BIOCHEMICAL TRACERS WORKSHOP

HOBART, TAS

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Table of contents **Page**

Abbreviations

AAD	Australian Antarctic Division
ACE CRC	Antarctic and Climate Ecosystems Co-operative Research Centre
C	Carbon
CMAR	CSIRO Marine and Atmospheric Research
CSIRO	Commonwealth Scientific and Industrial Research Organization
DHA	Docosahexaenoic acid (DHA, 22:6ω3)
EBFM	Ecosystem based fisheries modeling
EPA	Eicosapentaenoic acid (EPA, 20:5ω3)
EwE	Ecopath with Ecosim models
FA	Fatty acids
IASOS	Institute of Antarctic and Southern Ocean Studies
ICP-MS	Inductively coupled plasma-mass spectrometry
IMAS	Institute of Marine and Antarctic Studies
N	Nitrogen
OCE	Office of the Chief Executive
PUFA	Polyunsaturated fatty acids
SAFE	Sustainable Australian Fisheries and Ecosystems
SIA	Stable Isotope Analysis
TAFI	Tasmanian Aquaculture and Fisheries Institute
Utas	University of Tasmania
WAF	West Australian Fisheries
WfO	Wealth from Ocean – CSIRO national Research Flagship

Workshop – Biochemical Tracers for Use in Fisheries Research including Ecosystem Based Fisheries Management

December 3, 2008, at CMAR Hobart

Co-chairs: Peter Nichols and Mat Vanderklift

1. Executive summary

A highly focused workshop on – Biochemical tracers - was attended by approximately 50 scientists from CSIRO Marine and Atmospheric Research (CMAR) and the Wealth from Oceans (WfO) National Research Flagship and collaborating institutes. An overview of current methods with emphasis on stable isotope analysis (SIA), signature fatty acid analysis, with a range of applications and case studies was provided by 16 speakers. The workshop summary and presentations are available at http://www.cmar.csiro.au/biochemicaltracers.

Strong consensus was reached at the meeting that Biochemical tracers offer significant potential for use in Australian fisheries, including EBFM, research. The current capability in this area of CMAR (and collaborating partners) will be increasingly attractive as it is clearly apparent that these approaches are being adapted internationally and nationally. Australian research as presented at the workshop is at the cutting edge. Noteworthy was the strong endorsement and support of the CMAR / WfO participation in the area, including research leadership and the training of young scientists.

The Biochemical tracer capability within CMAR is currently homed in the SAFE stream within Wealth from Oceans (WfO). Given the outcomes of and support at the workshop and the enormous potential of the Biochemical tracer approaches, the workshop further recognized that the research be given enhanced priority as CMAR / WfO moves forward in restructuring current research activities.

2. **Introduction**

The WfO Flagship and CMAR are presently reviewing the use of biochemical tracers in their research portfolios. The call for projects in the 2008/09 cycle saw a number of proposals submitted. As part of the path forward, a review of this research area, including the holding of a workshop and provision of a summary report, was proposed. The workshop was held on December 3, 2008 and comprised 17 presentations followed by discussion on current developments, linkages, issues, research needs and opportunities. In excess of 50 scientists from CSIRO and external agencies attended.

A full listing of attendees and the workshop presentations are appended, with the presentations available at (XXX - obtain website details). A summary of the meeting is also available on the CSIRO Intranet at: http://intranet.csiro.au/intranet/communication/internalcomm/mondaymail/2008/MM081215/htm/biochemical.htm .

The following sections provide further details on the various research fields and discussions as presented and covered at the workshop.

3. **Stable Isotope Analysis**

The use of stable isotope analysis (SIA) in marine and fisheries ecology has burgeoned in the last decade, largely due to the ability to generate data relatively rapidly and cheaply, and to advances in quantitative methods. The technique is most typically applied in studies of diet and movement/migration, although there are other applications, such as the use of lead isotopes to age deep sea corals, mentioned in the workshop.

The application of SIA in studies of diet began in the 1970s, although the basis for this work was set somewhat earlier with the discovery that abiotic matter (such as rocks and air) and biota (such as plants) show consistent patterns in their ratio of heavy to light isotopes. In the 1980s and 1990s there was a steady increase in the number of studies using SIA – typically of carbon and nitrogen – to determine diets of consumers. The technique was especially embraced by marine ecologists, because of its utility for distinguishing between different potential sources of production, such as phytoplankton versus macrophytes. Until the late 1990s, most of the interpretation of these studies was qualitative inferences based on expected patterns of stable isotope discrimination (the difference in stable isotope ratio between diet and consumer). In the 1990's ecologists began using geometric mixing models to quantitatively estimate consumer diets. However, these models were relatively restricted, and from 2001 mixing models based on mass-balance were introduced, led by Don Phillips (based at the US EPA). Very recently, mixing models based on Bayesian inference have been developed. These mixing models have increased the robustness of inferences about diets that are derived from SIA, but there remain several limitations. Firstly, the usefulness of SIA relies on diets having

distinct stable isotope ratios – where this does not occur, the results will necessarily have limited resolving power. In this case, a multiple-tracer approach can be very useful, as highlighted during the workshop. Secondly, the application rests on assumptions about diet-consumer discrimination that remain incompletely tested – while progress has been made towards improved understanding, ecologists have not yet achieved a level understanding that will allow reliable predictions.

The application of SIA to the study of movement and migration of animals began in the 1980's, often using stable isotopes of carbon and nitrogen, and often focusing on marine organisms. In the last decade most of the applications have been by avian and insect ecologists, and have typically used stable isotopes of hydrogen and oxygen. This has been facilitated by good understanding of the spatial distribution of oxygen and hydrogen isotopes in rain and freshwater (see for example web sites devoted to this source of information at http://waterisotopes.org/ and http://ecophys.biology.utah.edu/Research/Isoscapes/models.html). Understanding of the spatial distribution of stable isotopes in marine environments has not progressed as far, but there are some broad trends that have been identified, such as a general decrease in $\delta^{13}C$ with increasing latitude. Greater spatial resolution data for the distribution of stable isotopes in marine environments is needed. Similar to the use of SIA in studies of diet, the application of SIA in studies of movement and origins began with qualitative inferences, and is moving towards quantitative inferences based on more robust statistical methods.

A range of applications of SIA were presented at the workshop, including applications to the study of diet and animal movement. Presentations at the workshop included:

- A general decrease in $\delta^{13}C$ of particulate organic carbon with increasing latitude, but a wide range of $\delta^{13}C$ in different size fractions
- Compound-specific SIA on amino acids can lead to important insights because it allows differentiation between 'trophic' amino acids (those that do transaminate after consumption) and 'source' amino acids (those that don't transaminate and therefore reflect the diet)
- $\delta^{15}N$ is positively correlated with size of many (but not all) species of fishes on the east coast of Australia, consistent with an ontogenetic increase in trophic position
- $\delta^{15}N$ of fishes shows some spatial variation, and might indicate long residence times in specific water masses
- SIA of whiskers of seals reflect the place where they are grown, because the tissue is inert and does not change

4. **Fatty Acid Analysis**

Australian fisheries biologists and biochemistry researchers have conducted signature fatty acid (FA) analyses across a range of environments, trophic levels and applications. Projects presently cover meso-zooplankton, abalone, Antarctic krill, squid, fishes including deep sea rattails, swordfish and other species and higher predators such as

whales, seals and penguins. Such studies are broadly at similar levels of development to those conducted by overseas groups.

Development and application of the signature FA methodology has resulted from the recognition that traditional dietary techniques may be problematic, e.g. gut content analysis does not supply sufficient information or may be biased. The FA methods has a number of features: prey species may have unique lipid class / FA compositions, many FA are readily transferred from prey to predator with minimal modification, constituent FA therefore represent, to some extent, a temporal integration of diet, the approach can be quantitative and allows temporal integration (cf gut content analysis) (quantitative fatty acid signature analysis, QFASA). In a broader context, signature FA are generally combinations of FA that are preserved, and or remain recognizable as they pass up the food chain. The methodology also complements other approaches.

The signature fatty acid approach was first developed and applied to marine food web studies in the 1960s. The enthusiasm of fisheries biologists to embrace alternate technologies, the advent of higher resolution GC columns and instrumentation, together with the opportunity for greater throughput of samples and advances in data treatment including statistical packages available has seen increasing uptake of the approach in recent years. Interestingly, previous CSIRO Fisheries research in the 1980s used FA profiling for a range of fish species from the northwest shelf environment, although this noteworthy application pre-dated the various method developments indicated above.

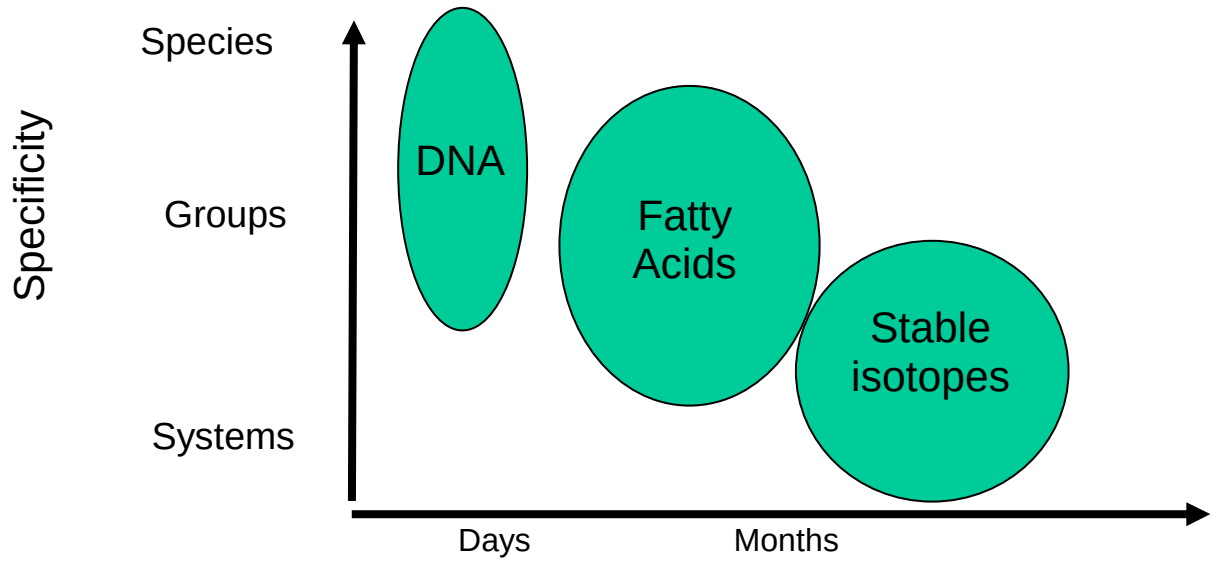
Specific topics presented and highlighted at the workshop included:

- Signature FA profiling of zooplankton from WA inshore and offshore coastal waters in winter 2007 did not reveal diatom signatures, nor differences between the two oceanic areas.
- Abalone diet was able to be distinguished as brown algae / detritus. Previous studies have been inconclusive, with the FA approach also complementing and in agreement with findings from SIA.
- FA and lipid class profiling of unique specimens of the southern ocean krill *Euphausia superba* collected in late winter revealed that the animals were in remarkably good condition, although levels of key polyunsaturated fatty acids (PUFA) were low compared to summer and spring specimens, as were levels in sea-ice algae cores. Laboratory feeding experiments also demonstrated that PUFA played a key role in larval development, with levels of 18:2ω6 and 20:4ω6 and the DHA/EPA ratio effect hatching success.
- The southern ocean squid *Moroteuthis ingens* was linked to a diet rich in those myctophids containing triacylglycerols (TAG), rather than the wax ester containing myctophids or krill as previously reported.
- Deep sea rattails from Californian oceanic waters linked to a possible carrion squid diet rather than potential benthic prey.
- Juvenile and smaller swordfish were shown to be consuming a diet of myctophid fishes rather than squid. It was also possible to distinguish various myctophid prey species based on their FA profiles.

- Comparison of juvenile and adult southern elephant fatty seal acid profiles revealed broad-scale dietary differences, with juvenile diet more similar to fish (myctophids) and adults to squid. Interpretations in terms of specific prey items can be problematic.

Collectively, the case studies presented at the workshop highlighted the broad range of projects occurring for the signature FA methodology. Whilst application of SIA has recently seen development of mixing model and similar approaches, such progress is less developed for signature FA. This is likely in part due to the more complex nature of the FA data set (SIA – two values; signature FA – typically 40 values). Iverson and colleagues have refined the fatty acid signature approach (FASA) to develop QFASA. Although this development has been applied to several marine mammals, it is clear that considerable research is still required for the approach to become more robust and more widely accepted and applied.

Several of the FA studies presented at the workshop were in combination with SIA. The use of multiple tracers is generally now recognized as a more powerful approach. A further presentation highlighted the potential for a combined SIA, signature FA and molecular biology techniques to be used as cost-effective tools for understanding change in marine ecosystems. In this proposed combined biochemical tracer approach, DNA markers are useful for obtaining precise identification of prey ingested over daily cycles, with stable isotopes and fatty acids providing a time integrated representation of diet over weekly to monthly intervals. A schematic indicating the specificity and time scale of three methods follows (Frusher et al. 2008).



5. **Other Tracers**

In addition to SIA and signature FA approaches, a number of other tracers were highlighted by CMAR scientists at the workshop. These included: a suite of trace elements now possible with the use of ICP-MS, amino acids, muramic acid, estradiol and pigments. Several of these approaches are at early stages of development, with others (e.g. pigments) in wide demand from a range of scientific projects.

Presentations at the workshop covered:

- Trace elements - cyanobacteria and the cocco-lithophorid *Emiliania huxleyi* have a demand for cobalt, where as diatoms (e.g. *Thalassiosira* spp.) prefer zinc over cobalt. A Southern Ocean transect across the Antarctic Polar Front showed declining bioavailable cobalt poleward. This corresponded to a decrease in *Synechococcus* sp.
- Trace elements - Analyses (total analysis, or location-specific) of hard parts of marine organisms (fish otoliths, squid statoliths, octopus styles, mollusc shells, diatoms / coccoliths, corals can be linked to temporal variation of life form via pattern of growth rings. Application can include distinguish habitat and long-term (climatic) changes.
- Amino acids - compound-specific SIA on amino acids allows differentiation between 'trophic' amino acids (those that do transaminate after consumption) and 'source' amino acids (those that don't transaminate and therefore reflect the diet).
- Muramic acid - measures how much bacteria is present in complex samples. In conjunction with pigment analysis can determine the community structure of microbial floc cultures. This could be widened to include FA profiling as specific microalgal groups can be distinguished using signature FA.
- Estradiol - Measurement of phenotypic expression of genetically modified organisms. Possible applications include: eradication of pest species e.g. carp and manipulated aquaculture species e.g. breeding, triploid.
- Pigments – biomarkers of algal groups. Considerable development has enabled classification of algal groups based on chlorophyll and carotenoid pigment profiles. Case studies presented demonstrated an estuarine community changing from dinoflagellate-dominated to green algae and diatom-dominated. In aquaculture, pigments provide information about the ecosystem and for determination of global ocean productivity, the pigments especially chlorophyll a can be estimated using remote sensing techniques.
- Molecular techniques – Two new technologies are: efficient blocking primers for suppressing PCR amplification of consumer DNA and next generation DNA sequencing.
- Molecular techniques - A case study with Australian fur seals showed: DNA analysis correlated well with hard part analysis for major fish species, with DNA detecting more rare species; taxa identified included: 55 fish to species or generic level, squid, octopus and cuttlefish at family level, crustaceans, nematodes, annelids, tunicates at ordinal or phyletic level; major dietary differences between locations identified; answered otherwise unanswerable questions on soft bodied prey; results are quantitative with correction.

The continuing development and application of these alternate tracer methodologies represents a clear opportunity for Australian scientists to become world leaders in coming years.

6. **Modeling including Future Needs**

Part of the rationale for the workshop was to bring together ecologists, chemists and modelers to engage in discussions about areas of overlap and common interest, and areas that would benefit from insights yielded by biomarkers. Several modelers participated and presented, and much of the workshop discussion focused around how the information provided by biomarkers can be incorporated into models. One obvious application is in Ecopath with Ecosim (EwE) models. Cathy Bulman highlighted that there are currently 12 EwE models in Australia, and that these models require time-integrated estimates of diets for consumers. The form the data is used is in a percentage of diet – a form that can be supplied by biomarker approaches, and that is the form supplied by SIA mixing models. This is also the form that data is required by In Vitro model. Atlantis uses a nutrient-based currency (which can also be calculated from % composition data).

Other models could benefit from data about prey use by predators (including how this can change in space and time), movements of animals, stock structure, and insights into the state of present and past ecosystems. There was substantial discussion around matching the resolution and form of data to the model requirements, and a general consensus that a better understanding of model requirements would enable practitioners to better match data to the modeler's needs. There was recognition that a spatially-explicit approach to the application of biomarker research would help in this matching.

Discussions subsequent to the workshop have followed up on some of these issues, and ideas presented include potentially modifying ecosystem models to represent biomarker data – specifically, modeling some of the physiological, as well as ecological, processes - including assimilation and modification of relevant compounds and elements. This approach would enable direct comparisons of model predictions with the data collected.

7. **External links**

Many projects presented at the workshop involved interagency and interdisciplinary collaborations. These aspects are becoming increasingly the norm in the development and application of the biochemical tracers approaches in not only fisheries research, but also in other fields. Inter-campus collaborations within CMAR (Hobart and Floreat), as recently commenced, are seen as a strengthening capability in this field.

Strong endorsement and support by the collaborating and other external agencies of the CMAR / WfO participation in the biochemical tracers research field was also apparent. It was emphasized that CSIRO is playing a key role in research leadership and training young scientists, and it was recommended that this role is continued.

8. **Conclusions and Recommendations**

The workshop brought together key practitioners and end users, demonstrated that biomarker methods already yield important insights, and highlighted that even greater value can be added by integrating the data and insights they provide with ecosystem models. Capability in this area within CMAR (and collaborating partners) is needed as it is clearly apparent that these approaches are being adapted at large scales internationally and nationally. The workshop highlighted that Australian research is at the cutting edge, and that CMAR scientists are playing a strong role.

There was strong endorsement by collaborators and external agencies present at the workshop of ongoing participation the CMAR / WfO in the area, including research leadership and particularly in the training of young scientists.

For the immediate future, efforts will be directed at pursuing avenues for funding the continued development of the methods in fisheries and marine ecology in Australia, and at avenues for funding the development of explicit links between data and ecosystem models. These should include those available under the Capability Development Fund, and those available through the Office of the Chief Executive (OCE), and also include allocation of appropriation funds to further seed this field, and external funding agencies.

The opportunity was emphasized for continuing application of biochemical tracers in existing and new case studies and methods development, including enhanced data treatment and modeling, including in collaboration with external agencies.

The ongoing support of WfO and CMAR to the field of biochemical tracers together with other developments proposed in this report will provide enhanced capability and outcomes to the Australian marine science community and a wide range of stakeholders.

9. **Acknowledgements**

The authors thank Tony Smith for his support and encouragement for the workshop together with his valuable contributions at the workshop. Edwina Hollander facilitated communication of the meeting and Bryony Bennett assisted with photography. Stewart Frusher provided the schematic illustrating specificity and time scale of the combined biochemical tracer approach. We are grateful to all presenters for their efforts in attending and contributing to the workshop.

Appendix

Speakers at Biochemical Tracers workshop - December 3, 2008, Hobart. Left to right: Gustaaf Hallegraaf, Mat Vanderklift, Stewart Frusher, Cathy Bulman, Mark Hindell, Patti Virtue, Peter Nichols, Tony Smith, Ed Butler, Michaela Guest, Jock Young and Lesley Clementson. Absent: Tom Trull, Rhys Leeming, Joanna Strzelecki and Simon Jarman. Photo courtesy of Bryony Bennett.

Workshop – Biochemical Tracers for Use in Fisheries Research including Ecosystem Based Fisheries Management

December 3, 2008, at CMAR Hobart

Co-chairs: Peter Nichols, Mat Vanderklift

Objectives (as per original communication to potential attendees)

- Examine and review the application of biochemical tracers including in EBFM
- Provide a consensus view on the utility of Biochemical tracers to fisheries research
- Propose representative pilot case studies for consideration of being conducted in the second half of the 2008/09 and or 2009/10

Attendees (speakers underlined)

CMAR
Peter Nichols, Mat Vanderklift, Jock Young, Tony Smith, Campbell Davies, John Volkman, Rick Phleger, Rhys Leeming, Ed Butler, Crystal Hassler, Lesley Clementson, Sue Blackburn, Hector Lozano-Montes, Fiona Parker, Jenny Skerratt, Cathy Bulman, Karen Wild-Allen, Joanna Strzelecki, Danny Holdsworth, Graeme Dunstan, Gary Critchley, Stephane Armand, Miriam Fluckiger, Anne-Elise Nieblas.

TAFI
Stewart Frusher, Michaela Guest, Jayson Semmens, Jenny Cobcroft, Katya Abrantes, Gretta Pecl, Christine Crawford.

AAD
Simon Jarman, Toshi Yoshida

IASOS/ACE CRC/Zoology/Plant Science
Patti Virtue, Tom Trull, Mark Hindell, Kathryn Wheatley, Gustaaf Hallegraeff, Ben Mooney, Andrea Walters, Kristen Karsh, Adam Barnett, Christine Jackson, Virginia Andrews Goff, Sophie Bestley, Leanne Armand.

WAF
Matthew Pember

Workshop – Biochemical Tracers for Use in Fisheries Research including Ecosystem Based Fisheries Management

December 3, 2008, at CMAR Hobart, Auditorium

Co-chairs: Peter Nichols, Mat Vanderklift

Speaker	Time (TBC)	Title
Morning		
Peter Nichols Mat Vanderklift Tony Smith	0900	Welcome and introduction
<u>Lower trophic levels</u>		
Tom Trull (or delegate)	0910	13C and 15N variations in Southern Ocean plankton – how processes at base of food-web contribute variability in trophic assessments
Joanna Strzelecki	0920	Fatty acids from mesozooplankton of 34º to 22º S parallel
Gustaaf Hallegraaf	0930	Lipid biomarkers to diagnose algal bloom mediated fish kills
Michaela Guest	0940	Evidence of abalone diet from combined fatty acid and stable isotope analysis
Patti Virtue	0950	Biochemical tracer studies with krill: overwintering strategies and key lipids determined for larval development
Discussion	1000	
Morning tea	1025	
<u>Modeling & other tracers</u>		
Jenny Skerratt	1045	(i) A modeler's perspective and needs for Biochemical Tracers
Cathy Bulman		(ii) Biochemical tracers in ecological modeling
Ed Butler	1055	Trace Elements as Tracers

Rhys Leeming	1105	Tracing nitrogen in food webs using compound specific isotope ratio mass spectrometry
Lesley Clementson	1115	Pigment tracers in fisheries research
Discussion	1125	
<u>Higher predators</u>		
Mat Vanderklift	1140	How can stable isotopes inform fisheries science
Jock Young	1150	SIA of top predators off eastern Australia
Peter Nichols	1200	Fatty acid profiling of top predators off eastern Australia and in the southern ocean
Rick Phleger	1210	Signature Lipids of Deep Ocean Rattail Fishes – a possible carrion diet?
Stewart Frusher	1220	Can molecular techniques be used as cost-effective tools for understanding change in marine ecosystems?
Simon Jarman	1230	The state of the art of DNA based dietary analysis
Mark Hindell	1240	New insights into difficult animals: using biochemical tracers in the foraging ecology of marine mammals
Discussion	1250	
Lunch	1315	*provided to all workshop participants*
Afternoon*	1400	Review of morning session towards providing a summary view on the workshop presentations and discussions including against the workshop objectives
Afternoon tea	1500	
Close	1600	

* Conference room A and B