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.

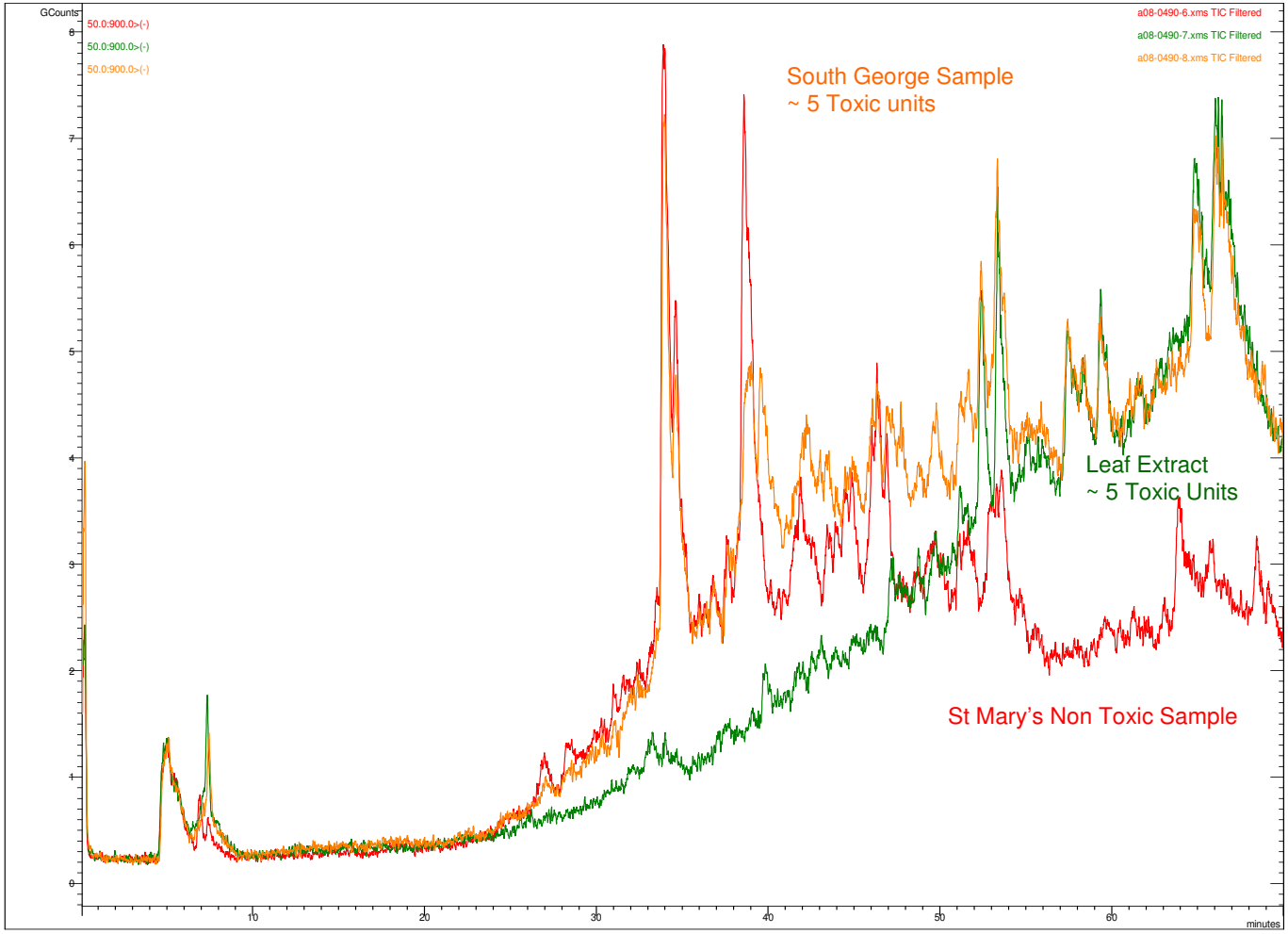
1) The toxin(s) was present in surface foam during all dry weather samples.
2) The toxin(s) has a relatively short half life, days to weeks.
3) The toxin(s) is primarily attached to fine particulate matter but some remains dispersed or dissolved.
4) The toxin(s) are not chealatable metals.
5) The toxin(s) 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.