CAPIM Newsletter

Centre for Aquatic Pollution Identification and Management

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Biomarkers at CAPIM: Director of Research and Biomarker Program Leader

Prof Ary Hoffmann is the Director of Research and the Biomarker Program Leader at CAPIM. Prof Hoffmann is also an ARC Laureate Fellow.

One of the challenges being undertaken by CAPIM is to develop new biomarkers for pollution detection and diagnostics. Biomarkers fall into a number of categories. They can be biochemical markers like changes in protein levels or gene expression that reflect an organism's biochemical responses to pollution exposure. They can also involve physiological, morphological or behavioural responses, like altered rates of metabolism or swimming speed exhibited by organisms exposed to stressful conditions, or altered levels of deformities and bilateral asymmetry in species developing under polluted conditions. Biomarkers aim to show the effects of pollutants before significant impairment of ecosystems occur. They are designed to be measured in one or a few species occurring in an environment but reflect the likely response of the entire biota. It is therefore important that biomarkers are extremely sensitive to pollutant conditions. Biomarkers are also ideally specific to particular types of pollutants; in that way specific changes can be diagnostic of particular pollution issues. If biomarkers are to be routinely applied for monitoring and management they need to be reliable as well as relatively cheap and simple to implement.

At CAPIM we are developing a series of biomarkers using local species of chironomid midges, snails, fish and algae. So far most of our development work has focussed on midges. We have explored the possibility of using altered levels of gene expression and patterns of biochemical metabolites as biomarkers. When midges are exposed to pollutants, the expression of many of genes is turned down, but some classes of genes that can help produce products that protect animals can be turned on. We have investigated some of these genes and shown that heavy metals can turn on expression of heat shock protein genes and the genes that produce metallothionein proteins. We are also testing whether there are novel classes of genes with altered expression patterns. At the metabolic level, we have compared whether midges exposed to different classes of pollutants (metals, pesticides, nutrients) exhibit different concentrations of metabolites including amino acids and carbohydrates. These data indicate that there is a specific signature of pollution type on metabolism. In both the gene expression and the metabolite comparison, we are detecting effects well before the midges suffer from mortality. This means that such markers could be used to detect pollution effects before organisms are drastically affected. However we have so far only completed laboratory studies, and the next challenge is to consider mixtures of pollutants under field conditions, which may also include other stresses like food limitation and temperature changes.

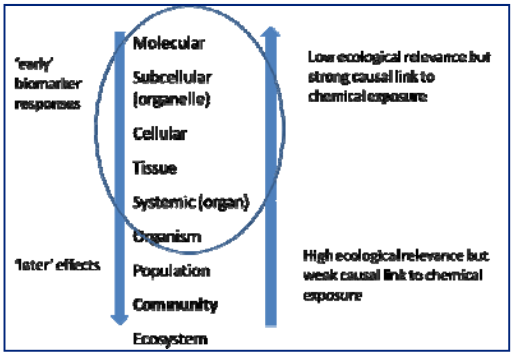

Figure 1. Levels of biological effects that can be measured in a Biomonitoring program. The circled effects are those generally measured as biomarkers.

We are exploring other types of biomarkers with the midges. These include changes in sex ratio of midges that have developed on sediment and different patterns of mouthpart deformities in some groups. In each case we are starting with simple laboratory tests and then extending to field situations. The metabolite changes and morphological work is being extended to other groups of organisms being studied at CAPIM. These markers are being integrated into our other chemical and biological approaches to detect pollution problems and increase the number of approaches we can apply to increase the "weight of evidence" needed before deciding on whether a site is impacted by pollution.

Prof Ary Hoffmann

Also in this issue:

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If you would like to contribute to the CAPIM Newsletter, or be added to the distribution list, please contact Cindy Goddard at cindy.goddard@unimelb.edu.au. Bio21 Institute, 30 Flemington Road, Parkville, VIC 3010. Tel: +61 3 8344 2522.

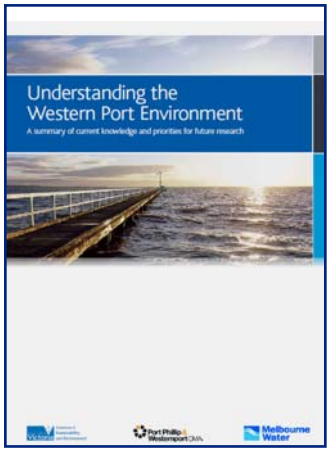
Western Port Environment Science Review

This month Melbourne Water will be releasing the Western Port environment scientific review – *Understanding the Western Port Environment: a summary of current knowledge and priorities for future research.*

Better Bays and Waterways (2009), the water quality improvement plan for Port Phillip and Western Port water quality, identified the need to undertake a strategic bay-wide assessment of our knowledge of the Western Port environment with a clear focus on informing future investment to protect and improve the health of the bay. In response, Melbourne Water and the Department of Sustainability and Environment led the development of the Western Port environment science review with involvement of an inter-agency advisory group consisting of representatives from the Port Phillip and Westernport Catchment Management Authority, Central Coastal Board, EPA Victoria, Parks Victoria, Department of Primary Industries, Department of Transport and South East Water.

CAPIM's Estuarine Program Leader, Professor Mick Keough, was the head scientist for the review and was supported by a team of experts from Victoria University, Phillip Island Nature Park, Flinders University, Victorian DPI, EPA Victoria, Deakin University, University of Tasmania, University of Western Australia and Museum Victoria. The research team was selected to integrate current international and national science relevant to Western Port. Input was also sought from a broad range of Western Port stakeholders, in particular through the Western Port Catchment Committee and a public seminar in April 2011, where the preliminary findings of the review were presented to an audience of over 200 people.

The review will be a useful resource to support natural resource management policy, strategic planning and on-ground works within the catchment and bay. It also provides a clear direction for future environmental research that will improve our understanding of how to manage key threats such as urban growth, climate change, pest plants and animals, and coastal development. Including co-funding through DSE and the Port Phillip and Westernport CMA via the Victorian Investment Framework, Melbourne Water has already commenced several high priority research projects, including improvements to EPA's Western Port hydrodynamic model, assessment of toxicants levels near major waterway inputs, confirmation of seagrass species using genetic markers and assessment of the nutrient inputs and fate within the bay. Further priority research projects will be commissioned over the coming months, including determining water quality thresholds to protect seagrass, understanding inputs of sediment from coastal erosion, the importance of certain habitats for maintaining fish diversity and an analysis of long-term data to investigate reasons for the recent decline in fish-eating bird species.



The Western Port Environment Study—a Summary of current knowledge and priorities review.

For further information contact Rhys Coleman, Waterways and Wetlands Research Manager, Melbourne Water/CAPIM Rhys.coleman@melbournewater.com.au.

Rhys Coleman

Linking Science and Policy

To better understand the presence and potential impacts of agrochemicals in the natural environment, it is important for both researchers and policy makers to work together. The Department of Primary Industries (DPI) works closely with CAPIM in the research of agrochemicals. Additionally, DPI also analyses the research information generated by CAPIM to inform policy development for the management of agrochemical use.

DPI through Future Farming Systems Research Division undertakes research of agrochemicals, while Biosecurity Victoria regulates their use. The regulation of agrochemicals in Australia is complex and involves all three levels of government, including: Commonwealth; State, and; Local.

At the Commonwealth level the Australian Pesticide and Veterinary Medicines Authority has responsibility for the registration of chemical products through to the point of retail sale. The state and territory governments are responsible for controlling the use of these chemical products. While, local government get involved when there have been reports of alleged spray drift that has potentially had an impact to human health and the environment.

There are well known differences between policy and research such as the narrower field of information that a researcher will focus on, unlike a policy maker that will use information from a broad range of areas that may include economic, social and environmental to formulate a policy position. However, with the increased focus in research of agrochemicals and their presence in the natural environment, both researchers and policy makers can benefit from better understanding the different disciplines. Working collaboratively will result in better outcomes by influencing all levels of government and research institutions, help identify end user information and future research needs as well as providing evidence to inform policy makers which should result in issues being addressed sooner. Finally research does not finish when it has been published. Research is an important part of the policy cycle by providing important information (evidence) to policy and decision makers who manage the use of agrochemcials in Victoria.

Robert Walters, Biosecurity Victoria

The impact of pesticides on the benthic macroinvertebrates in a rural lake

Lake Hawthorn is located in the Sunraysia region of Victoria near Mildura. As of 2007, the lake was only one of four lakes known in Victoria to support a population of the endangered Murray hardyhead (*Craterocephalus fluviatilis*) and these fish have been restocked in the lake over the past few years. In October 2010 CAPIM commenced sediment quality and toxicity monitoring in Lake Hawthorn as part of a DPI funded project to determine whether a locust control had any impact on aquatic ecosystems. Sediment was collected on three occasions within the lake. Sediments contained elevated concentrations of pesticides. The pesticide data and sediment toxicology data (using *Chironomus tepperi*) collected from Lake Hawthorn over the past year indicates that the pesticides present are toxic to macroinvertebrates. The results also indicate that the pesticide contamination is not dispersed homogeneously throughout the lake sediments. Therefore the CAPIM freshwater and biomarker groups commenced a study in September 2011 to a) determine the number of samples that should be collected to ensure that there is an adequate assessment of contaminants present in sediment and water and b) determine if the composition and health of benthic macroinvertebrates are affected by the presence of pesticides.

The lake was divided into six sampling locations. Between three and five sites within each location were sampled for sediment and surface water and a range of chemicals were measured (including metals, pesticides and nutrients). Benthic macroinvertebrates were sampled in a subset of sites at each location for species abundance and diversity and also for changes at the sub-organism level on a number of biochemical bio-markers. Sediments were also assessed for toxic effects on survival, growth and emergence of *Chironomus tepperi* using a laboratory-based bioassay and impacts on indigenous macroinvertebrates using field-based microcosms. The chemical and biological data are currently being analysed and it is envisaged that all samples will be processed over the next few months. Results on sampling strategically and the usefulness of a multiple lines of evidence approach for aquatic biomonitoring will be assessed and incorporated into future sampling studies.

Dr Vincent Pettigrove, CEO & Freshwater Program Leader and Bryant Gagliardi, at Lake Hawthorn.

*Claudette Kellar, *Sara Long, Maria Ballesteros, Lisa Golding, Bryant Gagliardi and Vincent Pettigrove
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Dr Claudette Kellar and Dr Sara Long

CAPIM Events in 2011: Participants Forum and Research Summit

In the final months of 2010, CAPIM held two major events at the Bio21 Institute, The University of Melbourne. The first was the Annual Participants Forum, whereby, government and industry were invited to attend a series of presentations which revealed key outcomes from significant research projects conducted by CAPIM. This event was well attended, providing key information to those who hold responsibilities in the field of waterways management.

To cap off the year, the Annual Research Summit was held on Friday 25th November. This was an equally important event on the CAPIM calendar as it provided an opportunity for staff, as well as students, to present information pertaining to current research projects, and their progress to date. This event was also well attended by staff, students, government and industry.

CAPIM would like to take the opportunity to thank and acknowledge, all of the speakers at these events, for their valuable contribution. These people include:

* Ary Hoffmann, Director of Research & Biomarker Program Leader
* Allyson O'Brien, Research Fellow, Estuarine Program
* Ana Miranda, PhD Candidate, EDCs Program
* Bryant Gagliardi, Research Assistant, Freshwater Program
* Cameron Amos, Research Assistant, Freshwater Program
* Claudette Kellar, Research Fellow, Freshwater Program
* Daniel MacMahon, Research Assistant, Freshwater Program
* David Sharley, Research Fellow, Freshwater Program
* Edward Nagul, MSc Candidate, Novel Chemistry
* Gavin Rose, Senior Research Scientist, DPI
* Ines Almeida, Research Fellow, Novel Chemistry
* Jackie Myers, Research Fellow, Freshwater & Biomarkers Programs
* Kallie Townsend, PhD Candidate, Freshwater Program
* Kathryn Hassell, Research Fellow, Freshwater Program
* Katy Jeppe, PhD Candidate, Freshwater Program
* Lisa Golding, Research Fellow, Freshwater Program
* Melissa Carew, Research Fellow, Biomarker Program
* Minna Saaristo, Research Fellow, Monash University
* Rhys Coleman, Estuarine Program and Waterways & Wetlands Research Manager, Melbourne Water
* Robert Walters, Manager, Legislation & Standards, Plant Biosecurity & Product Integrity, Biosecurity Victoria, DPI
* Sara Long, Research Fellow, Biomarker & Freshwater Programs
* Simon Sharp, Research Assistant, Freshwater & Estuarine Programs
* Steve Marshall, Research Assistant, Freshwater Program
* Valentina Colombo, PhD Candidate, Freshwater Program
* Vincent Pettigrove, CEO & Freshwater Program Leader

Attendees mingle at the Annual Participants Forum, held at the Bio21 Institute, The University of Melbourne, on 4th October 2011.

Like mother, like son...parental chemical exposure and offspring quality

I was recently awarded a University of Melbourne Early Career Researcher (ECR) Grant, to study the effects of parental exposure to EDCs on offspring quality in the estuarine fish, the Eastern blue spot goby.

Endocrine disrupting chemicals (EDCs) include natural and synthetic compounds that are capable of altering production, storage and function of hormones. Globally, over 200 chemicals have been identified as potential endocrine disruptors, and many are in widespread, everyday use such as pesticides and some detergents. EDCs regularly enter estuaries through sewage effluents and agricultural discharges, placing estuarine fishes at high risk of exposure. EDCs are especially harmful to the reproductive system, and in fishes, EDCs contribute to development of gonadal tumours, lowered fecundity and infertility in adults, and birth defects, retarded growth, and gonad malformations in offspring.

We will expose breeding adults to environmentally relevant concentrations of EDCs, and monitor their reproductive output over a given time period. All offspring produced from the exposed parents will be grown to maturity, and assessed for changes in various endpoints, including, but not limited to gonad development, expression of specific genes and proteins, hormone balance, growth, survival, and reproductive output. I am hoping to adapt some of the specialised techniques for yellowfin gobies that I learned whilst working in Japan for use with blue spot gobies during this study.

Dr Kathryn Hassell

This project aims to establish how EDC exposure in adult fish affects reproductive success and development in their ffspring, and represents the first lab-based, multi-generational study of EDCs in any Victorian fish species. It will substantially improve our understanding of the threats EDCs pose to estuarine organisms and provide important scientific evidence into the potential long-term reproductive consequences of environmental toxicants.

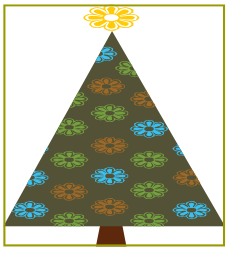
Dr Kathryn Hassell

Profile: Dr Maria Ballesteros

My name is Dr. María Laura Ballesteros. I am from Argentina and recently finished my PhD studying sublethal effects of Endosulfan, an organochlorine pesticide, in a native fish (*Jenynsia multidentata*) at the Universidad Nacional de Córdoba. This project investigated biochemical (oxidative stress enzymes, acetylcholinestarase and lipid peroxidation), histological (gills and liver) and behavioural (swimming activity) effects of endosulfan and their use as biomarkers in aquatic environments. My current postdoctoral research project is at the Ecotoxicology Laboratory of the Universidad Nacional de Mar del Plata, with a fellowship from the National Research Council (CONICET) from the Argentina government. I am working specifically on the dynamics of Persistent Organics Pollutants (POPs) in different environmental matrices (water, sediments, fish, etc.) from Mar Chiquita Lagoon (Córdoba, Argentina). As part of my postdoctoral appointment I am encouraged to carry out a short term research project overseas to gain knowledge and practical experience from working in a different country. In order to learn more about microcosms approach, I contacted to Dr. Vincent Pettigrove and I applied for a fellowship from CONICET to spend almost 3 months at CAPIM. During my short stay at CAPIM, I am have learned about the biochemical effects of bifenthrin (a synthetic pyrethroid pesticide) on *Chironomus tepperi* larvae as a model invertebrate species, using spiked sediments. I am also conducting a field-based microcosm study with sediments collected from Lake Hawthorn (Mildura, Victoria) and bifenthrin-spiked sediments in order to determine oxidative stress response of chironomid larvae exposed to these sediments. I am working with Dr Sara Long to develop new techniques to determine oxidative stress enzymes activity in chironomid larvae (from a range of chironomid species), which will be used as part of weight of evidence approach in future CAPIM biomonitoring programs, to assess the health of Victoria's waterways. Watersheds in Argentina, like many countries in the world, have contamination issues from different sources (including chemicals, household effluents and industrial activity), due to the rapid population increase and the extension of agricultural crops concomitant with the destruction of forests, being the main problems. Many river basins such as Río de la Plata, Río Negro and Suquía rivers have been widely characterized in recent years in relation to water quality, detecting the appearance of toxic substances, both organic (microcystins, organochlorine pesticides, hydrocarbons, phenols, etc.) and inorganic (ammonium, heavy metals). The Argentinean government knows the environmental situation but sometimes they give priority to other issues such as economic stability for the country rather than protecting the environment. There are also economic interests that prevent the government from taking more protectionist actions in this regard. I think that this research will foster a strong relationship between The University of Melbourne and Mar del Plata and Córdoba Universities and enhance opportunities for seeking international grants for further collaborative research.

Dr Maria Ballesteros, during her stay at CAPIM.

From all of us at CAPIM – We would like to wish you a very Merry Christmas and a Happy and Safe New Year !



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