Catchment studies in Georges Bay, Tasmania: base-flow water and foam toxicity to cladocerans and blue-mussels

Chris Hickey and Michael Stewart

A case of unintended consequences?

National Institute of Water
and Atmospheric Research
Hamilton, New Zealand

Society for Environmental Toxicology and Chemistry; Seville 24 May, 2010

NIWA
Taihoro Nukurangi

Overview

- Background
- The Problem
- Results
  - toxicity assessment
  - characterisation
- Forensic eco-toxicology
- Integrated risk assessment
- Conclusions
- Future studies

Background

- Helicopter crash in catchment (2003) – multiple pesticides detected
- Mass mortality of oysters in Georges Bay, Tasmania was observed in summer 2004 following a record floods
- Extensive mortality of other marine species - filter-feeders (clams, mussels, barnacles), prawns, crabs, sea urchins and a variety of fish species
- Freshwater species affected were noted as "rafts of dead frogs and other insects" in the Bay

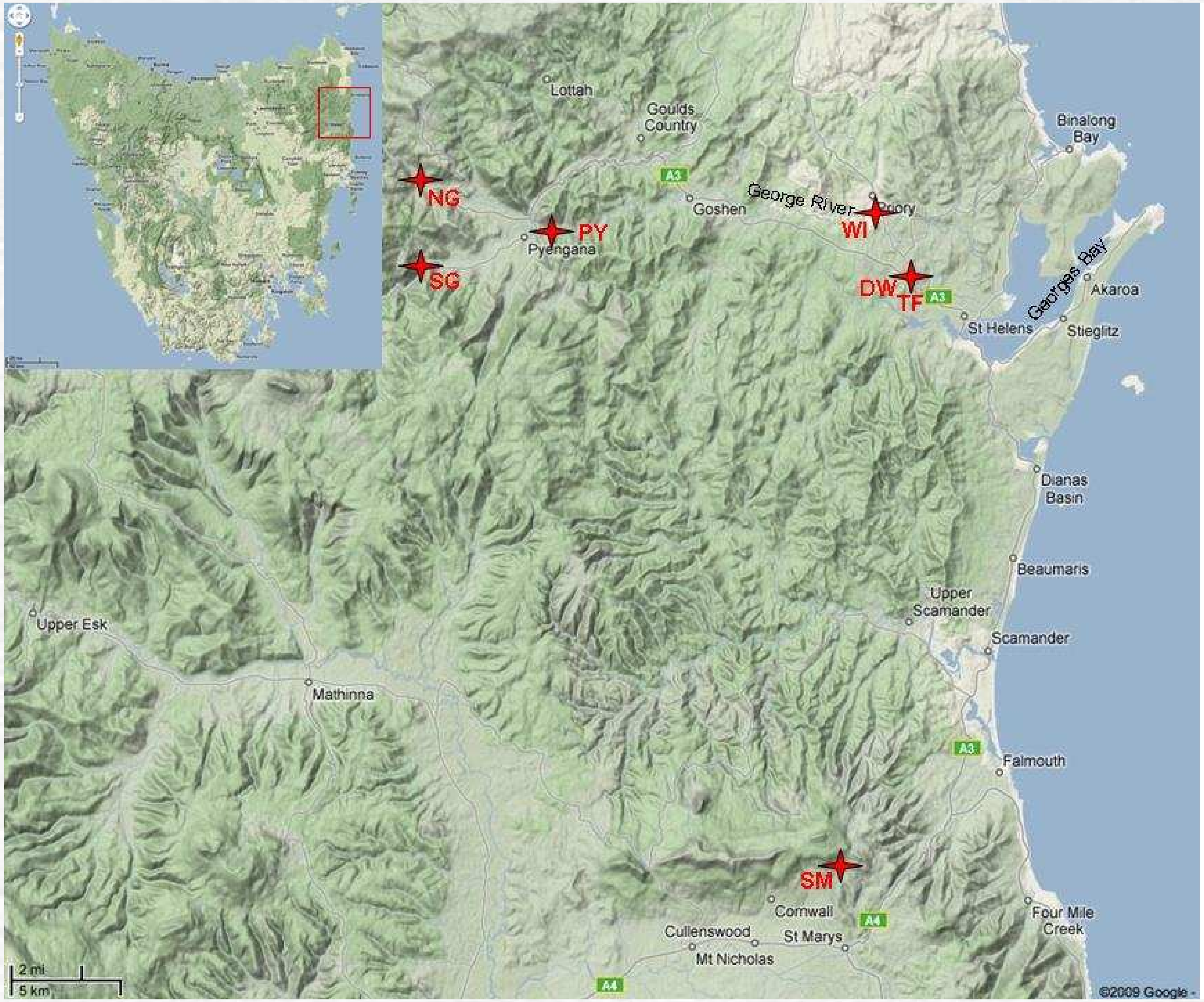
- Insecticides and herbicides have been measured in the catchment soils and waters - though their water concentrations have generally been below effects thresholds

- Human health issues – various and often rare at elevated frequency
- Oyster health decline – "novel" symptoms
- Wildlife issues
- High ecotoxicity effects measured in foam and storm-flow waters
- Observed elevated production of foams in river catchment

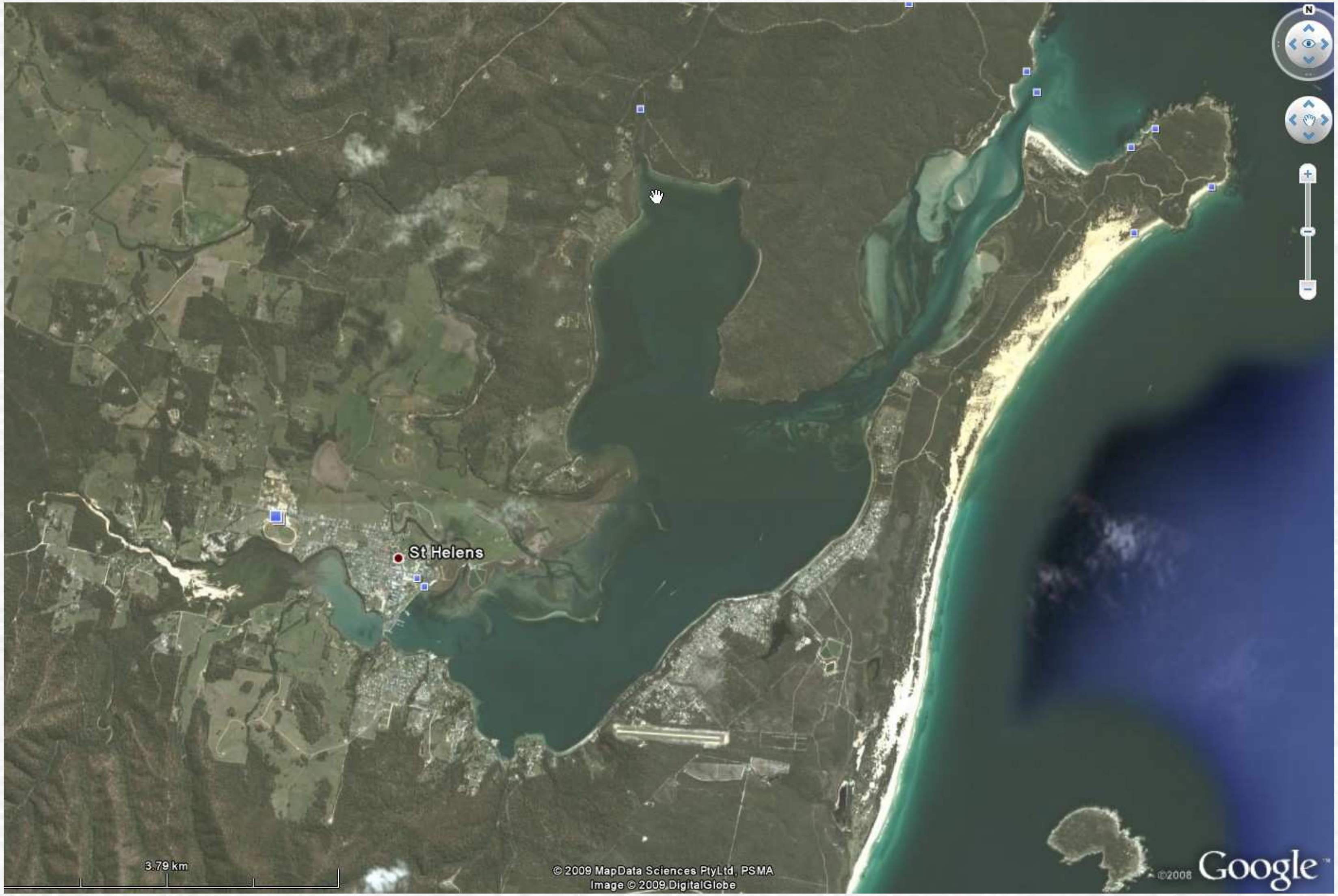
- Plantation forests of ***Eucalyptus nitens*** established and developing in catchment over this period

→ Multiple effects/targets – persistent over time

Background: Site

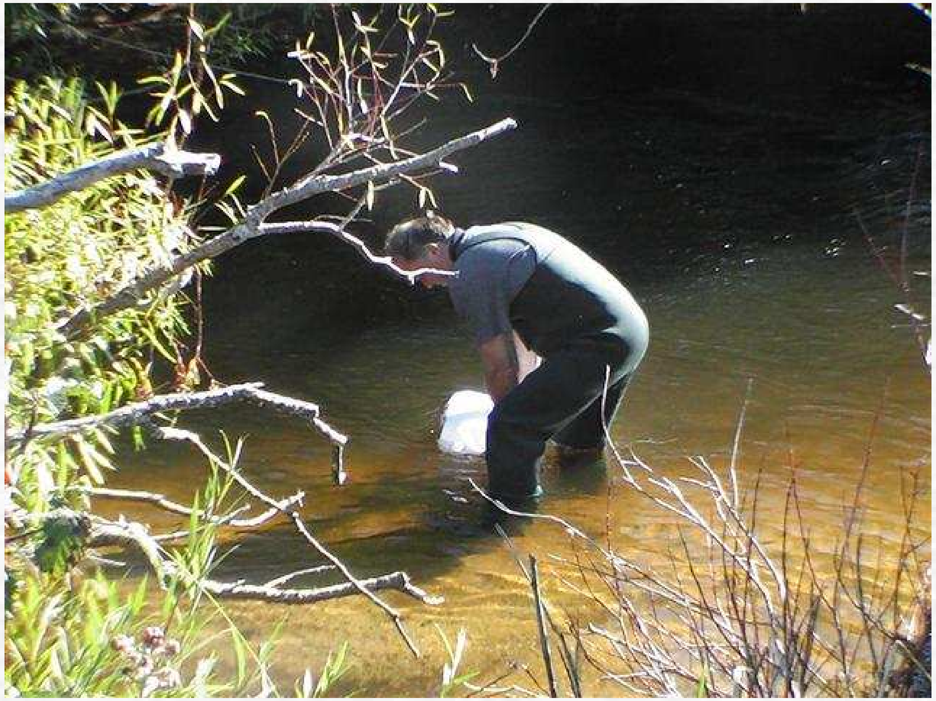


Background: Site



Background: Site

The Problem: Rivers



Drinking water intake pipe on George River at Priory

South George foam collection

Collapsed foam

Flocculated particulates in water-treatment system

More river foam: prior to filtration

The Problem: Bay

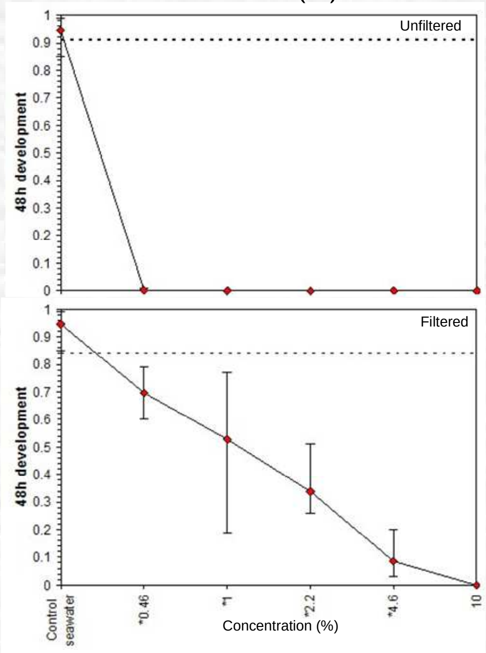
→ **Transport and wildlife exposure**

Study design: Ecotoxicity

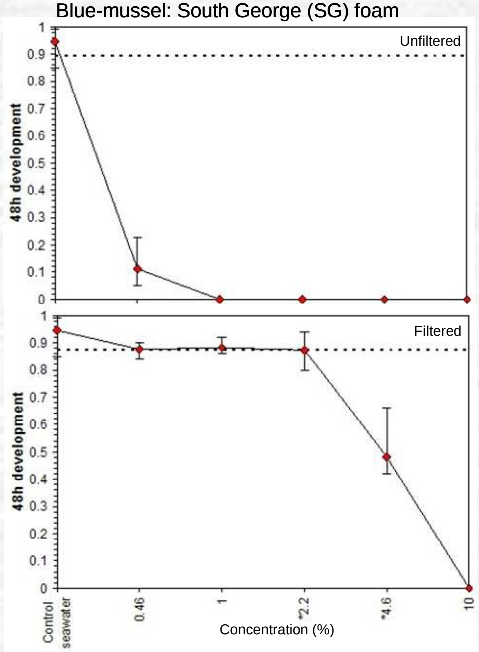
- Baseflow stream-water collection – multiple sites, including reference catchment (pre-filtered 50 µm)
- River foam collection at 2 sites (pre-filtered 140 µm)
- Toxicity assessment:
  - freshwater cladocerans (*Ceriodaphnia dubia*)
  - marine bivalve, blue mussel embryo-larvae (*Mytilus galloprovincialis*)
- Chemical characterisation:
  - organic content, suspended solids, particle counts, particle size distribution, pesticide suite
  - organic extract (EtOH) of foam and *E. nitens* leaves (catchment & reference site (Victoria))
  - bioassay directed fractionation: HPLC/mass spec → Molecular fractionation (LH20) → ms & NMR
- Toxicity identification:
  - filtration; add-back
  - screening and definitive bioassays of foam and leaf extracts (toxic units, TUs)
  - chemical standards
- Foam characterisation:
  - quantitative foam assay procedure
  - applied to chemical fractions

Results: Foam concentration-response

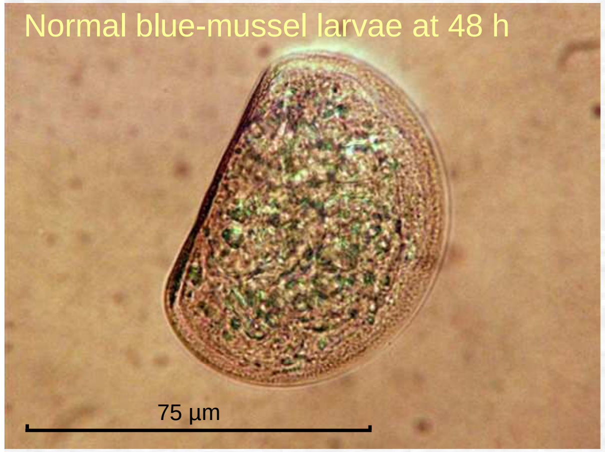
Blue-mussel: Water Intake (WI) foam

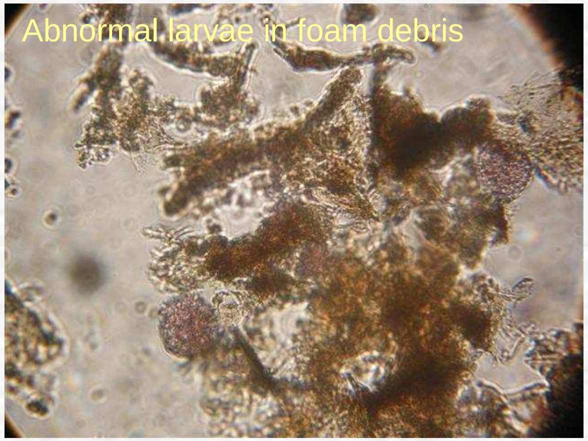


Blue-mussel: South George (SG) foam



Concentration-response relationships for filtered and unfiltered foam samples from Georges River catchment (South George & Water Intakes) to blue mussel larvae





→ Higher particle-associated toxicity in foams

Results: foam specific toxicity

Site/Sample	Code	Ceriodaphnia (CL) LC_{50} (%)	Blue-mussel (BM) EC_{50} (%)	CL Toxic units[a] TU_{50}	BM Toxic units[a] TU_{50}	CL TU/SS [b]	BM TU/SS [b]
Water Intake foam	WI_F	4.4	0.23	23	435	0.0047	0.091
South George foam	SG_F	<10	0.26	10	385	0.0038	0.15

Blue-mussel larvae markedly more sensitive

Calculated toxic threshold for BM larvae is 3x above base flow SS concentration

→ Source of foam and toxicity?

Forensic: Study design

Concurrent analyses:

1. Foam from St George River, Tasmania
2. Tasmania leaves – new growth *E. nitens*
3. Victoria leaves (reference)

Freeze dry → EtOH extracts

Bioassay analyses:

Blue mussel assays
0.1% EtOH
Screening
Definitive – dilution series

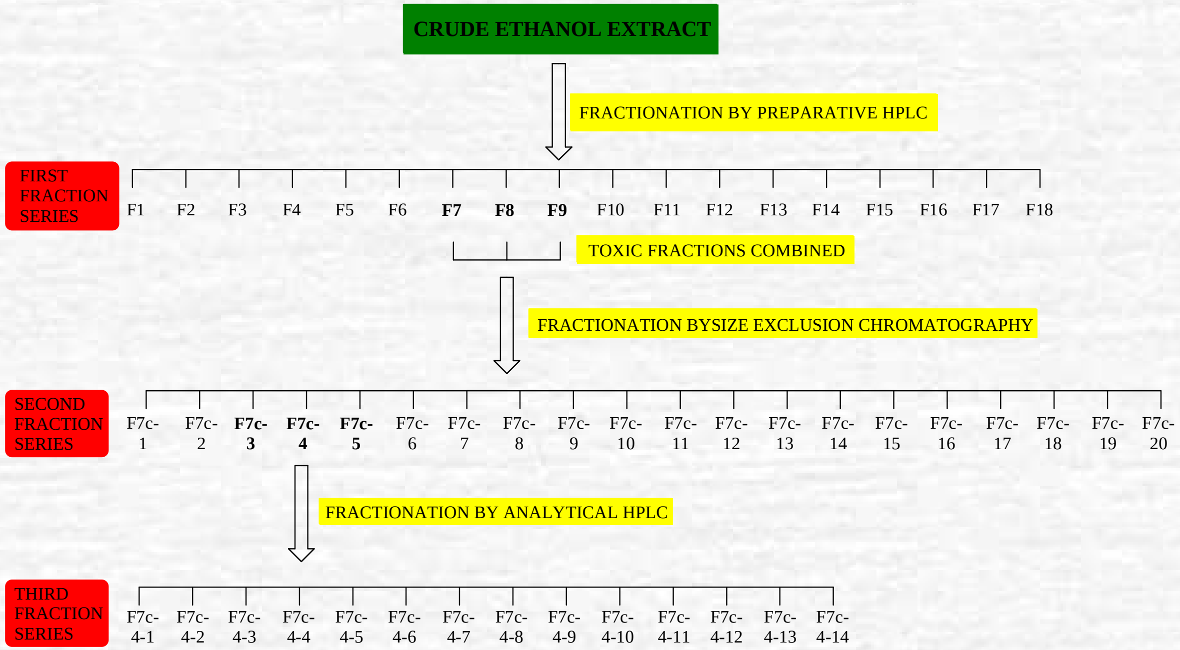
Foam assays: agitation

Chemistry

Preparative HPLC

Size exclusion LH20

Analytical HPLC



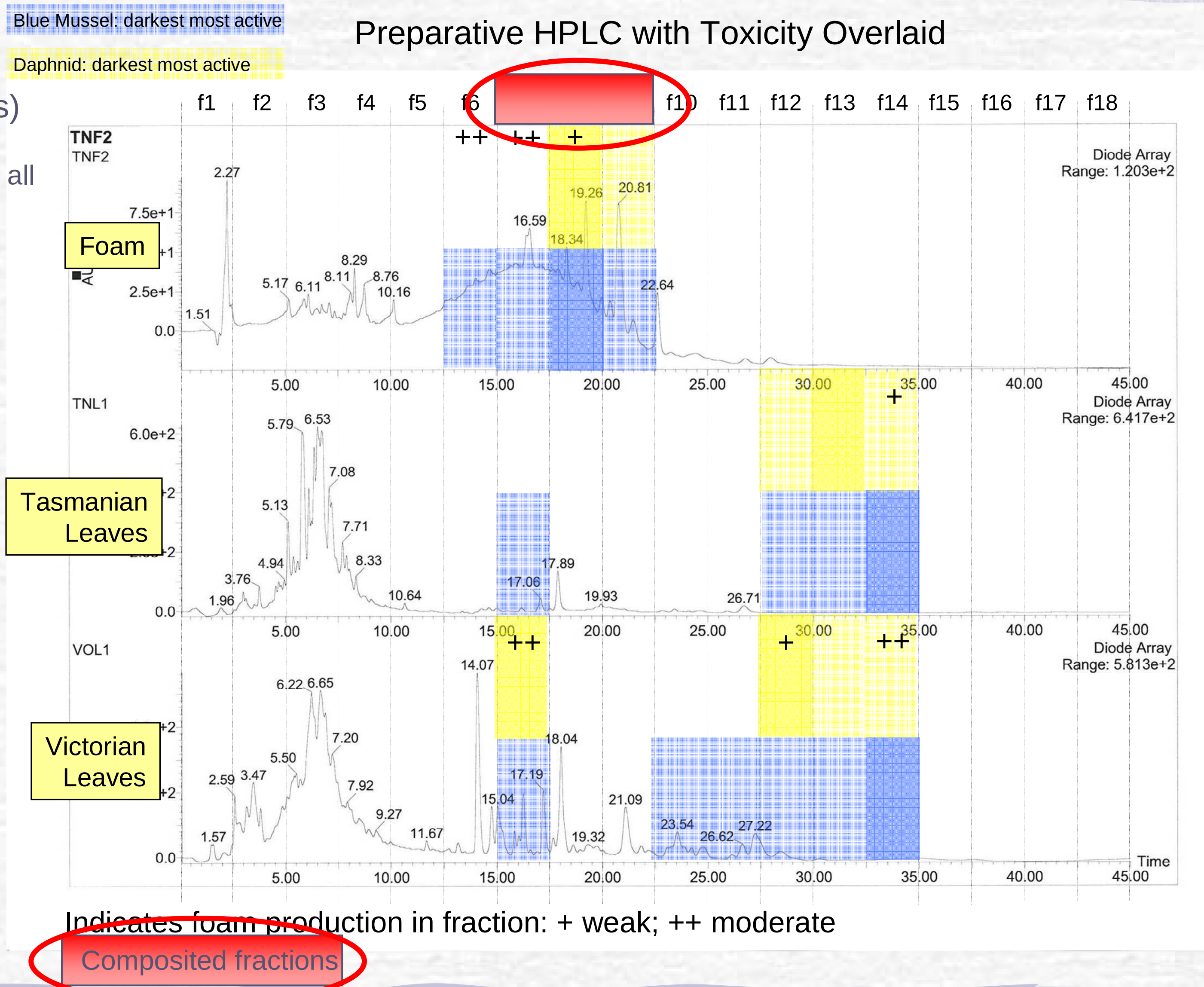
Forensic: Bioassay directed fractionation

Toxicity (TUs)
Parent
(% recovery of all fractions)

36 TUs
(78%)

21 TUs
(126%)

59 TUs
(57%)



Common toxic fractions for size exclusion chromatography

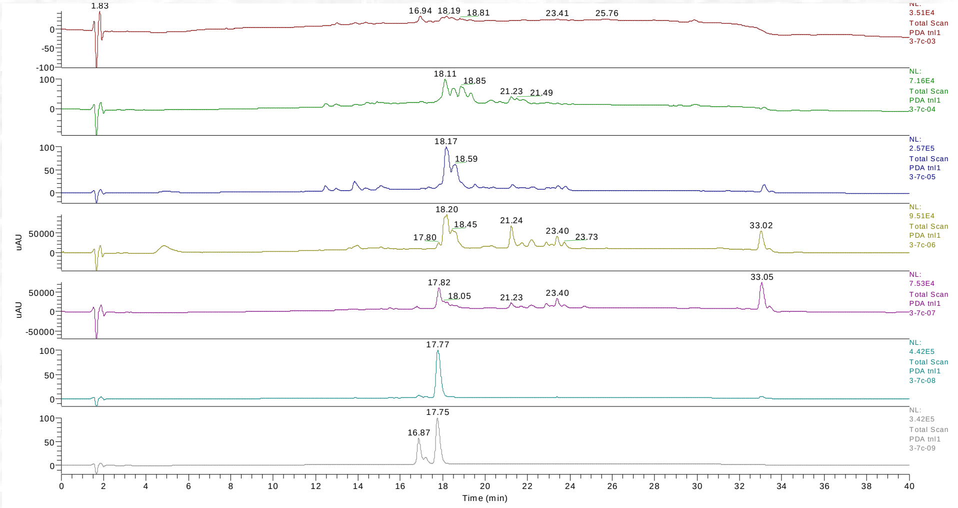
Forensic: Bioassay directed fractionation

Molecular weight fractionation (LH20)
(Parent: **HPLC f7-9, 5x concentrated**;
UV of HPLC/mass spec)

Tasmanian leaves

parent 256 TUs; f3-5 28%

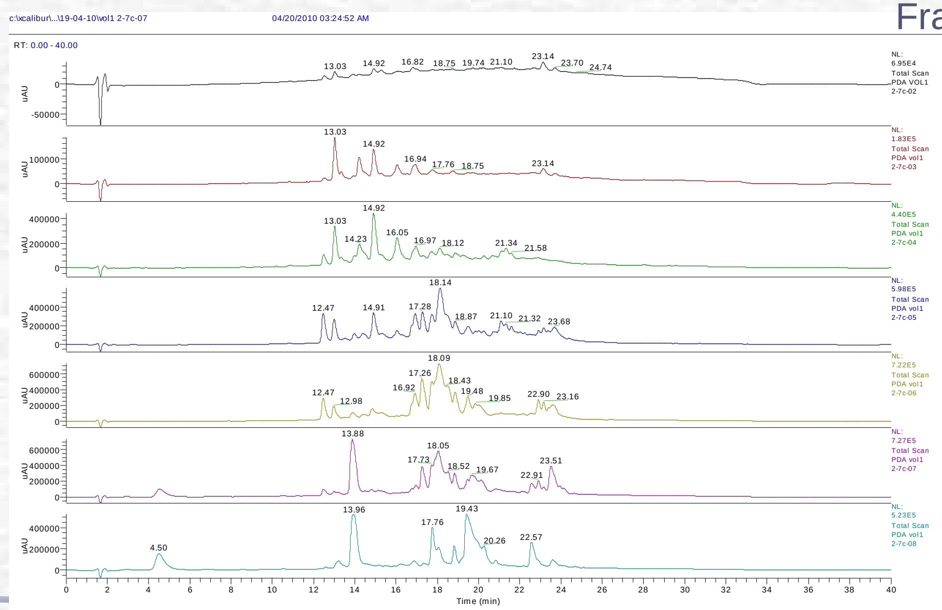
TUs	Fraction
	1
	2
1.4	**3**
11	**4**
59	**5**
	6
	7



Victorian leaves

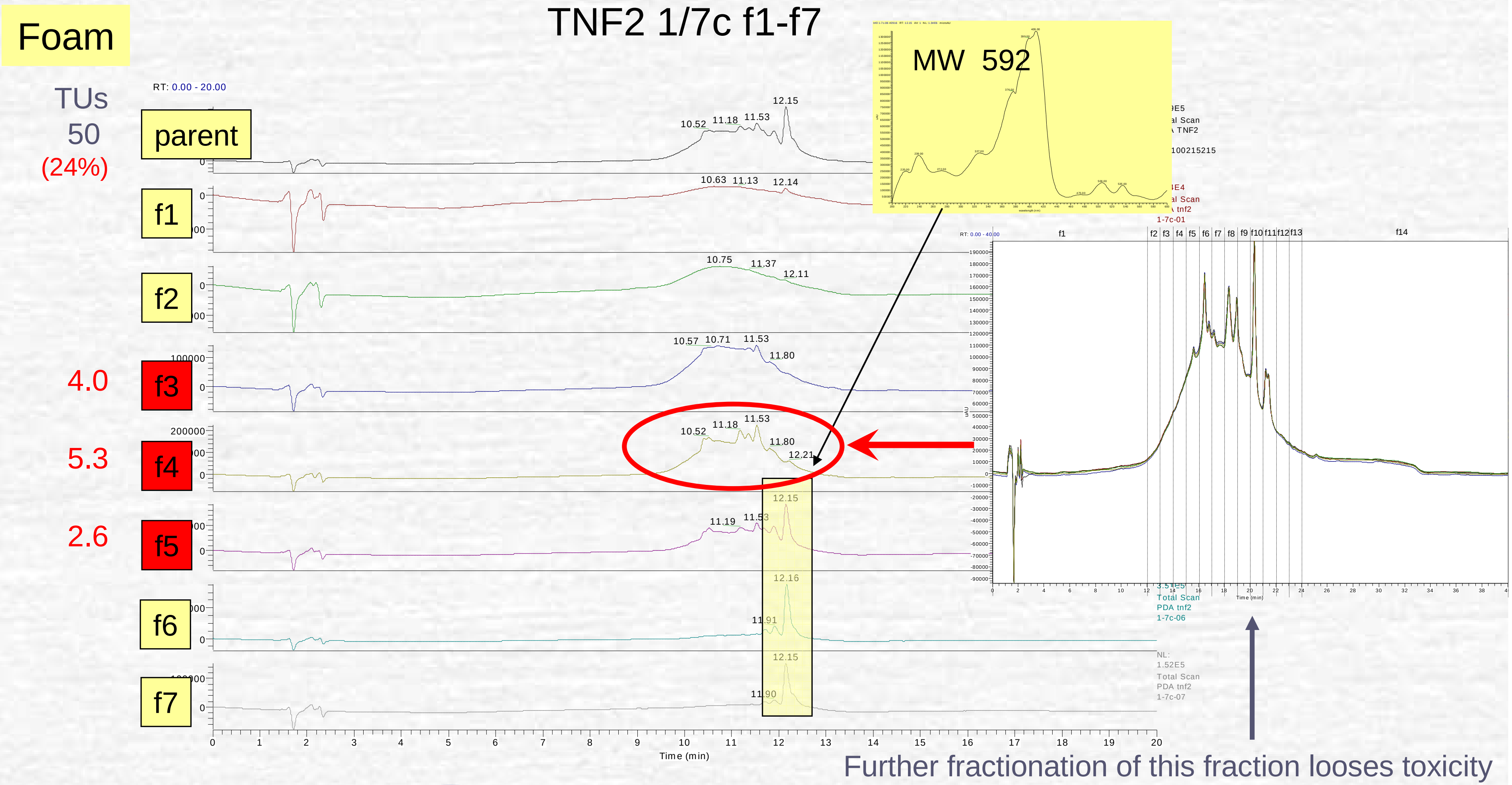
parent 370 TUs; f3-5 15%

TUs	Fraction
	1
	2
1.0	**3**
16	**4**
39	**5**
	6
	7



Forensic: Bioassay directed fractionation

Molecular weight fractionation (LH20)
(Parent: **HPLC f7-9**; UV of mass spec)



Toxic fractions of foam poorly resolved – multiple components

Forensic: Bioassay directed fractionation

Mass spectrometer molecular weight comparisons for HPLC fractions

Metabolite Group	Simple FPCs[a]			monoterpene euglobals		macrocarpals[b]	sideroxylonals [c]	
Molecular Weight (MW)	266	252		386		472	500	
Extracted m/z (-ve mode)	265	251		385		471	499	
HPLC Retention Time Sample[d]	Not detected	9.9 min	10.4 min	12.6 min	13.5 min	11.5 min	12.1 min	12.8 min
Tasmanian Leaves (crude extract)	-	+	+	+	++	++	++	-
F7	-	++	++	-	-	+	-	-
F12	-	-	-	-	++	-	-	-
F13	-	-	-	-	-	-	++	-
F14	-	-	-	-	-	-	-	-
Victorian Leaves (crude extract)	-	++	++	+	++	++	++	+
F7	-	++	++	-	-	++	++	-
F10	-	-	+	+	-	+	++	++
F11	-	-	++	++	++	+	++	++
F12	-	-	-	-	++	-	++	-
F13	-	-	+	-	-	-	++	-
F14	-	-	++	-	-	+	++	-
Tasmanian Foam (crude extract)	-	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-	-
F7	-	-	-	-	-	-	-	-
F8	-	-	-	-	-	-	-	-
F9	-	-	-	-	-	-	-	-
F10-F14	-	-	-	-	-	-	-	-

[a] Includes jensenone (MW 266) and grandinol/homograndinol (MW 252). [b] Includes 12 macrocarpals with MW 472. [c] Sideroxylonals A-C (MW 500).
[d] Only toxic fractions included, with the exception of F10-F14 for foam, which are included for comparison
- not detected; + low level detection; ++ high level detection

→ Indicated common toxic fraction

Fractions coded red are most toxic

→ Elimination of known compounds in toxic foam fractions; 400-500 MW

Forensic: Foam

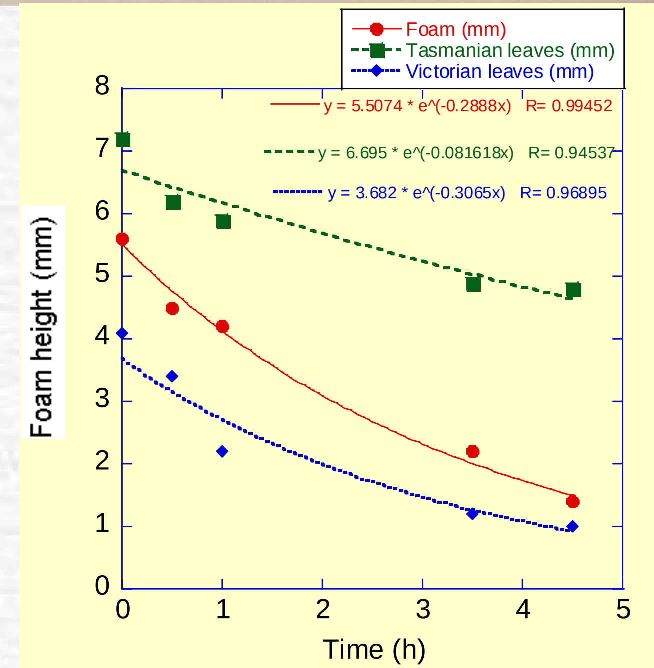
Primary (EtOH) extracts

Foam decay half-life:

Foam = 2.8 h

Tasmanian leaves = 12 h

Victoria leaves = 2.4 h



→ Marked difference in foam characteristics

Summary:

- highly toxic foams – particularly to bivalve larvae
- strong particle-associated toxicity
- a toxic effects threshold about 3x over base-flow river suspended solids
- strong toxicity for the whole extract for foam and both leaf sources
- discrete toxicity in only a few foam and Eucalyptus leaf chemical fractions
- concordant toxicity in both cladocerans and blue mussels for all toxic chemical fractions

cont...

Summary:

- higher number of toxic fractions in leaf extracts than foam samples
- an unknown mixture of common toxic components (MW 400-500) result in foam toxicity and some leaf toxicity
- foam "hump" reversibly looses toxicity with further purification → difficulty for field chemical characterisation
- foam-forming ability not concordant with high toxicity fractions
- Tasmanian *E. nitens* are chemically different and have markedly stronger foam-forming ability

→ Have we enough information for causation of field effects?

Potential causation: *Eucalyptus nitens* ?

Evidence?

- extensive plantations in catchment
- time concordance with effects and foam occurrence
- persistent and ongoing inputs
- foam has high organic content with plant debris
- foam and stream particulates have ability to reach target species
- common toxic fractions in foam and eucalypt leaves

Knowledge about plantations:

- selectively breed – for pest resistance
- chemically different from parent plantation leaves (this study)

⟶ Other information from literature…?

Literature

Toxins in transgenic crop byproducts may affect headwater stream ecosystems

E. J. Rosi-Marshall*[†], J. L. Tank[‡], T. V. Royer[§], M. R. Whiles[¶], M. Evans-White[‡], C. Chambers[¶], N. A. Griffiths[‡], J. Pokelsek*, and M. L. Stephen[‡]

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Communicated by Gene E. Likens, Institute of Ecosystem Studies, Millbrook, NY, July 31, 2007 (received for review March 5, 2007)

Corn (*Zea mays* L.) that has been genetically engineered to produce the Cry1Ab protein (Bt corn) is resistant to lepidopteran pests. Bt corn is widely planted in the midwestern United States, often adjacent to headwater streams. We show that corn byproducts, such as pollen and detritus, enter headwater streams and are subject to storage, consumption, and transport to downstream water bodies. Laboratory feeding trials showed that consumption of Bt corn byproducts reduced growth and increased mortality of nontarget stream insects. Stream insects are important prey for aquatic and riparian predators, and widespread planting of Bt crops has unexpected ecosystem-scale consequences.

caddisflies | genetically modified crops

Headwater streams are intimately connected with the adjacent terrestrial environment (1, 2). Thus, the proximity of crop fields and stream channels in the agricultural midwestern U.S. suggests that crop byproducts can enter streams. Much of the Midwest is planted in, or influenced by, row crop agriculture. In 2006, 33.1 million hectares of corn were planted in the U.S., and 35% of this was transgenic corn (www.nass.usda.gov/index.asp) modified to express the δ-endotoxin Cry1Ab, derived from *Bacillus thuringiensis* (hereafter "Bt corn"). Crop byproducts from Bt corn contain this toxin (3, 4), but until now the effects of Bt corn byproducts on stream organisms have not been examined. This is in sharp contrast to numerous studies examining potential effects on nontarget organisms in the terrestrial environment (4–8).

Crop byproducts are a component of the benthic detritus pool in agricultural streams (9), but quantitative information on the input, transport, and fate of these materials in the aquatic environment is lacking. During pollen shed, wind can transport corn pollen from 40 to 60 m away from source fields (10), and rain can dislodge and transport pollen away from crops (6). After harvest, crop byproducts remain on fields and may be transported to adjacent streams via wind and water. Once in stream channels, possible fates of crop byproducts include microbial decomposition, consumption by aquatic invertebrates, burial via sedimentation, or downstream transport (Fig. 1*A*).

We quantified inputs of corn byproducts to headwater agricultural streams, measured transport distances of these materials within streams, and examined the effects of these materials on stream-dwelling aquatic insects. We focused on headwater streams because of their dominance in the agricultural landscape, their tight linkage to the terrestrial environment, and their proximity to cornfields in the Midwest. Headwaters are also a logical starting point for assessing potential impacts of crop byproducts on aquatic environments because they serve as an initial conduit for transport to downstream water bodies. We measured inputs of corn byproducts to 12 typical headwater streams (Fig. 1 *B* and *C*) in an intensely agricultural region of northern Indiana in 2005 and 2006. The landscape in this part of Indiana is 90% row crop agriculture, and we believe that the inputs we measured are representative of the large number of streams in the agricultural Midwest. We then quantified downstream transport distances of these materials during baseflow conditions. Lastly, we used laboratory feeding studies to examine the effects of Bt corn byproducts on selected aquatic insect taxa commonly found in headwater streams.

Results

Beginning with autumn harvest and extending through the next growing season, we used stream-side litter traps to quantify litter inputs and found that the input of unharvested crop byproducts ranged from 0.1 to 7.9 g of ash-free dry mass (AFDM) m^{-2} of stream channel (Fig. 2*A*). We also found storage of crop byproducts within stream channels; benthic sediments within streams contained up to 6.4 g of AFDM m^{-2} of particulate corn byproducts. Pollen shed occurred during July and lasted ≈5–10 days at each site. Using pollen sticky traps placed in stream channels near the water surface, we found that corn pollen was aerially deposited into all streams, and annual inputs ranged from 0.1 to 1.0 g m^{-2} (Fig. 2*B*). Inputs of corn byproducts were highly variable among the 12 study streams for both litter and pollen, suggesting that potential impacts of these novel carbon sources could vary depending on the magnitude of the inputs to a given stream.

Using short-term releases of labeled material, we found that mean travel distance for leaves and cobs ranged from 0.38 to 180 m and that pollen traveled from 20 to 60 m (Fig. 2*C*). Despite the large range in size of byproducts, transport distances for all corn byproducts were strongly influenced by stream discharge ($r^2 = 0.69$, $P < 0.0001$; Fig. 2*C*). At site 2F, pollen was estimated to travel >2,000 m because of high water velocities, which contrasted with sites 1B and 1C, where pollen did not move because water velocity was near zero. Mechanisms for crop byproduct retention include deposition onto the streambed and adherence to benthic algal biofilms and macroalgae. Results from our estimates of transport distances for the various corn byproducts indicate that transgenic material entering streams is retained during base flow and thus is available for microbial processing, consumption by aquatic insects, or export during storms.

Decomposition of plant litter by microbes and physical abrasion generates food for local aquatic consumers and also facilitates the transfer of energy and nutrients from upstream to downstream reaches within a river network (11). We measured breakdown rates of Bt and non-Bt corn litter to determine whether the Bt δ-endotoxin influences rates of organic matter processing in our study streams. We found no difference in decomposition rates between Bt ($k = 0.020\ d^{-1} \pm 0.002$ SEM) and non-Bt ($k = 0.015\ d^{-1} \pm 0.003$ SEM) corn litter ($P = 0.95$; analysis of covariance), suggesting that

Author contributions: E.J.R.-M., J.L.T., T.V.R. and M.R.W. contributed equally to this work; E.J.R.-M., J.L.T., T.V.R., and M.R.W. designed research; E.J.R.-M., J.L.T., T.V.R., M.R.W., M.E.-W., C.C., N.A.G., J.P., and M.L.S. performed research; E.J.R.-M., J.L.T., T.V.R., M.R.W., M.E.-W., C.C., N.A.G., J.P., and M.L.S. analyzed data; and E.J.R.-M., J.L.T., T.V.R., and M.R.W. wrote the paper.

The authors declare no conflict of interest.

Abbreviation: AFDM, ash-free dry mass.

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Rosi-Marshall, E.J.; Tank, J.L.; Royer, T.V.; Whiles, M.R.; Evans-White, M.; Chambers, C.; Griffiths, N.A.; Pokelsek, J.; Stephen, M.L. (2007). Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* 104: 16204-16208.

Literature

International Paper Treads Monsanto's Path to 'Frankenforests'

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By Jack Kaskey

Aug. 28 (Bloomberg) -- **International Paper Co.**, the world's largest pulp and paper maker, plans to remake commercial forests in the same way **Monsanto Co.** revolutionized farms with genetically modified crops.

International Paper's ArborGen joint venture with **MeadWestvaco Corp.** and New Zealand's **Rubicon Ltd.** is seeking permission from the U.S. Department of Agriculture to sell the first genetically engineered forest trees outside China. The Australian eucalyptus trees are designed to survive freezes in the U.S. South.

Plantations of engineered trees would give International Paper a competitive advantage by providing a reliable supply of lower cost wood at a time when timberlands are dwindling because of development, said **David Liebetreu**, the Memphis, Tennessee- based company's vice president of global sourcing. Opponents are concerned that alien genes may contaminate natural forests, echoing objections to modified crops that Monsanto still faces.

"There is a potential to explode once they get these trees approved," said **David Knott**, who manages $1.3 billion as chief executive officer of Dorset Management in Syosett, New York. He said he increased his **stake** in Rubicon to 70.5 million shares this year to bet on ArborGen because it has a customer base of large landowners and little competition. "This could take off faster than Monsanto."

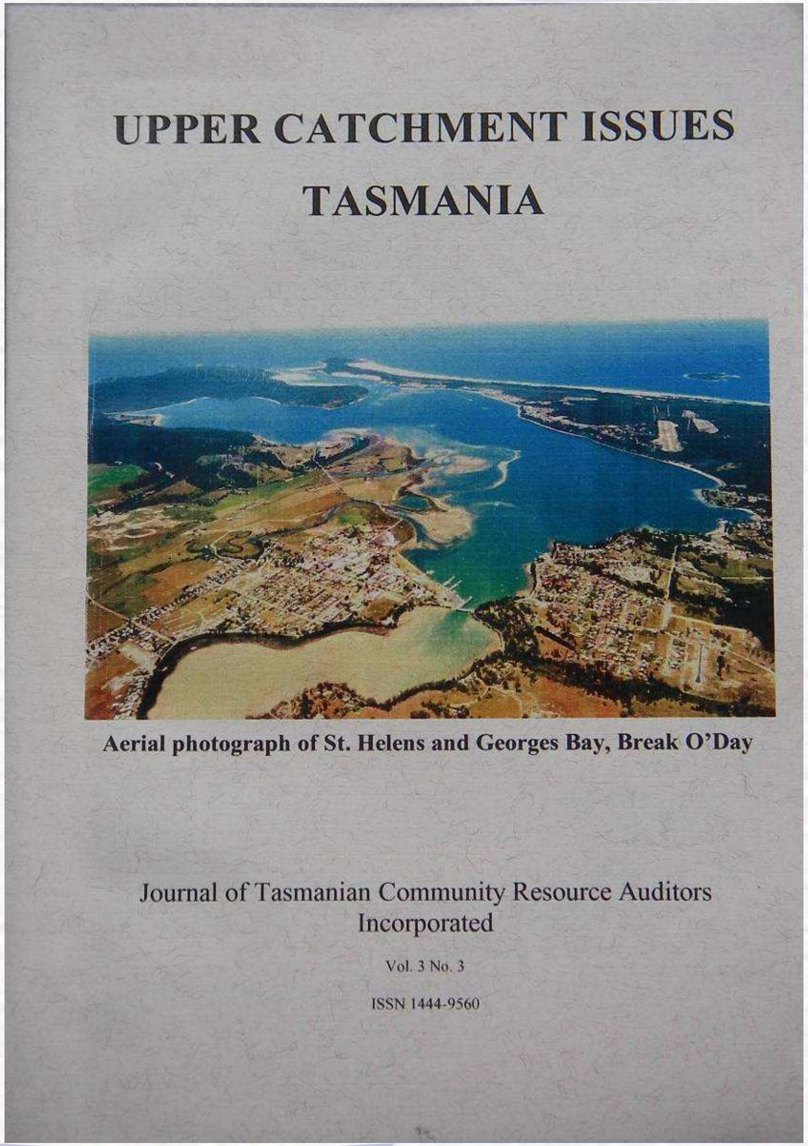
Monsanto's genetics, which were first sold in herbicide- tolerant soybeans in 1996 and insect-resistant corn the following year, were used in 88 percent of the world's 309 million acres of biotech plantings last year. Monsanto's sales of seeds and genetics quadrupled since 2002 to **$6.4 billion** last year.

ArborGen Sales

ArborGen may boost yearly sales to $500 million in 2017 from $25 million by following Monsanto's blueprint for commercializing engineered plants, said **Stephen Walker**, head of asset management at New Zealand-based Goldman Sachs JBWere Ltd., which owns Rubicon shares and holds no stock in International Paper or MeadWestvaco. The partners eventually might sell shares of ArborGen to the public, International Paper's Liebetreu said.

http://www.bloomberg.com/apps/news?pid=email_en&sid=aEHNB_XJRWGU#

Weight-of-evidence: Human health



Bleaney,A. 2007 'Risk Awareness and Incident Response Capability in Water Catchments in North Eastern Tasmania, Australia – A Community Based Audit', Upper Catchments Issues Tasmania, Vol 3 No 3, ISSN 1444-9560.

Weight-of-evidence: Wildlife issues

September 20, 2009 SUNDAY TASMANIAN — 9

Crisis time for species

Vet seeks action on habitat

By DANIELLE McKAY

MYSTERY diseases are threatening the existence of Tasmania's iconic native wildlife, a veterinarian expert warns.

Tumours, infections and ulcers are impacting on populations including echidnas, platypus, wombats, wallabies, frogs and bandicoots, Dr David Obendorf says.

He believes opportunistic diseases are attacking wildlife because of immune deficiencies caused by environmental damage and bioaccumulation of toxins.

Unless immediate action is taken, more native species will suffer the fate of the threatened Tasmanian devil, Dr Obendorf says.

Greens senator Christine Milne said it was time the Government took action to protect wildlife, which it relied upon heavily for Tasmania's global clean, green image.

Ms Milne called for a comprehensive assessment of Tasmania's wildlife, inside and outside protected areas.

The Department of Primary Industries, Parks, Water and Environment said research was under way into the chytrid fungus affecting Tasmania's frogs and a fungal disease affecting the platypus.

The department said monitoring had been enhanced with the employment of a wildlife health officer who was tasked with improving reporting measures, implementing disease management strategies and providing advice for conservation programs.

INFECTED: Staphylococcus bacteria eats into an echidna's foot.

STRESSED-OUT SKIN: A common wombat with mange.

FATAL FUNGUS: A mucormycosis ulcer on a platypus from Brumby Creek.

CAT PARASITES: Eastern barred bandicoot.

SHOCKER: A devil stricken with DFTD.

CHYTRID THREAT: The green and gold frog.

TASMANIAN WILDLIFE DISEASES

Source: Sunday Tasmanian, 20 September 2009

Conclusion: Potential ecotoxic causation: *Eucalyptus nitens?*

Very highly plausible

i.e., A combination of toxic components and foam-forming ability

Causation of other catchment health effects? → awaiting further studies

→ Unintended consequence of silviculture practices

Future

- sensitive (and simple) chemical analytical methods
- field measurements →

Should do:

- routine suspended solids within the George River catchment and Georges Bay → concentrations and mass loads
- toxicity monitoring of storm-flow waters → toxicity thresholds exceedance during events
- oyster "health" monitoring studies along exposure gradients relative to riverine inputs

→ This issue needs a lot more investigation!

Acknowledgements

- Alison Bleaney
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- Rick Krassoi
- Jim Harris
- Pete Long
- Graham

Questions?

ABC documentary reference:
www.abc.net.au\austory "Something in the Water?"

Contact: c.hickey@niwa.co.nz

NIWA Taihoro Nukurangi

Results: Foam characteristics

Site	Code	Particle count (particles/mL) [calculated add-back]	Suspended solids (g/m³)	Inorganic SS (g/m³)	Organic SS (g/m³)	Inorganic %
South George	SG		1.9	1.2	0.7	63
North George	NG		1.1	<0.5	0.6	<50
Pyengana	PY		2.7	0.8	1.9	30
Water Intakes	WI	21,060	2.6	<0.5	2.1	<20
Drinking Water	DW		10.9	3.3	7.6	30
Treatment Filtrate	TF		8300	4700	3600	57
Gardiners Creek, St Marys	SM	15,260	3.4	1.6	1.8	47
Foam – from WI	WI_F	60,150,600	4800	1600	3200	33
Foam – from SG	SG_F		2600	1100	1500	42
Add-back: SM_1x	SM_1X	53,213 [3.5]				
Add-back: SM_5x	SM_5X	164,500 [10.8]				
Add-back: WI_1x	WI_1X	41,440 [2.0]				
Add-back: WI_5x	WI_5X	144,620 [6.9]				

High particle content of foams