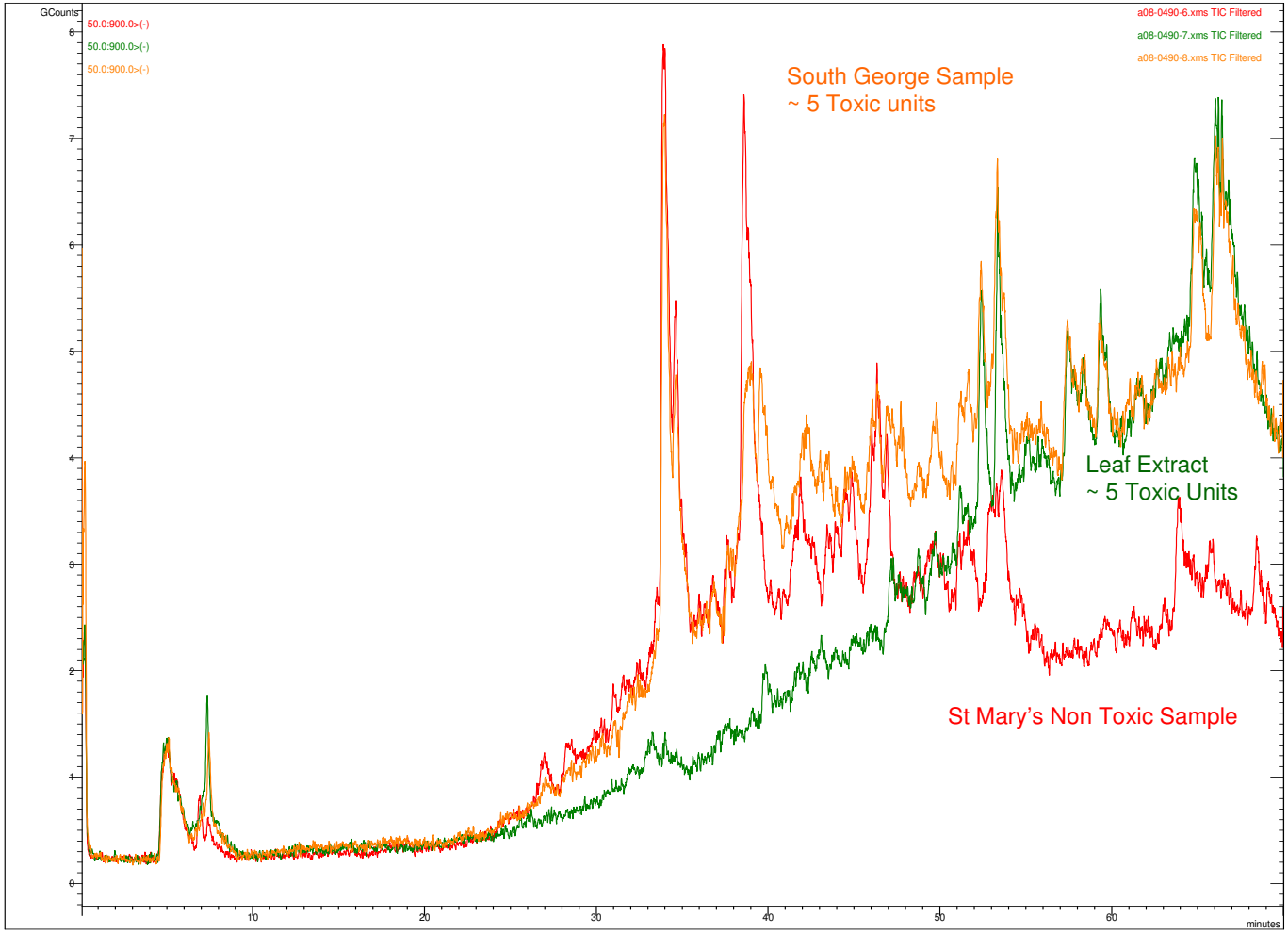
1) The toxin(s) was present in surface foam during all dry weather samples.
2) The toxin(s) has a relatively short half life, days to weeks.
3) The toxin(s) is primarily attached to fine particulate matter but some remains dispersed or dissolved.
4) The toxin(s) are not chealatable metals.
5) The toxin(s) are not volatile.
6) The toxin(s) behaves like an organic chemical.
7) The toxin(s) is methanol soluble.
8) During March 2005 the toxin(S) was enhanced by the addition of PBO (suggesting a pyrethroid type chemical was present).
9) By mid April 2005 this PBO enhancement disappeared, toxicity did not.
10) By Mid April 2005 PBO suppressed toxicity suggesting an organo-phosphate type chemical was present.
11) Subsequent tests had no PBO effect but a methanol soluble toxin remained.
12) Chemistry was unable to confirm what the PBO effecting chemicals were.
13) Methanol fractionation indicated multiple toxins were present.
14) The toxins were not proteins.
15) The toxins were not of blue-green algal origin.
16) The toxins were unlikely to be of bacterial or fungal origins.
17) The toxins affected multiple test targets (Cladocerans, Oysters, Sea Urchins and three human cell lines) at similar concentrations.
18) The toxin(s) are not found downstream of natural forests.

At this point the leaves of the monoculture *E.nitens* plantations were tested. After a number of supporting tests (but not conclusive tests) an add back style of experiment was designed.

The rationale for this experiment is as follows: If the methanol soluble dry weather toxin that was always present in foam samples was from *E.nitens,* then water downstream of *E.nitens* would have an overlapping section of its chemical signature when compared to reference water containing extracts added from *E.nitens* leaves. This overlap would not be present when compared to water downstream of natural Eucalypt Forests.

An experiment was run using South George water containing five toxic units (a measure of dilution factor required to reduce toxicity), reference water with leaf extract also containing five toxic units and surface water from a catchment downstream of natural forests containing no toxicity. The chemical signatures were obtained using LCMS in full wide scan. A graph showing the results follows.

Overlaid Chromatogram Plots



The red line is the non toxic St Mary's water and it tracks for at least half the graph with the toxic South George Sample.

However the non toxic signature departs from the toxic signature about two thirds of the way along the graph.

The green line which is toxic leaf extract converges with the orange line (toxic water) three quarters of the way along the graph with a near perfect trace of one over the other.

Both water and leaf spike contain approximately five toxic units and clearly have many chemicals in common at similar concentrations.

This last experiment strongly suggests one of the causes of toxicity in George River waters is from chemicals found in *E.nitens* leaves.

Due to the unexpected findings and the evidence of multiple contaminants it was decided that this pilot study needed to be completely and independently repeated. Funding for the repeat study was secured through an independent source and that study is being conducted by NIWA in New Zealand. Our findings have been confirmed (February 2010) and the additional work of putting those findings into environmental perspective is ongoing.

Methods to Alert Authorities

In 2002 a report was written for DPIWE (the author) advising that oysters in the George Bay area were hyper-sensitive to Tributyl Tin thus suggesting the presence of additional contaminants. A catchment investigation was recommended. In 2003 oysters suffered unexpected mortality following rainfall, DPIWE was again advised. In December 2003 there was a helicopter crash resulting in contamination in the upper catchment. In January 2004 extensive oyster mortality followed a six day flood in the catchment. The author advised DPIWE of the contaminated site and anomalies in the health of the human population and was advised that the site would be cleaned up. To the best of the author's knowledge this never occurred.

Throughout 2004 chemical measurement was undertaken to identify man made chemicals, however, a variety of problems with sampling and detection limits meant that this approach was not useful. In October 2004 an Adverse Experience Report was sent to APVMA. No investigation subsequently occurred.

In January 2005, a grab sample from the water column was found to be toxic. Tasmanian Health was immediately notified. In February 2005, samples from water column and skimmer boxes were analysed by the Tasmanian Government and by us (the Oyster Farmers, Dr Bleaney and the author), and the surface samples were found to be toxic by both parties. Later that month a briefing paper was sent to the Federal AMA requesting support for an investigation. This request was not supported. In April 2005 an additional Adverse Experience Report was submitted to the APVMA, again no investigation occurred.

During 2005 and 2006 a number of trips (4) were made to Canberra to see various members of parliament and advisors. The last trip was to see the Prime Minister's senior science advisor (Howard Government). At this meeting we were advised that this was a State issue and if Tasmanians wanted the problem fixed they needed to change their government. We were further advised that the Federal Government would not intervene even though it was clear the State Government was failing to meet the terms of their charter.

In August 2006 we (Jim Harris, Dr Bleaney and the author) were appointed as members to the Tasmanian Water Quality Initiative, part of the Australian Government Water Quality Monitoring Consultative Committee. The author advised the convenor of that committee of the toxicity issues and offered to do a presentation to the committee. This offer was not accepted and the committee was only allowed to address pesticide issues associated with the calibration of CSIRO's PIRI model of chemical contamination for Tasmania. After attending a few committee meetings it became obvious that the committee had no interest in the actual problems that were

occurring in the field. This led to the resignation of the oyster farmer Jim Harris, Dr Bleaney and the author from that committee.

In 2008, the paper on the effect of the river toxicity on human cell lines was presented at an ecotoxicology conference in Spain and subsequently published on-line. Tasmanian Health was advised of the paper and the web site on which it could be found.

In September 2009, Dr Chris Hickey presented his findings in Adelaide at the annual ecotoxicology conference (ASE). In December 2009 Dr Fiona Young's preliminary findings were published in an abstract at a conference in Canberra (ACTRA).

It was not until after the Australian Story in February 2010 that any response became apparent from the Tasmania Government.

Tasmanian Investigation

Introduction

The water cycle begins with relatively pure water arriving in a catchment via rainfall. As water moves through a catchment, it dissolves minerals from the soil, it dissolves organic matter, it interacts with plants, fungi and bacteria and eventually reaches creeks, streams, and rivers. It is then a habitat for many organisms and a resource for many others (eg. animals' water source). Thus water that we call "clean" contains many naturally occurring impurities (dissolved gasses, dissolved minerals, dissolved organic matter, living organisms and suspended solids).

Animals can deal with these natural impurities. Indeed, some of these natural impurities may be vital for some biological functions, like minerals and trace elements. Others may be potentially hazardous (like bacterial toxins), but at the concentrations at which they occur naturally, they are quickly metabolised by biological processes that occur in organs like the liver.

Thus, under natural conditions, clean water always contains impurities. Living things have evolved to take advantage of this fact and it is the primary mechanism via which nutrients and trace elements from soil become available to plants and animals.

However, water will become toxic when naturally occurring substances are present in unnaturally high concentrations or when man-made substances are present at concentrations that overpower our natural detoxification processes.

Toxicity tests, which are the basis of the following study, do not ask the question: "Are impurities/contaminants present?" Rather, they ask the question: "Are impurities/ contaminants present at concentrations which cause harm?"

ANZECC (2000) Guidelines ("the Guidelines")

The Australian and New Zealand Environment and Conservation Council (ANZECC) is an organisation made up of representatives from all States and Territories in Australia and New Zealand. It is ANZECC's charter to provide uniform environmental Guidelines that embrace the principles of conservation and sustainable development for each signatory State and Territory to adopt. Tasmania is a signatory to these Guidelines. The current Guidelines were released in 2000, replacing the ANZECC 1992 Guidelines. The current Guidelines recognise the complexities of the environment and require a much more investigative approach than the previous Guidelines.

The current Guidelines provide a hierarchy of evidence recognising that the most powerful evidence is biological effect and the least powerful evidence is chemical measurement. This is in direct contrast to the 1992 Guidelines where chemical measurement was the only requirement. The current Guidelines are based on toxicity testing and the adoption of safety margins to produce trigger values. A trigger value is the concentration below which no harm should occur. However, the Guidelines recognise that there is often insufficient reliable toxicity data upon which to make a sensible risk based decision. Thus, the Guidelines provide fall back positions so that trigger values for individual chemicals can be derived.

The Guidelines concentrate predominantly on conservative environmental protection so that biological impacts do not occur. However, when at the opposite end of the

spectrum, where a biological impact is present but the causal agent is unknown, a different set of procedures are advised.

If an investigator thinks that a biological impact is present, then the first thing to do is establish whether that is true. This is done through a procedure called "Whole Effluent Toxicity" or WET testing. The name was derived from testing complex mixtures of chemicals like sewage effluent. Despite its name, the test can be applied to any complex water matrix regardless of whether it is coming out of a man-made pipe or a natural pipe like a river. The test involves exposing aquatic organisms to the complex water matrix for a set period of time (e.g. 48 hours) and then observing what state of health those organisms are in at the end of the time period. It is as simple as filling fish tanks with the water in question and seeing if aquatic organisms can live in it.

If the organisms die after a set period of exposure, then a "Toxicity Identification and Evaluation" or TIE needs to be run. This is a vastly more complex set of tests where the toxic water is manipulated predominantly to try to reduce or remove toxicity. These manipulations provide clues as to what class of chemicals the toxin belongs. Once the toxin is isolated, it can be sent to a chemical laboratory for identification. The chemical laboratory might identify a number of chemicals in the toxin-isolated sample. Each of these chemicals can be added back to clean water allowing specific identification of which one or ones are responsible for toxicity.

Once the toxin has been identified, the cause of the contamination (usually a human practice) of the water can be addressed.

As already stated, the Tasmanian Government is a signatory to the ANZECC 2000 Guidelines and, as such, it has agreed to adopt the intent of those Guidelines which are to protect and improve the environment for existing and future generations. Nowhere in those Guidelines does it allow for deterioration or degradation of the environment. Similarly, the Australian Drinking Water Guidelines (ADWG) shares similar sentiment to the ANZECC 2000 Guidelines.

The roles and responsibilities of signatories to these Guidelines are for continuous improvement of catchments for the protection of the existing and future generations.

The Clients

The "clients" consist of Ian Coatsworth (Oyster Farmer), Jim Harris (Oyster Farmer), Dr Alison Bleaney (Medical Practitioner) and Dr Marcus Scammell (Marine Ecologist). These people have funded the tests conducted by the investigating laboratories. The investigating laboratories, in consultation with the clients, have determined what tests need to be run.

Background

This study was produced following the clients' observations that anomalous oyster mortality was occurring in the study area on a regular basis following rainfall. At the same time, anomalous observations were being made with respect to human health.

The largest of the oyster kills occurred following a helicopter crash carrying agricultural chemicals closely followed by a flood. It appeared to the clients that chemical contamination was a probable source of the oysters' and the peoples' problems. The clients brought these anomalies to the Tasmanian Government's attention in the expectation that the Government would properly investigate. To the

clients' surprise, the Government took an adversarial position and publicly discredited the clients.

It is the clients' view "that they were simply reporting the car accident". It was the Government's view that the client had to "prove the car accident existed'. Using this analogy, the clients have chosen to build the "ambulance" in order to bring the "car accident" to the communities' attention.

The Investigating Laboratories

Ecotox Services Australia Pty Ltd., Sydney, Australia.

Advanced Analytical Australia Pty Ltd., Sydney, Australia.

Australian Proteome Analysis Facility, Macquarie University, Sydney, Australia.

Chemical Safety and Applied Toxicology Laboratories, University of New South Wales, Sydney, Australia.

Australian Water Quality Centre, a business unit of South Australian Water, Adelaide, Australia.

Genetic ID (NA) Inc., Iowa, USA

Scientists from whom opinion has been sought.

Professor Joe Cummings, Genetics, Ontario University, Canada.

Professor Tyrone Hayes, Biology, Berkeley University, USA.

Why are the Clients doing this Study?

The purpose of the following investigation was to determine if the anomalous oyster mortality and the anomalous human illnesses could be caused by contaminated water in the George River System.

In Dr Scammell's experience, large scale oyster mortality does not occur naturally following rainfall (Dr Scammell's PhD was obtained from identifying oyster mortality caused by the antifouling agent, tri-butyl tin). The largest natural mortality event that Dr Scammell has observed in the last twenty five years occurred in the late eighties in the Hawkesbury River, NSW. This happened following six weeks of exposure to flood waters with oyster mortalities of less than 5%. By comparison, the mortalities following rainfall in the Georges Basin (since 2002) were typically 20% to 30% with a 90% kill following the helicopter crash and subsequent heavy rains.

Similarly, Dr Bleaney was beginning to observe increasing numbers of unusual diseases amongst her patients. She had not observed such an increase in unusual diseases in her thirty years of general practice. When Dr's Scammell and Bleaney analysed the incidence of notifiable diseases, a statistically significant increase had occurred from 2002 to 2005.

Tasmanian Departments were notified, resulting in the following responses:

1) the oysters were killed by freshwater;

2) the human health anomalies were a result of poor data collection and inadequate data analysis.

However, neither Dr Scammell nor Dr Bleaney could find evidence to support these explanations. As a result, the clients decided to do what the Government would not do: conduct an investigation in accordance with the ANZECC 2000 Guidelines.

Toxicity Data

In order to understand the following study, it is helpful for the reader to understand what the results say. The following is an explanation of how to interpret toxicity results. The table below gives the results of some WET tests using oyster larvae and sea urchin larvae as the test organisms.

Table 1: Skimmer box samples: testing for the presence of toxin(s).

Skimmer Box **Laboratory Controls**	**Oysters** **% survival** *(Mean +/- SD)*	**Oysters** **% normal larvae** *(Mean +/- SD)*	**Sea Urchin** **% normal larvae** *(Mean +/- SD)*
Sea Water	86.5 +/- 7.1	75.3 +/- 6.9	92.5 +/- 5.5
Artificial Sea Water	89.5 +/- 6.6	80.5 +/- 9.6	90.8 +/- 3.1
Samples			
South George	0.0 +/- 0.0	n/a	0.0 +/- 0.0
Pyengana	79.9 +/- 12.9	39.8 +/- 6.7	0.0 +/- 0.0
Water Intake	85.3 +/- 13.5	56.7 +/- 14.7	0.0 +/- 0.0

Firstly, two controls are run to check that everything is working properly in the laboratory. These two controls are growing the test organisms in clean seawater ("Sea Water") and growing the organisms in "Artificial Sea Water" (i.e. freshwater that has had salt added to it). Both these should have healthy test organism populations surviving above either 70% or 80% of the original 100% put in the water (depending on the test), and the two "sea water tests" should have similar results to each other. If the laboratory controls are below these figures, no further interpretation should occur because the test organisms have become ill for some unknown reason.

If the laboratory controls pass the check, then the results for the samples can be compared with them. So in Table 1, oyster survival in the South George sample is obviously different to the laboratory controls whereas oyster survival in the samples from Pyengana and the Water Intake site are sufficiently similar to the laboratory controls to be considered non-toxic.

Following WET tests, a dilution curve is often run to determine the relative toxicity of samples. The laboratory runs a dilution series using the original sample (with salt added) and dilutes that using clean sea water (when testing salt water organisms).

A typical dilution series would be as follows.

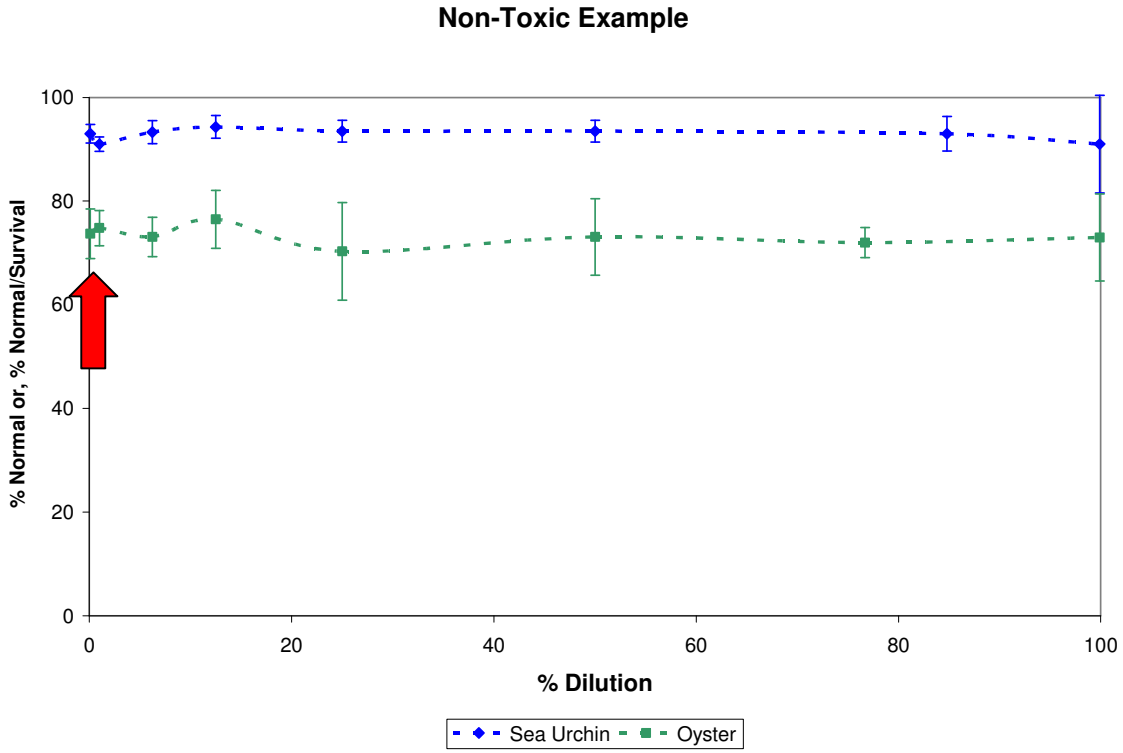
Sample	100%	50%	25%	12.5%	6.25%	0%
Sea Water	0%	50%	75%	87.5%	93.75%	100%

The number of organisms that survive in each of these dilutions can then be assessed and plotted as below.

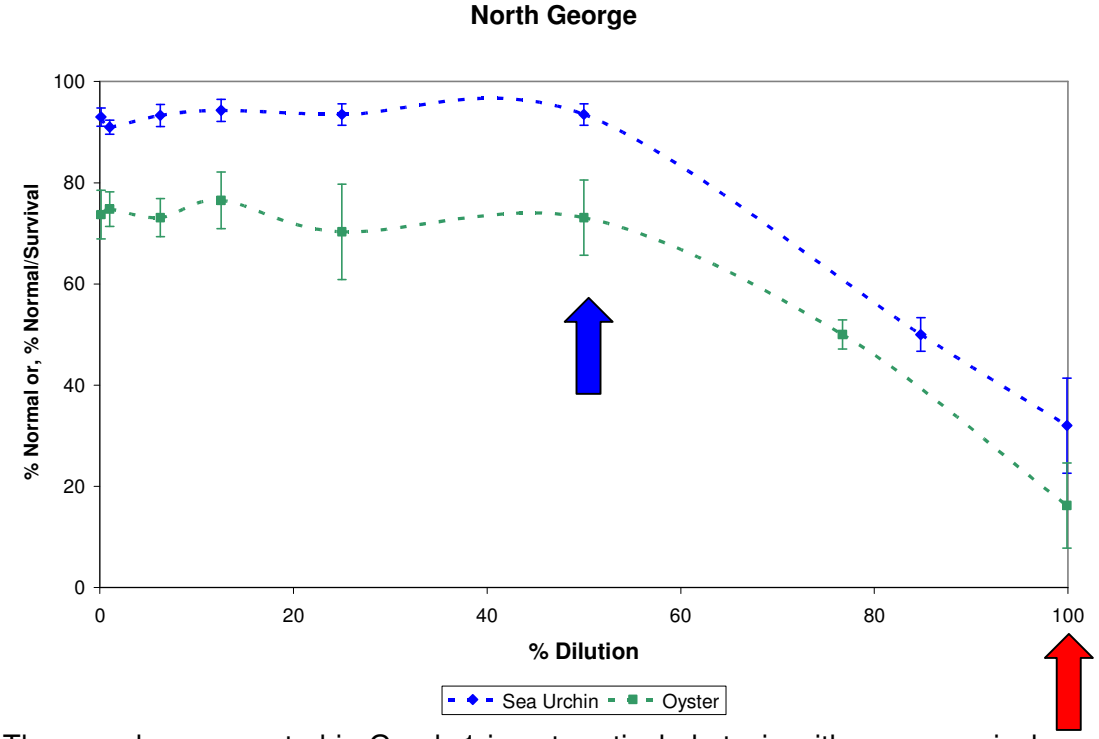
Consider initially a hypothetical non-toxic sample (Graph A below). While an investigator would not run a dilution curve on a non-toxic sample, were they to do so,

it would look like the graph below with all samples being similar to the laboratory controls (marked by the red arrow).

Graph A: Dilution Curves for a hypothetical non-toxic sample



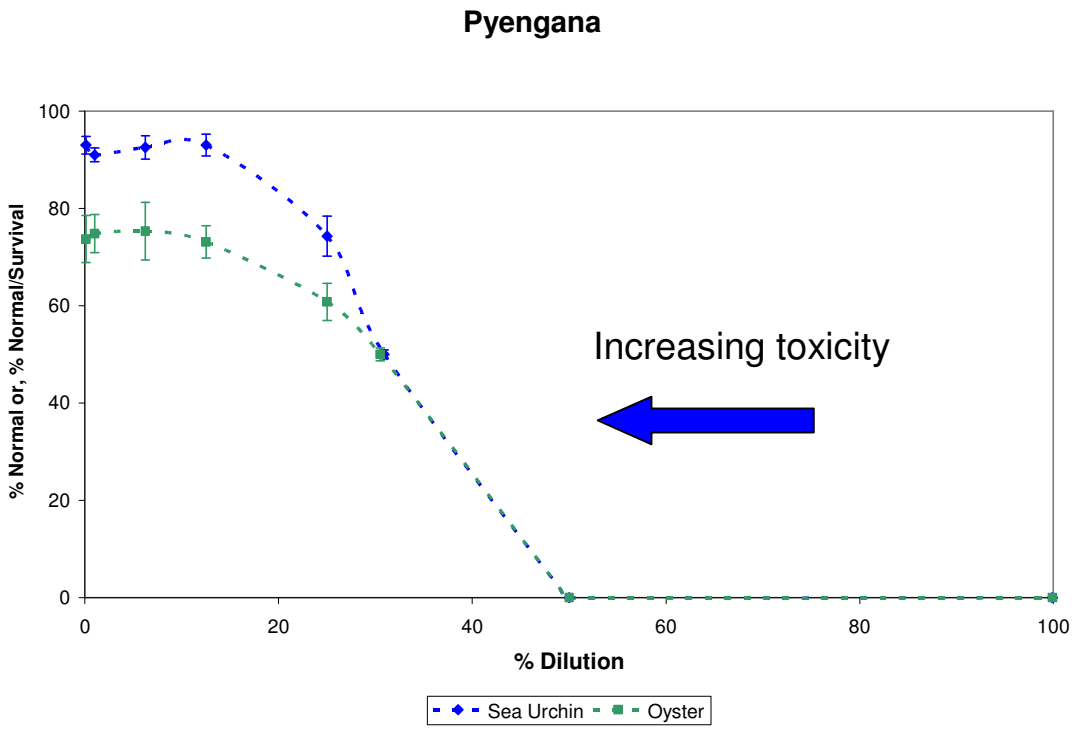
Graph 1: Dilution Curves for the North George Sample



The sample represented in Graph 1 is not particularly toxic with some survival even at 100% of the sample (red arrow) and no decrease in survival at 50% dilution (blue

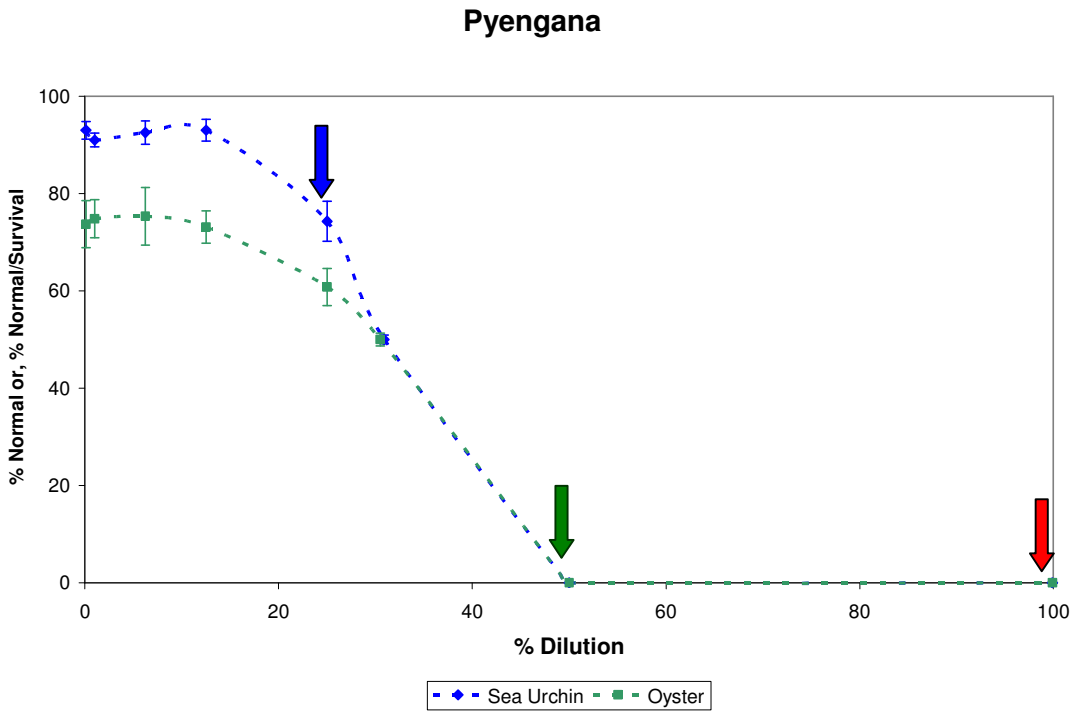
arrow) compared with the laboratory reference samples. The sample is slightly more toxic to oysters (the green line) than sea urchins.

Graph 3a: Dilution Curves for the Pyengana Sample



By comparison, the sample in Graph 3a is quite toxic as indicated by the movement of both lines to the left in the direction of the blue arrow.

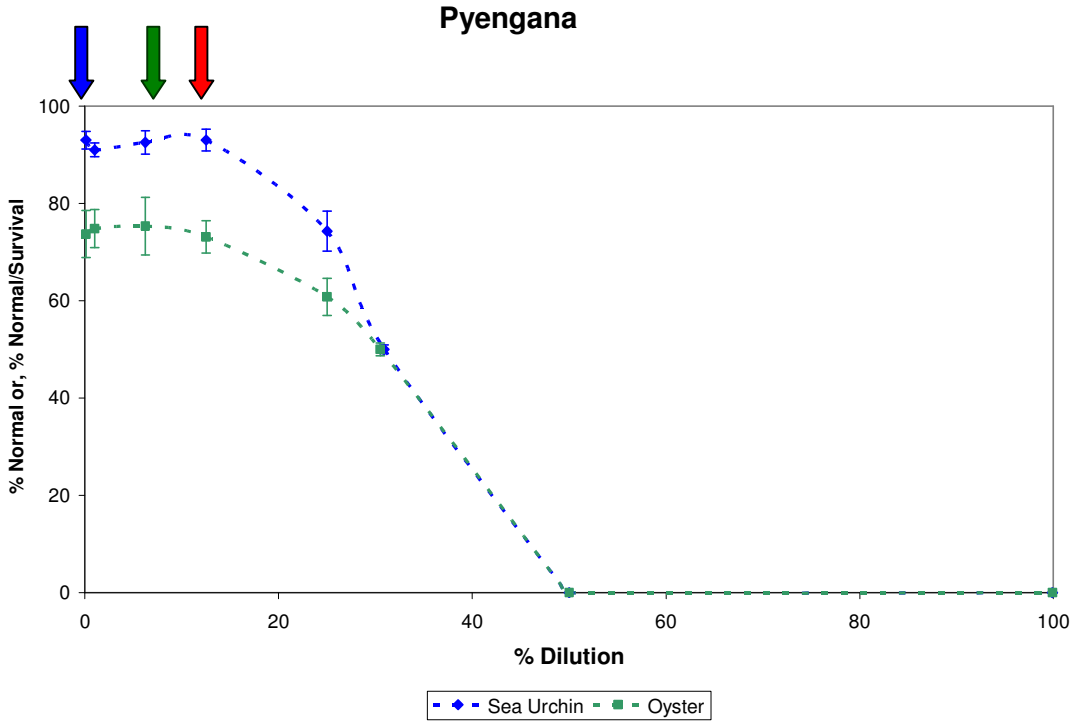
Graph 3b: Dilution Curves for the Pyengana Sample



This sampled produced 100% mortality at 100% sample (red arrow, Graph 3b) and 100% mortality at 50% sample (green arrow). It is still significantly toxic at 25%

sample (blue arrow) with the mean still being less than the laboratory controls (0% dilution).

Graph 3c: Dilution Curves for the Pyengana Sample



In the 12.5% sample (red arrow, graph 3c) and 6.25% sample (green arrow), there is no significant toxicity when compared with the laboratory control samples, 0% samples (blue arrow).

The graph shows approximately the same level of toxicity to both test organisms.

With dilution curves, the further the curve moves to the left the more toxic the sample is.

Sample Locations

Map of North East Tasmania showing approximate sampling locations

The exact locations are in the following table:

Location	Easting	Northing
North George	579594	5431274
South George	580199	5427434
Pyengana	585202	5428809
Upstream of Water Intake	601062	5428505

Sampling Devices

Two types of sampling methods are employed throughout this study. The first is called a "grab" sample. This is done by submerging a sampling bottle in the water. This method gives a representative sample of the part of the water column the bottle was opened in (i.e. the surface or 0.5m below the surface).

The second method is called the "Skimmer Box" (Photo 1). The skimmer box is a floating device that concentrates floating chemicals. It works in exactly the same way as a skimmer box in a domestic swimming pool. Surface water enters a skimmer box in a pool and is then sucked down by a pump trapping floating material in the skimmer box. In our device the river provides the current. Water enters the device between two floating booms and is directed into the front end of a plastic drum. Water leaves the drum by going down through a hole in the bottom, trapping foam and other floating chemicals on the surface. The purpose of the skimmer box is to provide a concentrated surface sample to assist chemical identification.

Photo 1: Picture of skimmer box at Pyengana after 24 hours in the water

The skimmer boxes were always given 24 hours to rinse and accumulate. The foam in the skimmer box was tested and found to be non-toxic C16 to C18 vegetative oils and fats. One of the anomalous observations is that the foam was always present in substantially larger concentrations than at reference locations. It is not known where this foam is coming from.

Tasmanian Investigation
Test Number 1

Sampling Details
January 17, 2005. Sampled for Dr Alison Bleaney by the St Helens Marine Farmers.

Locations Sampled
North George
South George
Pyengana
Upstream of Town's Water Intake pipe (Water Intake)

Purpose
To determine the baseline condition of the George River system, St Helens, Tasmania, with respect to the presence of toxin or toxins (referred to from here onwards as "toxicant(s) in accordance with ANZECC's terminology").

Investigating Laboratory(s)
Ecotox Australia, Sydney

Tests Conducted
Oyster development test
Sea Urchin development test

Choice of tests
Oysters were chosen because of the past history of repeated impacts on commercial oysters since the late 1990's.

Sea Urchins were chosen because of the application of the tests for predicting impacts on humans. The scientific community generally accepts that the Sea Urchin development test is an adequate model for predicting human cancer[1]. The French scientist who developed the human cancer model using Sea Urchin development received a Nobel Prize for her efforts.

Sampling Method
Two types of sampling methods were employed. The first method, grab sampling, involves submerging a sampling bottle in the water column. This method provides a sample that represents a normal water column sample and indicates if drinking (or being exposed to) the water in an untreated state would be hazardous (for the test organism). The second method, using a skimmer box, concentrates the surface water where lipid soluble toxicants are likely to accumulate. The purpose of the second method is to determine if lipid soluble toxicant(s) is present and to make it easier to then identify them chemically. If lipid soluble toxicants are present (using this second method), it does not necessarily mean that they are at sufficient concentrations in the water column to be of concern.

[1] J. Marc, O Mulner-Lorillon and R Belle (2004). Glyphosate-based pesticides affect cell cycle regulation. *Biology of the Cell 96* (2004), 245-249
J Marc, O Mulner-Lorillon, G Durand and R Belle (2003) Embryonic cell cycle for risk assessment of pesticides at the molecular level. *Environ Chem Lett* (2003) 1: 8-12

Results
Skimmer box samples indicated that substances toxic to Sea Urchins were present at South George, Pyengana and the Water Intake sampling locations. These substances caused 100% mortality at all sites compared with 8 to 10% mortality amongst laboratory control treatments (Table 1). Mortality was also significant for oysters when exposed to the South George sample. Only oyster development was inhibited for the other two locations (Pyengana and the Water Intake sample, table 1).

Grab samples indicated that significant concentrations of toxicant(s) were present with respect to both test organisms for the Pyengana sample, but the North George sample was only significantly toxic to Sea Urchins (Table 2).

Conclusions
Skimmer box results indicate that toxic substances were present on this first round of sampling. Grab samples indicate that, at the Pyengana location, concentrations of toxicant(s) were sufficiently high to be of concern with respect to the test organisms (drinking the water or being exposed to the water in an untreated state is therefore, likely to be hazardous).

Status
Result to be confirmed through re-testing. Pursuant to the Australian Drinking Water Guidelines, Tasmanian Health was notified. It was agreed that the Department of Primary Industry Water and the Environment (DPIWE) would participate in a repeat sampling of these locations.

Table 1: Skimmer box samples: testing for the presence of toxicant(s).

Skimmer Box **Laboratory Controls**	**Oysters** **% survival** *(Mean +/- SD)*	**Oysters** **% normal larvae** *(Mean +/- SD)*	**Sea Urchin** **% normal larvae** *(Mean +/- SD)*
Sea Water	86.5 +/- 7.1	75.3 +/- 6.9	92.5 +/- 5.5
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Samples			
South George	0.0 +/- 0.0	n/a	0.0 +/- 0.0
Pyengana	79.9 +/- 12.9	39.8 +/- 6.7	0.0 +/- 0.0
Water Intake	85.3 +/- 13.5	56.7 +/- 14.7	0.0 +/- 0.0

Table 2: Grab samples: testing for the significance of the presence of toxicant(s)

Grab Samples **Laboratory Controls**	**Oysters** **% survival** *(Mean +/- SD)*	**Oysters** **% normal larvae** *(Mean +/- SD)*	**Sea Urchin** **% normal larvae** *(Mean +/- SD)*
Sea Water	86.5 +/- 7.1	75.3 +/- 6.9	92.5 +/- 5.5
Artificial Sea Water	89.5 +/- 6.6	80.5 +/- 9.6	90.8 +/- 3.1
Samples			
North George	85.9 +/- 9.3	75.8 +/- 3.9	80.5 +/- 2.7
Pyengana	0.0 +/- 0.0	n/a	0.0 +/- 0.0

Tasmanian Investigation
Test Number 2

Sampling Details
February 14, 2005. Sampled by Rick Krassoi, Ecotox Services Australia for the Clients. Sampled by DPIWE for the Tasmanian Governments investigation.

Locations Sampled
North George
South George
Pyengana
Upstream of Town's Water Intake pipe (Water Intake)

Purpose
To confirm the presence of toxicant(s) within the George River system, St Helens, Tasmania, following the results of Test 1 which were reported to the Tasmanian Government.

Investigating Laboratory(s)
Ecotox Services Australia, Sydney (for the Clients)
Advanced Analytical Australia, Sydney (for the Clients)
Tasmanian Government Laboratories (for the Tasmanian Government)

Tests Conducted by Ecotox and Advanced
Cladoceran test
Oyster development test
Sea Urchin development test
Pesticide and Herbicide Screens
General Screen

Choice of tests
Oysters were chosen because of the past history of repeated impacts on commercial oysters since the late 90's.

Sea Urchins were chosen because of the application of the tests for predicting impacts on humans.

Cladocerans (fresh water fleas) are fresh water organisms and the most commonly used test organism for the presence of toxicants. If these prove to be adequate substitutes for Oysters and Sea Urchins, then they will be used in any further tests. Thus, salt manipulation of fresh water samples would no longer be required, thereby removing a potential source of laboratory error.

Sampling Method
Only the skimmer box method was employed for the Clients' tests. DPIWE employed the grab sample method as well as the skimmer box method.

Results
Comparison between the sensitivity of the three test organisms indicates that Oysters are the most sensitive to the toxicant(s), Sea Urchins are the next most sensitive and that Cladocerans are the least sensitive (Table 3). Of the four locations sampled, toxicity was found to increase with distance downstream, with the Water Intake location containing the most toxicant(s), followed by Pyengana, then South George, with North George being the least toxic (Graphs 1 to 4).

Advanced Analytical ran tests for man-made pesticides, man-made herbicides and general screens. They concluded that more than 400 substances were present in the water but none were man-made herbicides, pesticides or fungicides. Examination of the detection limits indicated that some of the suspected chemicals could have been missed at concentrations that might be toxic. After discussion, it was decided that lower detection limits would be targeted for the next round of testing.

Another group of samples were taken at Crystal Creek on the day after the Clients' sampling (grab samples of surface water and foam taken by DPIWE). These samples were not taken in the presence of the Clients or their representatives, so verification of the samples compatibility was not possible. However, the results, if compatible, may be useful to people reviewing this study. These samples were more toxic than North George but less toxic than South George (Graph 5).

Note: Recent examinations of Google Earth suggests plantations are established upstream of the Crystal Creek sampling site. However, the date of establishment is unknown.

Conclusions

Skimmer box results indicate that toxic substances were present on this round of sampling. While Cladocerans were the least sensitive of the three test organisms, they should still be adequate for the purpose of running a TIE (Toxicity Identification and Evaluation). Cladocerans are preferable because the majority of samples will be fresh water. The additional step of adjusting salt to allow the use of the most sensitive species, Oysters, adds possible scientific uncertainty to the tests, which it is preferable to avoid. Consequently, further tests will be on Cladocerans.

Results reported by DPIWE (see DPIWE web site) were similar to the Clients' for skimmer box samples. The Government did not identify toxicity for the majority of grab samples with the exception of the surface water and foam grab sample from Crystal Creek.

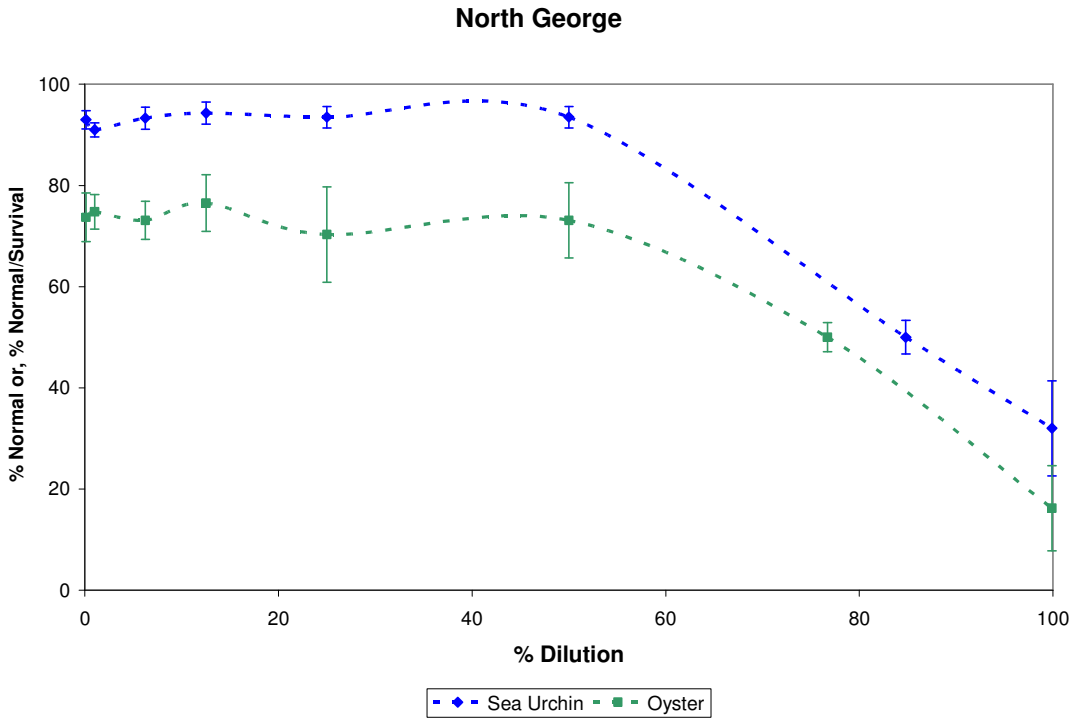
Status

Where comparable samples had been taken, similar results were being obtained by the Clients and the Government. Further studies will use the most resilient and therefore the most conservative test organism, the Cladoceran.

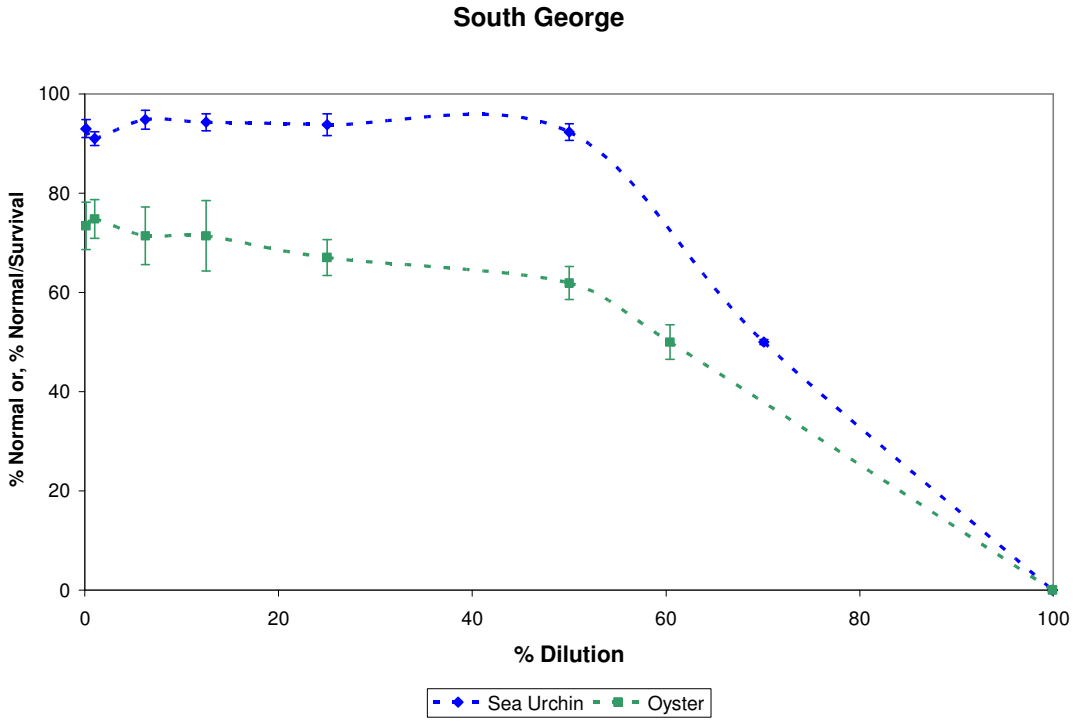
Table 3: Skimmer box samples: testing for the presence of toxicant(s).

Skimmer Box **Laboratory Controls**	**Cladocerans** **% survival** *(Mean +/- SD)*	**Oysters** **% normal/survival** *(Mean +/- SD)*	**Sea Urchin** **% normal larvae** *(Mean +/- SD)*
Sea Water	n/a	73.7 +/- 4.8	93.0 +/- 1.8
Artificial Sea Water	n/a	74.8 +/- 3.9	91.0 +/- 1.4
Fresh Water	100 +/- 0.0	n/a	n/a
Samples			
North George	100 +/- 0.0	16.2 +/- 8.4	32.0 +/- 9.4
South George	100 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0
Pyengana	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0
Water Intake	0.0 +/- 0.0	0.0 +/- 0.0	0.0 +/- 0.0

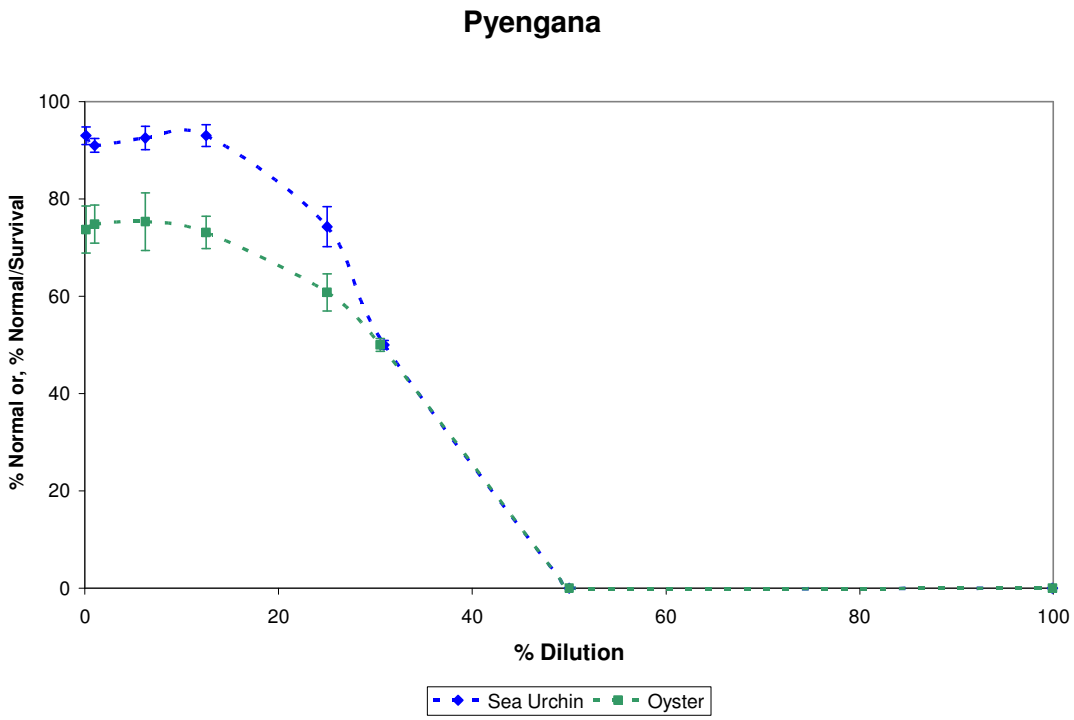
Graph 1: Dilution Curves for the North George Sample



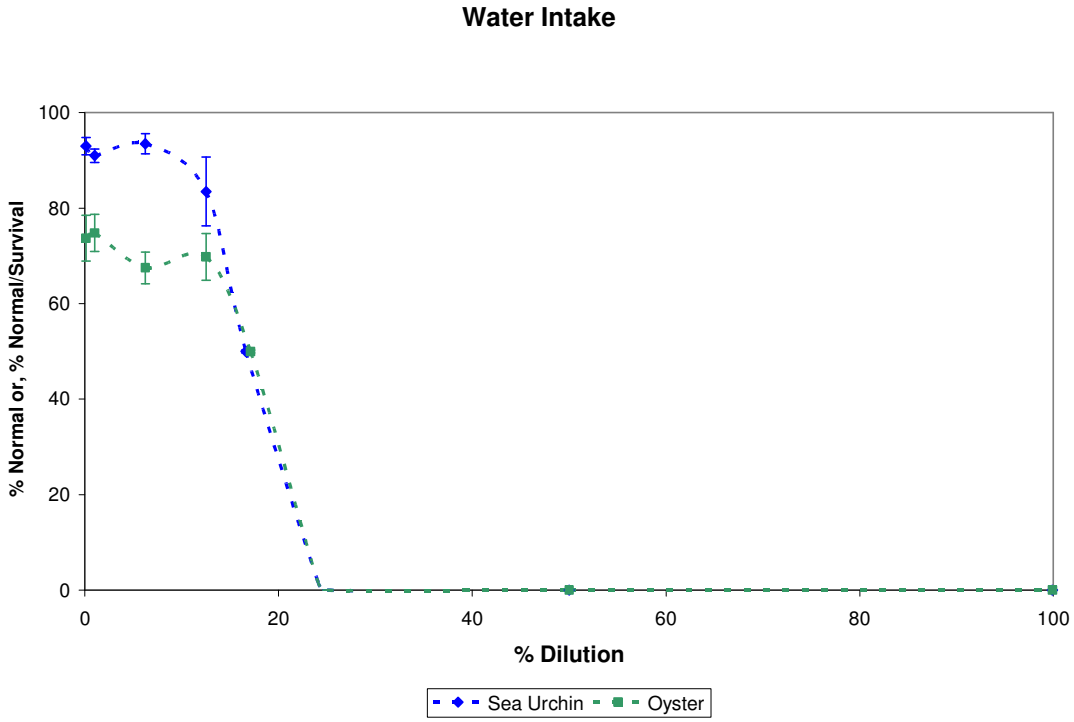
Graph 2: Dilution Curves for the South George Sample



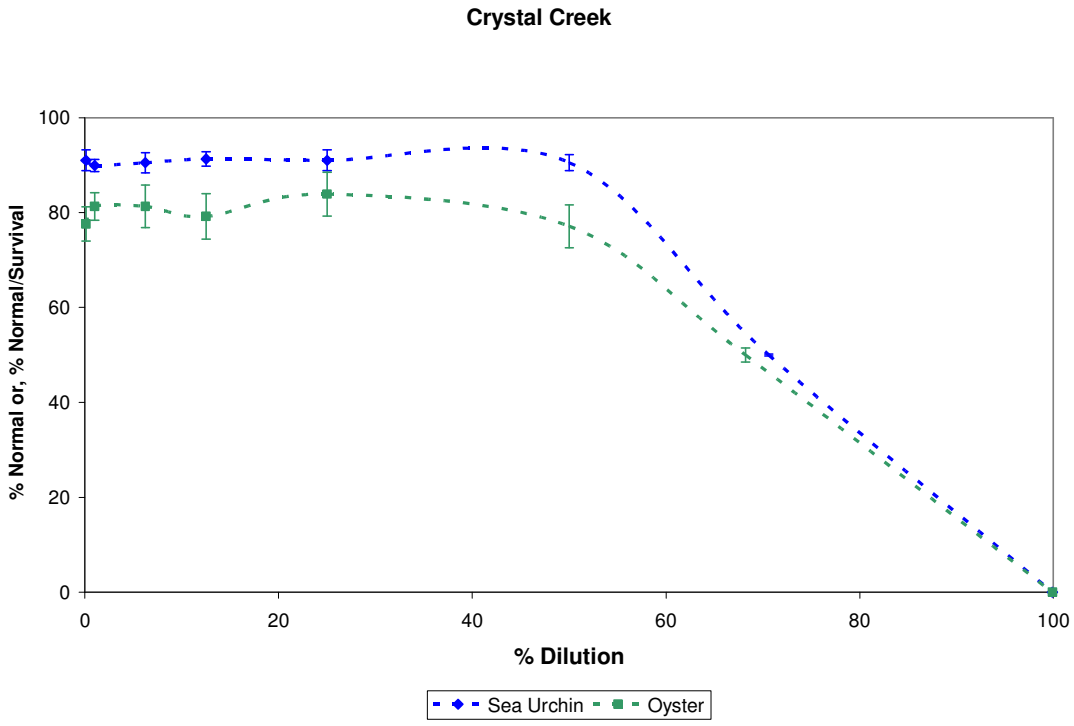
Graph 3: Dilution Curves for the Pyengana Sample



Graph 4: Dilution Curves for the Water Intake Sample



Graph 5: Dilution Curves for the Crystal Creek Grab Sample



Tasmanian Investigation
Test Number 3

Sampling Details
February 2 & 3, 2005. Sampled by Judy Marshall, University of Tasmania.

Locations Sampled
North George
South George
Pyengana
Moulting Bay

Purpose
To determine the baseline condition of the George River system, St Helens, Tasmania, with respect to the presence of toxicant(s) before and during rainfall.

Investigating Laboratory(s)
Ecotox Australia, Sydney

Tests Conducted
Cladoceran test

Choice of tests
The Cladoceran (fresh water flea) had already been calibrated against Oysters and Sea Urchins and was deemed to be of similar enough sensitivity to be useful. It was also chosen because it is a fresh water animal and therefore manipulating salt levels would not be required, allowing removal of a possible source of laboratory error.

Sampling Method
Two types of sampling methods were employed. The first method, grab sampling, involves submerging a sampling bottle in the water column. This method provides a sample that represents a normal water column sample and indicates if drinking (or being exposed to) the water in an untreated state would be hazardous. The second method, skimmer box, concentrates the surface water where lipid soluble toxicants are likely to accumulate. The purpose of the second method is to determine if lipid soluble toxicants are present and to make it easier to then identify them chemically. If lipid soluble toxicants are present it does not necessarily mean they are at sufficient concentrations in the water column to be of concern.

Results
Skimmer box samples indicated that toxicant(s) to Cladocerans were present at North George and South George prior to rain. These substances caused 100% mortality for the South George sample and 20% mortality in the North George sample. Toxicity persisted during early rain within the South George sample (100% mortality) but not for the North George sample or the sample from Pyengana, where no mortality was observed. No mortality was observed for the samples from South George or North George, which were sampled during the middle of the rain event (Table 4).

Grab samples indicated that significant concentrations of toxicant(s) were present with respect to Cladocerans for the Moulting Bay sample (100% mortality) taken the day following the onset of rain, but the South George sample, taken in the middle of the rain event, was not toxic (Table 5).

Conclusions
Skimmer box results indicate that toxicant(s) were present on this round of sampling. Grab samples indicate that at the Moulting Bay location concentrations of toxicant(s) were sufficiently high to be of concern with respect to the test organism (therefore drinking the water or being exposed to the water over time in an untreated state is likely to be hazardous).

Status
Skimmer box samples confirm that this method concentrates toxicant(s) and will be useful for the toxicant(s) identification.

Result confirmed that hazardous concentrations of toxicant(s) occur in the water column of the test area using grab samples. This is the third occasion when grab samples have produced positive identification of toxicant(s) from raw water. In Test 1, the North George and Pyengana grab samples were positive. In Test 2, the Crystal Creek surface grab sample tested positive. In this set of tests, the Moulting Bay surface grab sample has tested positive. A "TIE" in accordance with the ANZECC 2000 guidelines is justified.

Table 4: Skimmer box samples: testing for the presence of toxicant(s).

Skimmer Box Date	**Time**	**Location**	**Cladoceran Survival**
2/02/2005	21:35	North George pre-rain	80%
3/02/2005	2:25	North George early rain	100%
3/02/2005	10:10	North George during rain	100%
2/02/2005	22:20	South George pre-rain	0%
3/02/2005	3:40	South George early rain	0%
3/02/2005	11:10	South George during rain	100%
3/02/2005	12:05	George River early rain*	100%

** The George River site is at Pyengana*

Table 5: Grab samples: testing for significance of the presence of toxicant(s).

Grab Sample	**Time**	**Location**	**Cladoceran Survival**
3/02/2005	13:10	Moulting Bay Surface	0%
3/02/2005	3:40	South George 0.5m	100%