
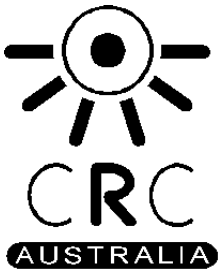


# PREDICTIVE AND RAPID DIAGNOSTIC TECHNOLOGIES FOR THE SEAFOOD INDUSTRY: A LITERATURE REVIEW.

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## EXECUTIVE SUMMARY

"An efficient measurement infrastructure is able to support Australian industry, and is essential for international competitiveness and investment attraction. Australian commercial transactions based on trade measurement have an annual value in excess of A$350 billion. Accurate, reliable and credible measurements assist fair and equitable decision making, promote consumer confidence, support trade and commerce, and ensure public health and safety."(

"In the food sector alone, where measurement of pesticide residues, and chemical and microbiological contaminants are technical barriers to trade, measurement efforts have contributed to ensuring market access for the Australian food export industry – worth around A$23 billion"[2]

Predictive technologies are becoming well established in the food industry to deal with issues of safety, spoilage and shelf-life. Large databases on microbial growth are established, are being added to continually, have been combined and linked and are increasingly accessible as web-based sites.

The Seafood Shelf-life and Safety Predictor (SSSP), available as freeware, is most commonly used for seafoods throughout the globe. It has been mainly based on microbial results and seafood species from the Northern hemisphere but it provides a good working structure in which to place results from the CRC to verify or increase the relevance and application here.

Information on specific spoilage organisms (SSO) under Australian conditions is lacking and it is therefore not clear which particular organisms, conditions or compositional characteristics are critical to minimisation of spoilage losses and to the mechanisms required to maximise shelf-life. The establishment of the relevant SSO should be part of each study in the CRC to build up a picture of Australian specific attributes.

The use of the concept of Icedays (equivalent days in ice) for expressing transit periods, shelf-lives and storage periods as a uniform time-temperature integral for chilled products can greatly improve communication and understanding along the production chain. In addition the use of the Quality Index, which is consistent with Icedays, similarly can improve understanding and aid in trouble-shooting.

A multiplicity of rapid diagnostic techniques were considered and discussed, although it is recognised that rapid is a relative term. Some of the techniques show considerable promise as they can either replace time-consuming classical analyses, reduce analytical sampling amounts, times and preparation and can provide results not previously possible at location.

Performance criteria affecting quality of analytical result include accuracy, precision, sensitivity (LOD and LOQ), selectivity, linearity, dynamic range, stability.
Performance criteria for the economics of analysis include, regulatory compliance, cost of purchase/ installation/ maintenance, analysis speed, running cost, training, suitability to purpose.

Many techniques produce multiple outputs as scans and large datasets which, in turn, require reference method comparisons and equivalent datasets for verification and validation, Consequently multivariate analyses, chemometric analysis and statistical assessment using several software

packages is required for model development and this is outside normal production staff capability. Once operational databases are established however, operation and maintenance is rugged, speedy and simple.

A Diagnostic Working Party has been suggested to carry forward exploration of specific industry requirements for diagnostics and to seek opportunities to modify and apply them locally. It would also maintain a watching brief on those technologies that are not ready for adoption at this stage but which may be ready during the life of the CRC. Devices based on nanotechnology and sensor chips are being developed with great rapidity.

Of the analytical techniques the use of near-infrared (NIR) spectroscopy and the electronic nose (E nose) hold out promise of being able to rapidly measure basic properties and changes in property attributes or classes of indicator compounds.
It is important that their utilisation is clearly formatted as each instrument has the capacity to form a valuable contribution to model development and application but cannot measure total concepts like quality or freshness, although their proponents often claim this.

Similarly colour, vision systems and image measurement using digital cameras and other optical devices also can be applied to sort, classify, grade, indicate parasites and identify many other useful properties including those related to individual or multiple quality attributes.

Lab-on-a-chip represents a miniaturisation of typical laboratory functions in sample processing and analysis, intrinsic control of environment and conditions lead to large improvements in speed of analysis and the footprint and ease of application suggest useful onsite applications. Application of this technology is dynamic with application driven outcomes proceeding at a great rate suggesting this is a methodology with great potential.

The aim to enable more diagnostics to be done onsite can be related to a desire for more accurate, functional and timely application at the boat, farm, processor, store or distributor where immediate decisions can be made, this is counter argued by the need for systems that provide regulatory compliance and achieve acceptable detection limits for critical chemical measures and the need for highly reproducible results and scientific understanding when establishing new degradation or quality models.

Issues of quality chain management and how diagnostics fit into the chain structure were also raised.

## *Recommendations:*

- Present this review to participants and survey spokespeople in person to obtain responses, ideas and survey opportunities.
- If required conduct a separate review on rapid microbiological methods to make recommendations on techniques this should include consideration of a standardised approach such as recently adopted by the meat processing industry.
- Seek to obtain results that can be added to the models in SSSP so it covers Australian seafoods with greater certainty
- Plan microbial activities in CoolFish so that SSO are established

- Promote the concept of Icedays and Quality Index establishment through training and information dissemination.
- Establish mechanisms to encourage use of and ready availability to multivariate methods
- Establish what NIR is used for at present in seafood analysis and develop applications where rapid non destructive analysis is of value
- Liaise with users of electronic noses e.g., FSA and others to identify specific areas where it may meet a need
- Explore opportunities in which digital cameras and RGB values would be of use in selection and control.
- Seek industry sectors that could benefit from imaging to detect parasites.
- Inform CRC participants of the Alert kit for histamine and develop Lab on a Chip application that meets regulatory compliance.
- Canvas industry and contract co research analysts for opportunities to increase efficiencies with Lab-on-a-chip technologies in the areas of PCR techniques for traceability, microbiology, and convert capillary electrophoresis methodology for toxin identification and degradation product detection.
- Monitor progress of real time PCR techniques for hepatitis and noroviruses being developed in SEAFOODplus and inform participants and analysts.
- Establish a Diagnostic Working Party to share information, evaluate techniques, conduct comparative trials on methodology and to help plan analytical aspects of project work.
- Diagnostic Working Party to be clearing house for information on new technologies as they become ready for trial.
- Hold a one-day satellite workshop on rapid diagnostic technologies at either Seafood Directions or the Inaugural CRC Conference
- Develop policy of industry involvement in development of rapid diagnostics
- Consider whether an aim is to devolve rapid diagnostics to parts of the chain and to consider the role of laboratory analysis
- Estimate the costs involved in industry outsourcing. This would involve exploration of the potential savings in either reducing overall cost or in standardising information for a production chain and retaining across industry benefits in analysis volume, instrument purchase and operation costs when considering meeting regulatory compliance
- Develop ideas of quality chain management models suited to the CRC
- Present this review to participants and survey spokespeople in person to obtain responses, ideas and survey opportunities to further garner differences in production or site limiting applications for rapid diagnostic technology.
- Seek to obtain results that can be added to the models in SSSP so it covers Australian seafoods with greater certainty.
- Plan microbial activities in CoolFish so that SSO are established.
- Promote the concept of Icedays through training and information dissemination
- Establish mechanisms to educate and encourage use of and ready availability to utilise multivariate methods and secondary analysis techniques.
- Educate and promote understanding of analytical regulatory compliance and encourage establishment of inter-laboratory or between industry analytical quality control to ISO 1725 and NATA specifications.
- Ensure analysis in research is conducted to international standard and encourage and support development of international interactions that can enhance Australian analytical quality control and between agency continuity.

# PART A - INTRODUCTION

## Broad Concepts

## Importance of measurement

"An efficient measurement infrastructure is able to support Australian industry, and is essential for international competitiveness and investment attraction. Australian commercial transactions based on trade measurement have an annual value in excess of A$350 billion. Accurate, reliable and credible measurements assist fair and equitable decision making, promote consumer confidence, support trade and commerce, and ensure public health and safety."[2]

"In the food sector alone, where measurement of pesticide residues, and chemical and microbiological contaminants are technical barriers to trade, measurement efforts have contributed to ensuring market access for the Australian food export industry – worth around A$23 billion"[2]

Before proceeding to outline current status in these fields it is necessary to both discuss what they are and the operating environment for them.

- **Predictive technologies** are those that provide, from measurement or observation, an estimate of the current state of some aspect of a product and allow through inferred deduction determination of future outcomes when a product is consumed or utilised for some purpose.
- **Rapid diagnostic techniques** provide timely assessment information on product attributes and processes to assist decision making process in areas such as food safety, quality and processing requirements and other important issues.

Preferably the predictive technologies are also rapid in execution.

## *Data and analysis*

Both predictive and rapid technologies generally require the accumulation of large data sets before they can be brought to bear on an issue. Therefore, any method should be preferably aimed at providing results that can be used to add to the body of information on a subject or product with a view to building up the largest possible related data set.

This approach has been greatly facilitated and made possible by the development in the last 20 years of multivariate data analysis and software packages for this purpose. Furthermore, the invention of benchtop and handheld instrumentation and miniaturisation, linked with the microchip and other advances in technology have also brought down the cost and increased the measuring capacity of many instruments. Small kits and test strips have been developed for some uses.

### *Considerations*

The advent of sophisticated instruments that rapidly provide a huge range of data has also created a dilemma for proving new rapid measurements as they often need to be calibrated against the traditional methods that are approved in standards and regulations. These traditional methods are often tedious, time- consuming and very costly. It is possible to generate hundreds of results from

e.g., rapid spectroscopic methods such as NIR (near infrared) in a short time, whereas to achieve the comparative set of results by the classical method for comparison e.g., for moisture determination, fat extraction, Kjeldahl analysis etc takes much, much longer. This has been a hold up in rapid methods becoming accepted as standard.
Relationships between rapid diagnostic measures and purchaser preferences frequently require sensory consideration and are significantly enhanced where these measures have been incorporated in research understanding. Standardising sensory procedure of assessment therefore is particularly important in development of  other instrumental or diagnostic complementary devices.

## The main aims for predictive and rapid technologies

The main aims of any production system are to seek to control all the factors within known limits so that the product and process meets customer or end use requirements. This is the evolving approach that lies behind the shift from end-product inspection to management of the system so that a measurable high degree of confidence can be placed in the product as to quality, safety, shelf-life, customer satisfaction and other issues that make the product fit for purpose and profitable.

With seafoods, this aim can be considered under the rubric of Quality Chain Management. Safety is implicit in this approach. Therefore the predictive and rapid diagnostic technologies that are chosen must fit readily into this aim and to serve it.

### *Predictive Technologies*

These arise from an accumulation of results from which a relationship that describes them often results. As more information is obtained the reliability of the relationship increases or another is formulated that better describes the results, influential variables are identified, standardised and monitored allowing more consistent product control. When fully understood, relationship modelling allows prediction from single point monitoring to determine a likely outcome. This may be, what is the current state of the product, what will it be in the future or under a different set of circumstances how intervention can assist.
For seafood this is commonly a prediction of its grade, or the end of shelf-life, or for how long it will remain safe to eat, or retain some set of properties or what the yield or other features will be after processing, packaging, distribution, storage and display.

These predictive relationships can be used to test out new situations by computer modelling and eliminate the less likely from the more likely prospects. The more data that is available the better the selection can be. The wider the range of circumstances measured the greater the likley prediction value.

### *Rapid Diagnostics*

Rapid diagnostics are concerned with finding ways to perform a useful measurement to estimate key properties or important factors in for example chain management such as temperatures, transit times and heat flows. The value of the rapid diagnostics is that timely information can be fed back into

prediction to avert disaster, to modify a process or to confirm success. Rapid methods thus provide a check on procedures, material, processes, equipment and personnel at every stage.

These rapid methods may supplant standard technologies or they may form part of a completely new approach. They tend to fall into two major types, although these may overlap.

1. Measurement of a property that indicates compliance to standard, regulation, to customer requirements or to market access. These often relate to safety e.g., contamination, harmful bacteria, toxins, pollution, adulterants, and to market specifications and preferences e.g., weights, sizes, product forms, processes, packaging etc. Although the boundaries for these properties may be hard to set, once that is done the results for them are fairly straightforward to interpret

2. Concern with issues commonly called quality, freshness, shelf-life, desirable and undesirable properties, customer preference, internal company standards and specifications all of which may relate to those listed in (1). These are properties that are much harder to define as they are generally concerned with the synthesis of individual properties and are more subject to circumstance, market influence and prevailing or individual opinion.

Both these types of diagnostics may be done internally by quality control (QC) or quality assurance (QA) staff and many measurements form part of the HACCP procedures. Elementary measures such as weights, temperatures, pH, colour, pressure and, in some cases, basic microbiology are routine internal matters but nutritional, microbial, contaminant and environmental analyses are typically outsourced. Ingredient source quality is increasingly required to be traceable and certified.

Judgments on quality are generally internal matters for routine production but during the development phase of a product, or to solve problems, external research or commercial consultants are often involved.
Maintenance of onsite calibrations for secondary analytical methods is also increasingly outsourced with multiple organisational inputs improving data set sample numbers and range leading to better calibrations and instrumentation provided under licence utilising lease arrangements.

## Need for predictive and rapid diagnostic technologies

The need for predictive and rapid diagnostic technologies is not in question. Consumers, and government agencies as their proxies, need to know that seafood is safe to eat. They also require it be nutritious, true to label, free of contaminants, from unpolluted areas, increasingly from sustainable fisheries and farming practices, that it is grown and harvested humanely, can be traced to origin, is consistent in quality, provides value for money, is hygienically processed, transported and stored correctly and is of the quality and freshness they prefer. These are tall orders but are achievable by cooperation along the chain and by having rapid methods for support and predictive tools to manage the properties throughout its passage along the chain.

Quality in final product is only achievable when constancy and understanding of ingredient characteristics is met and adequate process control can be established.

It should be noted that processor ingredient requirements are not always at the premium fresh market particularly where novel product improvement is undertaken. Processing can form a valuable second tier approach for medium grade product that cannot achieve premium quality status in a fresh or fresh chilled market.

The main protocols of HACCP, QA, and QC and other internal and external requirements are employed for the routine matters and measurements are taken of properties. Many results merely reflect the current status and provide little interpretative explanation, nor do they have much predictive power on their own. This needs to be taken into account when choosing a particular method.

The means of meeting the need must be in accordance with sound practice and must also not be expensively prohibitive.

Rapid instrumentation and methodology represent electronic mechanisms that should be non-intrusive and designed to enhance the flexibility, reliability, precision, accuracy or fitness to purpose of an attribute measure. Sensory index method scores, chemical or environmental monitoring for product or models for best production practice are examples of utilisation. Instrumentation, sensor, physical or chemical measures should be sufficiently rapid and accurate to purpose that their utilisation enhances the understanding of conditions surrounding the product (ie environmental or process sensors or directly monitor product attribute indicators.

Improving sensor technology suggests that revision of available technology that may assist the seafood industry should be ongoing, attributes of interest should include fitness to purpose, purchase cost, operational cost and overall monitoring value.

## Modern approach to analysis

There is a desire to design analyses that can be used online or at the line. Online measurements are non-invasive and often involve electromagnetic radiation adjacent to, and at both ends of, the visible spectrum e.g., near infra-red (NIR), through to magnetic resonance and fluorescence. Currently most of the measures are not online but are at line as they either need work up of a sample, or are too slow or both. Online analyses often may not result in a readout but in an action when a particular pre-set value for a variable is transgressed. An example of this lies in the sorting of cooked peeled prawns by colour as an online process operation, this has been fully commercialised and is in wide use. Similarly automatic weighing and sizing devices may both perform these operations and retain records.

Many methods use forms of spectroscopy and unlike classical approaches in which the spectrum, either absorbed or reflected, is measured at some peak wavelength(s) the whole spectrum can now be analysed. It is mathematically cut into small pieces each of which is effectively treated as a measurement for statistical analysis by multivariate analytical methods. At times the frontier lies not in the instrumentation and measurement technology but in the development of the multivariate statistical techniques and appropriate algorithms to deal with results to gain the most from them. Many instruments are computer controlled and have inbuilt software for data analysis but combinations of results from different techniques require analysis by a range of different softwares and are generally done on more powerful separate computing systems.

# Part B - PRIMARY CHEMICAL MEASURES

## Introduction

Primary measurement is typically associated with considerations of food safety however increasingly international trade and multinational purchasers are insisting on primary measurement of detrimental characteristics of seafood. International trade has tripled over the past twenty years. Trade is a prime driver in the generation of wealth in most of the countries around the world. Trade is vital to Australian seafood sustainability, growth and development.

"Our trade strategy is about ensuring, in a difficult and uncertain trading environment, that our exporters achieve greater access to overseas markets as quickly, as broadly, and as deeply as possible."[1]

"The government puts a high priority on an ambitious trade policy agenda because better market access for Australian exports is critical to generating wealth, creating jobs and raising living standards for all Australians. This is especially important in regional Australia where exports account for one in every four jobs."[2]

### *Technical trade barriers*

"As tariffs are progressively reduced, trade facilitation measures and other reforms directed at addressing barriers to trade in goods and services – including technical measures and customs rules and procedures – will assume even more importance"[2]

In the future, technical barriers to trade (TBTs) are likely to increase in prominence owing to three main reasons;[3]

- Traditional domestic industry protection is being reduced and dismantled. TBTs provide a convenient camouflage for the protection of the domestic market, particularly since there is a legitimate reason for their use.
- TBTs can be used by exporting countries as a method for protecting their market share by raising the cost of rival countries to enter the market.
- The cost involved in complying with TBTs is capital-intensive which gives an advantage to industrialised nations over developing countries.

Australia is currently poorly placed to take advantage of TBTs in export markets and is vulnerable to technologically superior rival economies. By improving Australian scientific capabilities, WA industry will be both better positioned to protect current export and domestic markets, and will gain easier entry for expansion into importing countries.

### *Maximum Residue Limit*

The maximum residue limit (MRL) is the maximum allowable residue of a particular chemical in a particular commodity. The primary purpose of maximum residue limits (MRLs) is to ensure that public health is maintained through application of the minimal amount of pesticide or veterinary chemicals to food products. The MRLs are recommended on the basis of appropriate residue data collected from supervised trials and is assigned according to dietary intake studies.

Even though the logic behind the imposition of food safety standards is clear, there is considerable room for flexibility and discretion in the application of the specific standards pose a serious threat of abuse of such measures for protection of domestic markets.[3]

Many countries, including Australia and the USA, have "positive" MRL lists. This means that the lists contain chemicals that are allowed in a particular commodity below the limit (MRL) stated. Some countries, such as Japan, have a "negative" list, where chemicals that are listed are not allowed in a particular commodity.

At the moment in Japan, a commodity that contains a chemical without an MRL may be distributed unless they pose a health hazard. To be regarded as safe, products without an MRL listed in the present list are usually compared to the Codex Alimentarius MRL or the exporting countries MRL, whichever is stricter. However, the Government of Japan is in the process of changing the way it controls farm chemicals. A provisional "positive" MRL list will be implemented in 2004. All products sold in Japan must comply with these MRLs. For products without MRLs in the provisional list, a three year transition period will apply where the rules will remain as previously stated, no later than May 2006. After the three year period, however, all chemicals that do not have an MRL on the Japanese provisional list will be illegal and a zero tolerance stance will be taken.[4,5] The current MRL list contains around 200 chemicals, compared to 418 for Australia. The Japanese provisional list now contains around 650 chemicals and will be the most comprehensive in the world.

Food additives has always been considered under a "positive" list system, ie only additives that have been approved by the Ministry for Health, Labour and Welfare may be used in foods and beverages sold in Japan.[5]

### *Zero Tolerance*

Zero tolerance for a chemical usually means that no detectable levels of the chemical must be found in the commodity. Consequently, a chemical may be present in a commodity as long as it cannot be detected by the laboratory analysing the sample. In the case of Japan, imported foods are tested by either the Central Customs Laboratory or the Department of Food Safety, Ministry for Health, Labour and Welfare.

The detection level for a chemical is dependent upon a number of factors;

- Scientist experience
- Analytical technique
- Analysis Method
- Analytical instrument

The better the technology and capability of the science the lower the detection level. Consequently, for Australian products to be marketed in countries with a "positive" chemical list, the products must have levels of contaminants that are undetectable by that countries laboratories. Thus, to protect Australian export interests, Australian laboratories must be able to achieve these detection levels or lower.

## $SO_2$ and Preservative identification.

Sodium sulfite preservation represent the primary traditional preservative utilised in the seafood industry particularly in the prawn or crustacean industries. Preservative concentration allowances for utilisation has dropped significantly and their levels are a regulatory requirement for many markets. Alternative preservatives and synergistic adoption are seen as mechanism of maintaining current shelflife quality characteristics along with identification of new preservative compounds or natural products.
Reference methodology for preservative identification utilises wet chemistry glass distillation procedures or HPLC or LCMS instrumentation to accurately measure primary compound concentration, non certifiable screening can be performed using rapid test kits however training in utilisation is required and certified confirmation recomended.

**Test kits for rapid estimation of SO2 in crustacea**

Sue Poole and Steve Slattery have recently completed an assessment of rapid diagnostic test kits in 2003 titled Evaluating effective quality monitoring methods for the Australian seafood industry that was funded by FRDC and is attached as an Appendix (Appendix 1) due to its direct value.

*Testing dip solutions*
Test kits from 6 different suppliers for estimating sulphur dioxide ($SO_2$) in dip solutions were tested for linearity and accuracy of response on standard solutions and on prawns dipped in solutions of known concentration were tested in the laboratory and compared to results obtained with the standard laboratory Monier-Williams distillation method (Poole and Slattery 1999) The three most accurate kits were tested by industry personnel in WA, SA and QLD at four different companies. The Palintest Sulphite Test (Tablet method) Kit, Titrets Sulphite Test Kit (10-100ppm $SO_2$), Titrets Sulphite Test Kit (50-500ppm $SO_2$) and Hannah Instruments Sulphite Test Kit were evaluated both on board vessels and in the processing factory. Samples of the test solutions were returned to the laboratory for evaluation by the Monier-Williams method. The accuracy of these kits was consistent with the previous trials conducted in the laboratory with the Titrets sulphite test kit being the most accurate but was more difficult to use. The Hannah Test kit followed this closely and was most preferred of the three. The Palintest kit performed badly.

Most evaluations were done with the researcher present but when the evaluation was conducted by industry without the researcher present the results obtained from the kits were unreliable. Staff should be trained in the use of the kits and regular backup testing of samples be done by an independent laboratory.

*Sulphite kits for testing prawn flesh*

$SO_2$ levels in prawns should be below 30mg/kg for the whole edible portion but a dip applies solution to the surface. Test strips suffer from two disadvantages (a) if applied directly they can only measure surface concentrations and (b) if applied to an homogenate of the edible portion they only measure the level of free $SO_2$, not the total $SO_2$, some of which is bound in the matrix. The reference Monier–Williams distillation method used for regulatory purposes measures total $SO_2$ both free and bound.

Three kits were tested on homogenates the Alert Sulphite Test Kit

Merckoquant Sulphite Test Strip and the Boehringer Mannheim Sulphite Test Kit. The Alert Sulphite Test Kit was the most accurate but only on 54% of measurements, Merckoquant 17% and the Boehringer kit only 11%. None of the kits were recommended.

### *Recommendation*

**No Kit can be recommended, monitoring of new kit developments or rapid instrumentation in this are continue as this appears a medium term need by industry**

## Total Volatile Basic Nitrogen (TVB-N), Trimethylamine N-oxide degradation and Biogenic amines index, ethanol and formaldehyde

### *Background*

Certification for product entry in international markets frequently requires chemical analysis to be conducted in a NATA certified laboratory with appropriate international clearances prior to sale or distribution. It should be noted that slight variations in equipment and methodology are believed to cause significant differences in result for these measures and as such research in this area must be validated for international conformity. Research efforts in Australia should also be conducted under analytical conditions that ensure that consistency and applicability is not compromised due to small differences in analysis methodology.

The microbiological and enzymatic catalytic degradation of proteins, nucleotides and amino acids and other non-protein nitrogenous compounds in seafood forms volatile bases such as ammonia, dimethylamine (DMA) and trimethylamine (TMA). These provide a mechanism for indirect measurement of the state of the seafood.

Total volatile bases can only be used to identify latter stages of seafood degradation (Botta et al., 1984). International import regulations, typified by the European union regulations 95/149/EC, stipulate a maximum value for total volatile bases in a range of seafood species. (Botta 1995). The measurement of TVB is specified in 95/149/EC and covers a dynamic range of 5mg/100g to 100mg/100g estimated by tedious distillation procedures. Due to international regulation this measure will continue to be used as a quality measure in many markets.

Marine seafood naturally contains TMAO as an osmoregulator and in elasmobranchs it is believed to counteract the deleterious effects of high amounts of urea. Trimethylamine (TMA) is the main product of microbiological degradation of TMAO.

In some species Gadiformes, Myctophids and others in frozen storage the enzyme TMAOase forms formaldehyde and DMA in equimolar amounts. Formaldehyde is extremely charged, highly mobile and very reactive and it denatures and cross-links the myofibrillar proteins and disrupts their ability to rehydrate after thawing, resulting in tough fibrous product. As DMA is virtually non-reactive its level serves as indicator of total formaldehyde production and thus is related to both texture change and storage life.

Biogenic amines index (BAI) is a third marker of interest in seafood. BAI is based on the fact that the amount of biogenic amines histamine, putrescine and cadaverine increase steadily after the death of fish due to bacterial action on amino acids.

It should also be noted that 'biogenic amines', such as serotonin, cadaverine and histamine, have clear toxicological profiles. They can be psycho-active, vaso-active and even carcinogenic and mutagenic. Thus the production of biogenic amines such as histamine in fish or seafood remains highly regulated and these anti-nutritional characteristics provide an additional characteristic of health concern.

## *International and Australian research capability*

Most universities with food or shelf-life capability will have conducted TVB analysis as part of undergraduate programs , however the use of this measure as a marker for seafood degradation is not particularly good. The value of TVB tends to come in the latter stages of seafood freshness when some quality and value aspects are already compromised.

Analysis of TMA and DMA breakdown products and biogenic amine products can occur by several reference methodologies including HPLC ion chromatography, Capillary Electrophoresis with UV (Liao et al. 1999), amperometric (Zhang L. et al., 2002) or fluorescence detection (Kovacs et al. 1999, Oguri et al.,) with OPA derivatives (Male K, Luong JHT, 2001) and headspace GCMS, these require specialist facilities that may not be universally available. The capital and running costs in chemicals and technician time of instruments with sufficient sensitivity means they tend to be limited to specialist research or regulatory laboratories. These methodologies are what is required for certified regulatory compliance.

It should be noted that these regulatory or standardised analyses mean that a fresh or chilled product is spoiled before the results are available, particularly if the product is stored at high temperatures At temperatures of 20$^{o}$C and with normal bacterial levels, histamine levels can exceed the 50ppm limit within 20 hrs so rapid turn around of samples is required for them to be useful to companies.

The methods are well suited to frozen samples where analytical time is available.
Knowledge and standardisation of methodology is important, histamine levels can vary locationally in larger fish with higher levels found in anterior sections of scrombroid fish such as tuna and mahimahi; a standardised sampling technique is needed.

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## *Rapid Diagnostic Technology*

## *Test Kits (ELISA) for histamine determination in fish*

Sue Poole and Steve Slattery have recently completed (2003) an assessment of rapid diagnostic test kits titled Evaluating effective quality monitoring methods for the Australian seafood industry that was funded by FRDC and is attached as an Appendix (Appendix 1) due to its direct value.

Test kit utilisation was also investigated by Staruszkiewicz W and Rogers, PL. (2001) and presented at the 4th World fish inspection and quality control conference in Vancouver and was utilised in the determination of histamine analysis of tuna and mahimahi. The test kits were tested for pass/fail compliance at 50ppm, a typical level for regulatory practice, and also for their ability to define an accurate concentration value when compared to AOAC HPLC fluorometric detection methodology.

Test kits tested all utilised an ELISA (enzyme linked immunosorbent assay) in a colour based conjugate as their means of determination. with varying levels of simplicity and accuracy, some false positives and significant biases were noted (21ppm) it was suggested however that these kits can form a useful on site pass or fail capacity with minimally trained of inspectors where handling is suspect.

It was also noted that production of the test kits ceased for three of the kits tested during the limited time of testing by these analysts. This variable supply would provide a significant erosion of confidence for industry that may have already invested in the training of staff in their utilisation. All kits required some level of instrumentation investment when quantitation was attempted, typically in the form of a microtitre plate reader (approx $15000.00).

***Australian capacity***

The seafood CRC has multiple partners that could benefit from improved test kit or test kit verification. Their use as a crude screening tool for safety compliance in lieu of later validated certification has some merit. Their advantages are they are relatively simple, available onsite, fast and can provide a degree of product safety reassurance. Disadvantages are a general lack of direct validation for specific product matrixes, not sufficiently accurate or precise for product degradation studies, cannot be easily used in in-line production systems.

Some degree of focus is required on new and emerging techniques that improve these characteristics of speed, flexibility and accuracy in application of traditional and kit based chemical analysis such as lab on a chip technologies and artificial nose technology.

***Commercial products***

The Alert Histamine Test Kit was part of the Rogers and Starukiewicz (2000) study and as it was available in Australia it was tested by the Innovative Food Technology laboratories of QDPI & F and demonstrated to industry (Poole and Slattery 1999). The kit is an ELISA based method in which a developed colour can be read visually against a standard or automatically with a well plate reader. This versatility means it can be used by QA personnel at a processors, after proper training, with the absolute minimum of laboratory equipment

The kit was found to be both accurate and reliable and was tried out at 11 industry and government establishments and was demonstrated at workshops. It had an accuracy better than the standard capillary electrophoretic method at low levels below 10 ppm which means it is useful for following the development of histamine in controlled experiments.

The susceptibility of these tests to matrix induced errors based on alterations in species, product or presevatives ect has not been identified though this may have been acheived. Indications of variability

by Rogers and Starukiewicz (2000)suggest that close to standard identification requires certified laboratory verification given that biases have been reported.

The cost per sample is cheaper than traditional instrument techniques and after a small amount of training it is easy to use and could be incorporated into QA systems currently in use by processors.

***Commercial Product - Veratox for histamine kit from Neogen.***

The test is a competitive direct ELISA that provides exact concentrations in parts per million (ppm). Histamine is extracted from a sample using a quick water extraction process. Free histamine in the sample and in controls competes with enzyme-labeled histamine (conjugate) for the antibody-binding sites. After a wash step, substrate reacts with the bound enzyme conjugate to produce blue color. A microwell reader is used to yield optical densities. Standard optical densities are used to form a standard curve, and sample optical densities are plotted against the curve to calculate the exact concentration of histamine.

**Fig Veratox test kit for histamine**

Product Specifications
Lower limit of detection: 2 ppm
Range of quantitation: 2.5 ppm - 50 ppm
Controls provided: 0, 2.5, 5, 10, 20 and 50 ppm
Testing time: 20 minutes
Antibody cross-reactivity: Specific for histamine
Tests per kit: Up to 38
Approvals: AOAC-RI #070703

***Recommendations***
The seafood CRC has multiple partners that could benefit from improved test kit or test kit verification. Their use as a crude screening tool for safety compliance in-Lue of later validated certification has some merit. Their advantages are they are relatively simple, available onsite, fast and

can provide a degree of product safety reassurance. Disadvantages are a general lack of direct validation for specific product matrixes, not sufficiently accurate or precise for product degradation studies, cannot be easily used in in-line production systems.

## Lab On a Chip Technology (LOC)

### *3.3.1 Background*

Miniaturization of sample analysis was first introduced in the 1990's by (Manz et al 1990) whereby sample pre-treatment, separation and detection were minimised and incorporated into a microfluidic device. The rapid development of this technique has seen adoption of commercial products such as the Agilent product "Lab On A Chip"Analyzer 2100 and the establishment of numerous chip production and platform companies that supply ready to use components and application packages.

Miniaturisation produces advantages over traditional analysis in the form of portability, durability in extreme or remote operation and speed of analysis, the shrinking and containment of analysis into a single device simplifies operation for specific analysis outcomes and allows a lower level of operational training once the platform is established. The advantages of miniaturisation include low reagent consumption, low waste production, small sample size and increased speed of analysis.

In depth review papers have been produced covering theory, fabrication of devices, sample introduction, fluid manipulation, sample pre-treatment, and detection methods (Marle,L et al 2005). Challenges remain in the full investigation of areas of sample introduction or the interface between the sample and the instrument, matrix interference and high LOD's {what is an LOD} and the ability to obtain and present a representative sample.

Analytically the use of LOC devices presents on two levels, already established and validated product, and research platform and chip development for novel or high threat, specific need analytical development. Cost in application also varies accordingly with established instrumentation significantly cheaper to implement than developing instrumentation, an added benefit is greater international recognition with validated methodology.

Not all applications can go down the established platform pathway and the value of a development capacity in chip design is important for specific needs. Even in this area "off the shelf " separation or combination systems are available with a multitude of end point detectors as the determination agent.

The low cost of these devices and the increasing range of off the shelf products suggest that this is an area worth investing in for screening or establishment of good quality practice in the storage, handling and processing of seafood. Comparable detection limits and increased speed and the ability to locate in a processing facility makes this technology very attractive and forms the future of the high end rapid diagnostic techniques market.

***Commercial instrumentation***

The Agilent LOC Bio-analyser 2100 represents the most commercially developed product on the market with numerous validated applications presented in its method descriptions including the analysis of biogenic amines using their electrophoresis platform. The adaptability of electrophoresis separation platforms suggests that both AMP and DMP degradation product analysis should also be easily achievable.

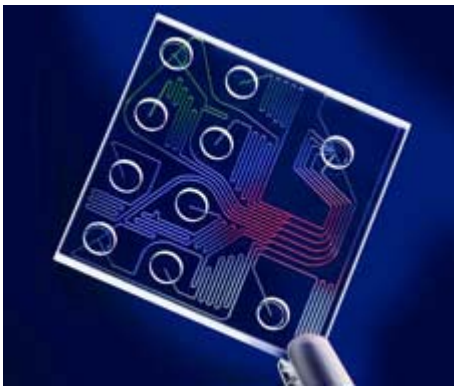

Fig
Silicone chip showing microfluidic channels

Fig
Agilents Bioanalyser 2100

***Australian Capacity***

Utilisation of LOC technology for the determination of seafood quality issues has many applications, Chemistry Centre (WA) have suggested application in the determination of Biogenic amine production and TMAO degradation products for adoption of species shelflife studies.

LOC technology also has applications in rapid microbiological and toxin identification and quantification as suggested by both Flinders University and CCWA.

Chemistry Centre WA has also suggested its utilisation in speeding up and providing onsite testing for traceability and truth in label determinations.

The Seafood CRC has significant research capacity in this area, Flinders University has nominated a project that aims to create an analytical LOC platform as per below, staff capability and facilities for chip manufacture are strong in this research group.
Chemistry Centre (WA) as a partner or as an individual organisation also has a strong certified method background with the capability for testing the ruggedness in application of innovative instrumentation. CCWA also have a project proposal with Agilent through CRC Care that will see a close working association with the further development of the Agilent LOC bio-analyser 2100 and extension of current applications to industry in Australia, CCWA would seek to extend this arrangement into the Seafood CRC with other collaborative partners.

*Flinders University LOC project*

Project Aim - Dr N H Volcker, Dr JG Mitchell, Dr. A Ellis

The aim of this project is to develop a miniaturised and portable biosensor platform for the rapid detection of a range of seafood-borne pathogens and their toxins. We aim to develop a platform that will feature lab-on-a-chip technology in the form of microfluidic channels for sample loading, transport, prepurification and incubation. Microfluidic channels will deliver the solution to be analysed to an optical biosensor platform where molecular recognition of the analyte molecules are transduced into colour changes that can be measured by a miniaturised CCD spectrometer.

*CCWA and partners LOC projects*

Project Aim – To establish rapid, on site methodology using commercially available lab on a chip platforms for the rapid determination of a range of useful determinants for the seafood producer these would include, aiding shelf-life index determination through onsite testing for microbiogical composition and load in combination with monitoring of histamine, other biogenic amines and TMAO degradation products for specific species destined for high value markets.

CCWA would also seek to provide onsite, rapid identification of seafood species in fresh and fillet or manufactured product utilising the same LOC platform, utilisation of identification fingerprints would be compiled for construction of a national onsite applicable rapid certification and traceability database for high value seafood product.

### *Recommendation*

**Given the low cost of the platform and the suggested ruggedness and diversity in operation sugested research in lab on chip applications be supported where value in application can be demonstrated.**

## Heavy Metals

### *Background*

Heavy metal concentration in seafood is regulated for food safety, effects of heavy metals on pregnant women and children and management of contamination in ecosystems remains a challenge internationally. Adoption of regulatory non tariff barriers to trade in major export markets is increasing, resulting in continual lowering of acceptance limits. This places increasing pressure on exporters to control these compounds of concern in their products or face rejection at delivery with high commercial cost.

Differences in toxicity between various organometallic species highlights the importance of metal speciation analysis. Unlike total metal analysis, speciation provides useful data to better understand the distribution and possible transformations of these toxic organometallic species within the marine environment.

Speciation assists in the understanding the disposition of methyl-mercury in fish or tri-butyl tin in molluscs and can significantly improve production yield or growth characteristics.

### *Seafood CRC research capacity*

The Seafood CRC has significant capacity for research in this area, complementary species, sampling, and export market regulatory knowledge and environmental management skills are presented among research providers, collaboration with the Chemistry centre (WA) gives access for these professionals to NATA certified instrumentation and world's best practice and analytical expertise. This powerful collaborative outcome represents a significant opportunity for industry to gain management

knowledge and product understanding where their product value may be jeopardised by these contaminants.

## *Rapid diagnostic Methodology*

***ICP-MS, with microwave digestion for heavy metals analysis.***

Rapid screening for total heavy metals can be achieved via microwave digested ICPMS. Total values for heavy metals are a regulatory requirement in many export markets with lower compliances causing serious difficulties for many seafood exporters particularly in the areas cadmium in prawns. Totals analysis in seafood represents a reasonable indicator of environmental or processing contaminants levels and when monitoring of low term trends occurs. It can provide a useful indicator potential toxic contamination at source but suffers form lack of discrimination when considering true toxic potential.

**Fig Agilent ICPMS instrumentation**

**Fig Microwave digester**

***Analytical principle***

Acid digestion of sample is rapidly achieved in a sealed inert chamber using microwave assisting pressure digestion.

The sample to be analysed is dispersed as a vapour in a stream of argon gas. The gas plus sample is injected into the core of a radio-frequency argon plasma which has an electrical temperature of $7000^{O}K$ (approx). The energy of the plasma is transferred to the sample and first dissociates the sample then sequentially atomises and ionises the constituent elements present in the aerosol. The plasma core containing the ions is extracted into a reduced pressure region through an orifice in metal (usually nickel or platinum) cones placed directly in the plasma. A portion of the plasma passes through an additional orifice into a region of significantly reduced pressure. A system of electrostatic lenses extracts the positive ions and vectors them though a quadrupole mass filter, which is controlled via alternating RF and DC fields in the quadrupole to allow transmission of ions of one selected mass to charge ration at any specific time. Cycling of the quadrupole facilitates passage of any selected ion with mass to charge of less than 250amu at specific times during the cycling program. An ion detection system registers the transmitted ions. Each naturally occurring element has a unique and simple pattern of nearly integer mass to charge to charge ratio corresponding to its stable isotopes,

thus facilitating identification of the elemental composition of a sample. The number of registered ions from a particular sample depends on the concentration of ions in that sample and quantification is achieved with reference to aqueous calibration standards.

The Octopole Reaction System consists of an ion guide, mounted on axis with the quadrupole for higher ion transmission. The octopole is located inside a cell that can be pressurized with a reaction/collision gas such as hydrogen or helium.
The difficult Argon based polyatomic interference are dissociated by collision with the added gas within the cell, enabling otherwise interfered analytes to be determined. Simple gases like Hydrogen or Helium are used to minimises side reactions and hence possible new interference.

***Gas Chromatography-Inductively Coupled Plasma-Mass Spectrometry (GC-ICP-MS) - Organic speciation of heavy metals***

Gas Chromatography-Inductively Coupled Plasma-Mass Spectrometry (GC-ICP-MS) provides a solution to separate and quantitate organometallic compounds at ultra-trace levels and represents world's best practice in this area. The main advantage of using ICP-MS is the superior sensitivity obtained for metal analysis when compared to other available GC detectors such as FPD, FID and ECD. Coupling the ICP-MS with a GC allows simultaneous separation and measurement of multiple organometallic compounds in a single analytical run.

***Commercial instrumentation.***
Smaele et al 2007 found that by coupling an HP 6890 series GC to HP 4500 bench-top ICP-MS, the lowest limits of detection (ever reported) were achieved for a selected group of organometallic compounds, measurements confirmed during analysis at CCWA. The ability to detect organometallic compounds at ultra-trace level is important for maintaining export market confidence and better assist in managing contamination issues. New trading opportunities may arise due to lower detection limits.

The ICP-MS is capable of analysing high matrix samples, mainly due to the efficiency of the sample introduction system in decomposing the sample matrix. Reproducibility is excellent, demonstrated in a test done by (McCurdy et al.2004) on the reproducibility and long-term stability of organo-tin analysis by GC-ICP-MS. Accurate and reproducible results are vital for certification purposes. Almost no sample matrix is introduced into the ICP-MS, thus maintaining tuning for weeks or even months. Due to its high efficiency in decomposing the sample matrix, the ICP-MS produces minimal interferences when compared to other common detectors generally used by laboratories.

Fig Agilent GC and ICPMS for organometallic determination

***Hand held XRF***

Traditionally field portable XRF systems used radioactive isotopes as their source of X-rays, they were expensive to own and operate and created regulatory burdens for their owners. Use of isotope sources made site-to-site travel difficult due to the requirements for transporting a radioactive source. Utilisation of x-ray tubes as source for XRF handheld systems has advanced the applicability of these instruments such that third generation instruments are now available, with increasing worldwide sales of $100 million US in 2006 indicating market utilisation is active it would suggest that continued investment in instrument improvement will occur.

Current detection limits in the 5-10ppm range suggest that these instruments do not yet have the detection limits capable for compliance analysis in seafood however they may be very useful as a screening tool given the speed and simplicity of this tool, monitoring of this product in its fourth generation version could prove more adequate given the level of investment in this area.

Collaborative efforts internationally with manufacturers for this relatively unexplored application could be fruitful.
Thermo Fisher Scientific, Bruker Biosciences, Innov-X Systems, Spectro, and Oxford Instruments are some of the leading participants in this market.

***Commercial product***

With its Alpha Series™ Innov-X Systems has pioneered a handheld XRF analyzer that utilizes an X-ray tube instead of radioactive isotopes. This battery powered point-and-shoot XRF system eliminates burdensome radioactive sources and provides on-the-spot quality data about elemental composition. The single X-ray tube replaces multiple isotopes used in source based systems to offer simultaneous analysis of 20-25 metals including all eight RCRA metals and the EPA priority pollutant metals.

It generally provides superior detection limits (DL) compared to isotope systems. Moreover, the testing time never increases with an X-ray tube because there is no source decaying. The testing speed after 4-5 years is the same as when the analyzer was purchased.
Features include
On-the-spot screening of up to 25 Toxic Metals including Pb, As, Hg, Cr, Cd, Tl, and 20+ other toxic elements with results in seconds.
The Innov-X handheld XRF is used by the US Food and Drug Administration for frontline screening of imported foods and consumer items for heavy metal contamination.
The top performing portable XRF for metals in soils based on a 2005 EPA evaluation. Meets EPA Method 6200 for analysis of metals in soils and sediments.

**Fig Innov-X handheld XRF**

***Seafood CRC Applications***
The Seafood industry is confronted with non tarrif barrier to trade that challenge the ability of exporters to find and supply high value markets.
Collaboration with industry and research groups within the CRC have the ability to reopen these markets where toxicity arguments are prevalent and provide certified product that reinforces our high quality product character.
Understanding of heavy metal disposition in seafood species, feed quality attributes and environmental impacts of encroaching industry their pollution characteristics, water quality, sediment and food chain interactions will enable the seafood industry to better manage this problem.

***Recommendation***
That the Seafood CRC invest in the maintenance of source quality attributes in seafood.
That processing and heavy metal distribution effect in species be researched
That a watch be placed on hand held XRD instrumentation with a view to utilisation when detection limits reach a level appropriate for seafood. The value of handheld XRD be investigated for screening value.

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## Organic residue analysis - Agricultural residues, Growth stimulants, Antibacterial's, Endocrine Disruptors fire retardants,

Analysis of organic residues is a highly diverse specialised field and beyond the scope of this review to individually classify.
Needless to say improvements in instrumentation have sped up analytical establishment and operation of instrumentation this is juxiposed by an ever lowering of detection limit requirements as the toxicity of many of these compounds is better understood.
Each analyte represents unique analytical issues to obtain valid results, instrumentation is expensive and typically not rapid though once established can achieve rapid turn around characteristics.

The analysis for pesticide and veterinary chemicals in seafood has traditionally been a challenging and time-consuming process. A wide variety of sample preparation processes are required before a sample is fit for introduction to an analytical instrument and, even then, results are usually confounded by interfering non-target chemicals. The positive identification and quantification of a chemical residue in seafood is important to establish the cleanliness and safeness of the seafood product when it reaches the consumer, either in the domestic market or when exported to other countries.

Residues of concern can be:

1. Insecticides, Herbicides
   Carbaryl, Ivermectin
   Organophosphorous pesticides - Dichlorvos, trichlorfon, malathion, diazinon
2. Feed Additives
3. Anaesthetics
4. HormonesAntibacterial Agents
   B-Lactams, Nitrofurans. Macrolides, "Phenicols", Quinolones, Sulphonamides, tetracylines
5. Theraputants
   Formalin, malachite green, Trifluralin
6. Chemicals associated with structural materials
   Paints (TBT), plastics, flame retardents
7. Fertilisers
8. Disinfectants
   - Chloramine T, Quaternary ammonium compounds
9. Environmental Contaminants - POP's
10. Organochlorine Pesticides
11. Polychlorinated Biphenyls (PCB's)
12. Endocrine disruptors

***Extraction instrumentation and methodology***
Extraction mechanisms can be via analysis of consumptive seafood or may involve sampling of its source environment. This can take the form of direct species sampling using designated species sampling regimes or it may utilise indicator species. Water environment may be direct sampled or

sampled using passive sampling devices (POCIS) that are essentially artificially targeted accumulators.
Frequently detection limits are challenging traditional organic phase concentration has been replaced with solid phase extraction from bulk source samples, Online solid-phase extraction (SPE) is another new technique that is becoming essential in the fast determination of residue contamination in seafood. Previously, laborious processes were required in the laboratory to remove co-eluting and interfering chemicals. With online SPE, faster and more accurate analyses can be performed by automatically concentrating the sample extract and removing interferences. This system is now in regular operation along with the high-sensitive triple quadrupole mass spectrometer at the Chemistry Centre (WA).

**Fig 1**
**Passive sampling (POCIS)**

**Fig 2**
**Solid-phase extraction**

**Fig 3**
SPE **Automated extraction and analysis**

**Fig 4**
**Accelerated Solvent Extraction (ASE)**

The use of new technology, such as the new highly-sensitive triple quadrupole mass spectrometers, provide better specificity and detection levels now required by export markets and the Australian health authorities. By using high technology instrumentation such as these, seafood products which, previously, have been turned back or prevented from entering new export markets owing to residue contamination at extremely low levels, are now able to be analysed regularly and with the appropriate analytical quality requirements.

Typical organic residue instrumentation includes:

Headspace GCMS

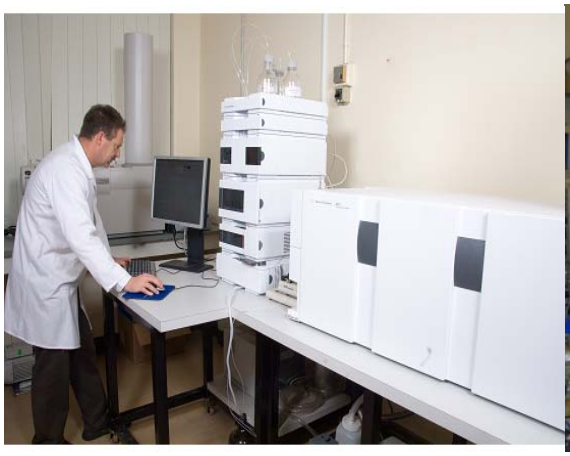


**Fig LCMS – Triple Quadrupole**

Traditional GCMS

**Fig LCMS - Trap**

## *Recommendation*

The Chemistry Centre (WA) as an participant in the CRC with significant analytical capacity is actively seeking co researchers and industry who wish to collaborate in this area, the Chemistry Centre has indicated its desire to significantly contribute to the CRC in this area both financially and intellectually.

I would recommend that this occurs where particularly detrimental characteristics for industry are already occurring and impacts on markets are already occurring.

It has been noted in other countries that increased utilisation of aquacultured product has introduced problems in feed quality and supply. Issues relating to imported feed quality for aquaculture are increasing in prevalence.

A chemical analysis guidance committee be constructed to ensure full benefit of all participants capacities in this area be considered.

## RAPID DIAGNOSTICS FOR MICROBIOLOGICAL ISSUES

### Introduction

A report published earlier this year by the US Centers for Disease Control and Prevention reveals a 50 percent increase in E coli infections since 2004, and a monstrous 78 percent increase in Vibrio infections - caused by eating raw shellfish - over the past decade.

The center estimates that 76 million Americans get sick and 5,000 die from foodborne hazards each year in the United States.

Bacteria likely to cause food safety issues in seafood are *Vibrio (cholerae, parahemolyticus, vulnificus*), *Aeromonas*, *Clostridium (botulinum, perfringens*), *Listeria monocytogenes*, Enterobacteriaceae (E. coli, Salmonella, Shigella, Yersinia enterocolitica, Staphylococcus aureus, Campylobacter).

Rapid Bacteriological Methods are being developed to ensure businesses have optimal access to methodology that ensures their workplaces and products are food-safe. A number of these rapid

technologies have been approved for use by AQIS, hence enabling approval of export premises. The following is a description of standard and rapid methods approved by AQIS for use in the meat industry which may be applicable to the seafood industry. Note that in some cases, importing country approved testing regimes may differ from those approved by AQIS. Also, often rapid tests can only be used as indicators as approved methods may need to be carried out in a NATA accredited laboratory.

The seafood industry may consider undergoing the development of an Australian Standard for Seafood Production. Successful accreditation of the Australian Standard for Meat Production has been completed. This auditable standard now lists all approved tests, protocols and procedures and has been implemented in relevant legislation, thereby aligning export and domestic standards.

### *Microbiological analysis restrictions*

Typically microbiological assessments are conducted externally from production facilities due to limitations in laboratory capacity. Turn around constraints imposed by sample transport and selective plate growth intervals mean that analysis is not completed in less than 48hrs.

## *Diagnostic Methodology*

#### *Rapid Microbiological test kits*

Sue Poole and Steve Slattery have recently completed an assessment of rapid diagnostic test kits in 2003 titled Evaluating effective quality monitoring methods for the Australian seafood industry that was funded by FRDC and is attached as an Appendix (Appendix 1) due to its direct value.

#### *DNA/RNA specific probes – Luminex suspension array systems.*

Investigators have increasingly turned toward molecular technologies to meet the need for rapid multiplexed species level detection, the use of microarray systems has enabled the simultaneous identification and quantification microbiological species based on the application of specific DNA and RNA probes. Recent introductions of suspension or microarray systems introduced improvements in flexibility cost effectiveness and faster hybridisation kinetics than planar arrays (Dunbar et al 2006).

The Luminex sytem was primarily developed in a clinical environment (Dunbar et al 2003) although other applications are in development Ellison and Burton 2005: Spiro et al 2000; Spiro and Lowe 2002; Baums et al 2007.

#### *Principle*

Any molecule or chemical group that can be recognised by reactive or chemical group can be recognised by reactive or complimentary functional groups can be immobilised on the surface of microspheres. For DNA or RNA hybridisation assays DNA id amplified with a biotin labelled primer and the amplicons are hybridised to capture probes bound to the microsphere surface. With Luminex xMAP technolohy systems the beads have verying ratios of red and infrared fluorophores, giving a unique spectral address to each set of beads. The beads are coupled to a reporter molecule to generate fluorescence. Intensity is read utilising two lasers. The red laser identifies the spectral address of the colour coded beads the green laser registers whether the probe has captured a target. There are 100 microsphere targets available allowing detection of many targets in a single sample well (Diaz and Fell 2005; Diaz et al 2006).

In the study of Baums et al 2007, Luminex probes were designed for faecal indicator organisms including *Bacteroids fragilis* group, *E. Coli* and *Shigella* spp *Enterococcus* Spp *Bacteroids Distasonis* and *Enterococcus faecalis*.

Hybridisation time is approx 1hr with reading taking approx 0.47sec per well in a 96 well plate platform thus the Luminex system has high potential to deliver rapid high throughput detection of multiple pathogens.

***Methodology in validation or development for E coli and enteric organisms***

Example 1

Recent work conducted at Michigan State University, by scientists Yang Liu, Shantanu Chakrabartty and Evangelyn Alocilja has developed a prototype nanotechnology-engineered biosensor. The sensor is designed to help processors detect multiple pathogens faster and more accurately than current devices.

The nano-biosensor works as a molecular transistor, triggered by the presence of specific pathogens on an immunosensor. The transistor works by processing data through fundamental logic gates. The logic gates operate by converting binding events between an antigen and an antibody into a measurable electrical signal using polyaniline nanowires as the transducer.

The logic gates are created by patterning antibodies at different spatial locations in an immunosensor assay. Immunosensors are biosensors that use antibodies to recognize the presence of a pathogen.

In this study, B. cereus and E. coli were used as model pathogens. The tests were validated by taking measurements with different pathogen concentrations, the change in conductance across the gates can be modeled as a log-linear response with respect to varying pathogen concentration.

Example 2

RAPID E. coli 2 and coliform bacteria test
24hr instead if 48hr by AOAC 966.24

**Reference**
**24 October issue of the Nanotechnology journal**

***Methodology in validation or development for Salmonellae***

*Genquence Salmonella assay* DNA hybridization technique reduction from 42hrs to 24 hrs

**3.4 Total Viable Count (TVC)**

Australian Standard AS 5013.1-2004 Food microbiology - Examination for specific organisms - Standard plate count AOAC 990.12 - Petrifilm™

***Methodology in validation or development for TVC***

Biophage has developed a new impedance spectrometer PDS96 Biosensor, which can be used to simultaneously measure the growth of up to 96 cell or bacteria cultures in a highly efficient and easy

to use automated system. The biosensor has a small footprint suitable for portable applications and benefits from requiring only small sample volumes of less than half a millilitre.

Bacterial activity detection is targeted, as well as the analysis of the total bacterial load, which could be particularly useful for the seafood industry.

When combined with biological recognition probes, such as phages or antibodies, the biosensor provides unambiguous detection and quantification of living pathogens and coliform bacteria.

The instrument measures extremely small electrical variations induced by cell growth at the bottom of an array plate. Each array plate contains 96 sample wells with a small gold detecting electrode deposited on the bottom of each well and a larger gold counter electrode attached to the side.

As cells attach themselves to the detecting electrode, their plasma membranes induce small variations in the properties of the electrical signal passing through the sample well. These changes are then measured and analysed before being displayed on the screen monitor.

Each measurement takes approximately five seconds and samples can be taken and logged for over 12 hours, allowing the system to easily follow the activity of a substance on a living system over time.

*2.1.2 Other Rapid Bacteriological Methods applicable to the Seafood Industry.*

*2.1.2.1 ATP Activity*.

A further rapid method is based on ATP activity.  Merck luminescence.

*2.1.2.2: Vibrio spp*.

An improved fluorogenic assay for the rapid detection of *Vibrio parahaemolyticus* has been developed.

## *Recommendations*

Recent developments in DNA and RNA identification of microbiological species suggest that this may be an area that can significantly lower turnaround times for these essential safety and shelflife significant microbiological species, development of 96 well microarray systems such as the Luminex's bead based microarray show particular promise. Combinations with capillary electrophoresis lab on a chip instead of gel based electrophoresis also suggests that this may be a technology that can be adopted in the production environment. Development of a combined Lab on a chip and microarray methodology utilizing Luminex's micro-sphere immobilization  probe carrier is highly possible with huge implications for speed flexibility and cost effectiveness.

2.2 Rapid Methods for Viruses Posing Food Safety Risk.

*2.2.1: Astroviruses (Norovirus)*

An international method for norovirus detection in shellfish has been developed and ISO accredited, it is available on the Canada Health or Cefas website.

Viruses cannot be cultured in vivo so you must have a test that works on the tissue itself and bacteria are no indicators of viral presence. Detection by ELISA of the viral protein coat is not sensitive enough and real time PCR is the only current option as a method.

Real time PCR methods that lead the world have now been developed for Hepatitis A and Noroviruses in the SEAFOODplus project (www.seafoodplus.org). Mengo virus has been used as a surrogate to validate the methodology.

Participants in its development are members of CEN and ISO working parties and the results are being confirmed by ring tests and will be moved into standards as soon as practical and they are close to having reference methods.

***Rapid Methods for Parasites Posing Food Safety Risk***

Parasites of food safety issues for the seafood industry are nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes). Once considered more a market problem due to unsightly appearance and less of a hazard because cooking destroys them the popularity of raw seafood as sushi and sashimi has renewed interest in their detection. Since they are also killed by freezing most of the fish presented as sushi and sashimi has been once frozen.
Imaging spectroscopy can be used to create automated surveillance of whitefish fillets to supplant the manual inspection by candling (Heia et al. 2007).

***Rapid Methods for Biotoxins Posing Food Safety Risk***

*Shellfish Toxins.*

The presence of toxin producing phytoplankton in shellfish growing areas, resulting in possible accumulation of the toxins in shellfish flesh, and subsequent food poisoning episodes, is a major management issue for shellfish quality assurance programs. Symptoms of toxic shellfish poisonings (TSP's) will vary according to which algal biotoxin has been accumulated and consumed. There are currently four main TSP's: amnesic shellfish poisoning (ASP), neurologic shellfish poisoning (NSP), paralytic shellfish poisoning (PSP) and diarrhoetic shellfish poisoning (DSP). To manage the risk afforded by toxin producing phytoplankton, shellfish programs generally have management strategies based on phytoplankton enumeration and/or direct measurement of biotoxin levels. A major review of all issues associated with Australian shellfish biotoxin issues was completed in Nov 2001 Australian Marine Biotoxin Management Plan, Cawthron Instituite report 645 this remains a definitive report in this area of interest.
A further risk to the seafood industry is associated with grazing fish eating microalgae that produce ciguatera. Fish containing ciguatera can cause serious illness, ciguatera poisoning is a major risk factor in some tropical commercial fisheries.

## *Australian Research*

Project title:

Assessment of the mouse neuroblastoma assay as a screening tool for detection of ciguatera toxins in Queensland reef fish

Researchers:
Ian Stewart1, Sue Poole2, Geoff Eaglesham3, Glen Shaw1,4, Ross Sadler4

Collaboration:
This is a jointly funded research project between
1 Griffith University (School of Public Health), 2 QDPI&F (Innovative Food Technologies) and Qld Health (3 Qld Health Forensic and Scientific Services and the 4 National Research Centre for Environmental Toxicology)

Objective:
To assess the applicability of the neuroblastoma assay in conjunction with HPLC-tandem mass spectrometry for determination of ciguatoxin in tropical reef fish species

The standard method for identifying and quantifying ciguatoxins in suspect fish is by high performance liquid chromatography and tandem mass spectrometry. This process is complex and expensive, requiring a multi-step extraction and sample clean-up, so is unsuitable for rapid screening or high volume throughput. We are assessing the utility of an in vitro technique, the mouse neuroblastoma assay, to detect ciguatoxic fish. Ciguatoxins function at the cellular level by prolonging the activity of voltage-gated sodium channels in nerve cells; the assay utilises the sensitivity of the mouse neuroblastoma cell line to sodium channel modulators. We will determine the reliability of the assay by using a ciguatoxin standard, suspect fish that have been implicated in human poisoning incidents, and extracts from non-toxic fish as negative controls.

Timeframe: May 2007 – June 2008

Project progress:

- reference methodology (HPLC-tandem MS) has been standardised using pure ciguatoxin standard
- appropriate cell-line has been established and neuroblastoma assay set up and shown effective with pure ciguatoxin
- extraction methodology has been refined and optimised for determining ciguatoxin in fish flesh samples
- current work is focussed on assessing fish samples implicated in CFP outbreaks
- next work will compare results the CiguaCheck kit directly with standard reference method

Project 2 about to commence

Project title:
Establish the reliability of insect bioassays for the qualitative detection of ciguatoxin in fish

Researchers:
Ian Stewart1, Sue Poole2, Geoff Eaglesham1, Glen Shaw1,4, Ross Sadler4

Collaboration:
This is a jointly funded research project between
Qld Health (1 Qld Health Forensic and Scientific Services) and2 QDPI&F (Innovative Food Technologies) and the 3 National Research Centre for Environmental Toxicology)

Objective:
Determine whether insect bioassays are suitable for the detection of ciguatoxins in tropical reef fish and their potential for use as a screening tool for ciguatoxic fish

Timeframe: June 2008 – June 2009

## *Rapid test kits*

Sue Poole and Steve Slattery have recently completed an assessment of rapid diagnostic test kits in 2003 titled Evaluating effective quality monitoring methods for the Australian seafood industry that was funded by FRDC and is attached as an Appendix (Appendix 1) due to its direct value.
The CiguaCheck kit for detecting ciguatoxin was found to be completely unreliable. False positive results were prevalent when using the kit, with 82% of randomly purchased fish samples demonstrating a positive result for ciguatoxin.

## *Rapid Enumeration of Toxic Phytoplankton*

Traditionally methods to assess the presence of toxic phytoplankton involve microscopy. These methods are time consuming and may have difficulties due to size differences in target species, fragility of target species, issues associated with fixative methods and difficulties in identification. Also, toxicity within a species varies dependent on various environmental and genetic factors.

Hence, there has been considerable research effort in using DNA/RNA probe technology to identify specific toxin producing species within a total phytoplankton population.

Two methods currently are being trialled
rRNA species specific probes, labelled with fluorescent dye so cells can be counted under a microscope (*Alexandrium* and *Pseudonitzschia* in New Zealand). (Cawthron Institute method ISO approved).
Immunochemical particle analysis based on flow cytometry, the probe contains antisera to antigens present on the surface of the cells, label also with fluorescent dye and cells are counted using flow cytometry. see microbiological applications.

*Proposed projects*
Creation of a DNA/RNA fingerprint of toxic phytoplankton species based on traditional electrophoresis determination techniques for rapid screen identification of species of concern.
Development of Microarray specific probes for phytoplankton to enable rapid and cheap identification of toxin producing species. CCWA and partners, possibly Flinders Uni and traditional identification group. Adaptation to lab on a chip platform using existing commercial platforms for rapid onsite testing.

***Rapid Shellfish Biotoxin Identification Methods (PSP, ASP, DSP and NSP toxins).***

Instrumental techniques for biotoxin measurement in Australian shellfish programs are generally conducted in New Zealand, as there is no one Australian laboratory accredited to undertake all toxin analyses. This results in long queues based on the amount of time it takes to get the samples to New Zealand. The actual tests themselves (generally HPLC, LCMS or mouse bioassay) will take less than 12 hours generally.

There has been the development of rapid, indicator tests which can be used on site to measure biotoxin production. These tests are being used as indicator tests for some Australian shellfish growing areas.

Jellett Rapid Testing Ltd. developed and manufactures the ONLY lateral flow rapid test that has been accepted by the USFDA into the National Shellfish Sanitation Program of the USA (www.jellet.ca).

PSP Jellett : approved by USFDA and new Zealand for use under certain conditions. Some analysis indicates there are some problems with use.
ASP Jellett
DSP Jellett.

# PART C - NON SENSORY - PHYSICAL CHARACTERISTIC AND SECONDARY ANALYSIS ASSESSMENTS.

Physical characteristic assessment is typified by instrumentation and diagnostic methodology that is generally based on indirect or multiple component effect measurement and is usually calibrated against sensory or other critical primary chemical compositional analysis.

As techniques that are not directly standardised against primary compounds but are based on chemometric or sensory associations levels of error are increased as the matrix becomes more diverse or complex and the variability's of trained panel assessment ad to error. Subsequently data set size must be large and suitably representative over the whole dynamic range of the attribute assessed against.

Frequently chemometric and statistical approaches are used to develop statistically acceptable regression relationships between attributes and measures. Because they are not based on primary compound elucidation and reference they have a greater scope for error that can result in less satisfactory prediction outcomes when applied to model development.

## Colour, Appearance and Imaging

Colour is a purchaser's most powerful tool for assessing quality where taste and touch are denied. People build a database of memories that matches the products look to its taste, texture and freshness and they act on their previous experiences when purchasing.

The scientific analysis of colour allows the producer to more accurately provide consistent product and makes the purchaser's job easier. A manufacturer can adopt new ingredient sources or introduce new products with confidence that the colour is matched to existing or time proven market expectation. (Pomeranz, Y. Meloan, C E. 1971.)

Colour is a property of the light that surrounds it and relationship between that light and the reflected or absorbed characteristics detected by the human eye. An object exhibits a particular colour because it reflects light. An apple is red because it reflects red light, when white light falls on an apple, the pigments in the skin of the apple absorb most of the light, however the pigments do not absorb wavelengths in the red region of visible light and these wavelengths are reflected and absorbed by the eye and the apple looks red.(Williams, JE.*et al.*. 1980)

Seafood colour is influenced by species characteristics that may relate to season of catching, health of fish, nutrition or diet, physiological maturity or lifecycle position, location, mechanism of capture post capture handling, processing conditions and storage.

Colour is a particularly important characteristic for several species of fish and crustacean. Prices for both tunas and salmonids rely on the flesh colour and for crustacea the colour of the carapace is an indicator for acceptability in both raw and cooked state.

The use of multivariate protein or nutritional sources in aquaculture feeds can result in unacceptable product variation, and aquacultured product colour can be a good indicator of dietary variation and vitamin or carotenoid deficiencies.

Pigment degradation in seafood can be as a result of both oxidative and enzymatic degradation pathways. Because colour pigments are susceptible to both these mechanisms of alteration, measurement of product colour is a useful mechanism for tracking product change and identification of critical conditional points in manufacture, particularly in freezer efficiency models or process establishment trials.

Polyphenol oxidase or other enzymic degradation catalysts are promoted with cell rupture and can result in melanin compound formation, other oxidative degradation pathways of pigments may be tracked using this rapid diagnostic instrumentation, pigment breakdown and rate of formation of degradation byproducts, intermediate compound ratios and tipping factors and final melanin or endpoint can be extrapolated using colour determination scales.

## *Rapid Diagnostic Techniques*

### *Colour Fans*

The simplest is a set of colour chips or swatches such as the Roche SalmoFan$^{TM}$ a colour fan used for salmon. Cheap, portable and in common use it serves a purpose but does not cope well with samples of different translucence or intensity.

### *Hand held Colorimeters*

There is a range of handheld products that determine tristimulus colour, using characterisation of colour into a three dimensional image (Fig 1) based on the x,y,z coordinates of a, b and L allows researchers to visualise readings within a coordinate grid that assists in the understanding of subtle colour change in product, association of colour change with compound identification or sensory attribute change allows increased product understanding. Conditional optimisation is possible through rapid utilisation of this detector in onsite or field applications.

The most common is the Minolta colourmeter a handheld portable instrument which analyses the object according to the CIE colour space in three coordinates L, a and b, in which the L value is reflectance, the a value is the red- green axis and the b value is the yellow-blue axis. A very useful instrument for many applications it has not been found so useful with fresh seafoods but is more successful with cooked products. It works well on relatively uniform materials with a non-transparent, flat surface but less well on non-uniform samples particularly those that are translucent. Translucent products like fish flesh scatter incident light which results in the instrument working in the grey colour space rather than the red colour space. Thus it does not always differentiate between samples that are obviously different to the naked eye.

The Minolta CM700d colour determinator (Fig 2) is a hand held, highly portable, non destructive reflectance based instrument with a readout designed to give a three dimensional projection of an objects colour.

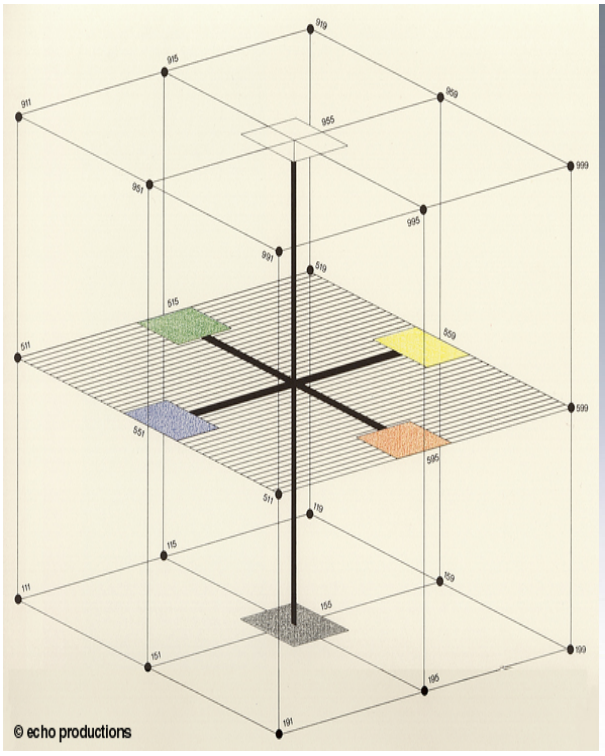



**Fig 1 Tristimulous colour projection of L,a,b readings.**

**Fig 2 Minolta CM700d**

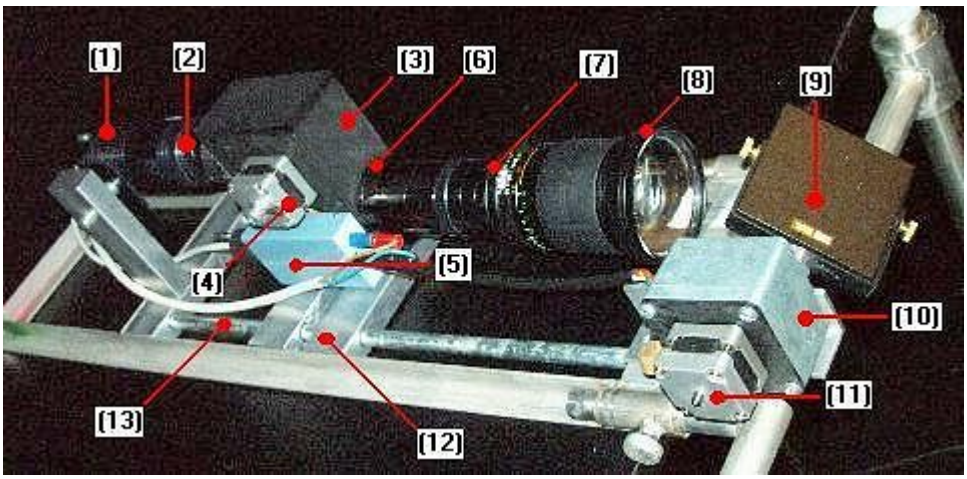
**“L” value** is a measure of the reflectance of the sample. 100% reflectance would indicate a sample that absorbed no light on its surface its L value would be 100 and it would appear white, In the opposite 0 % reflectance would indicate a sample that absorbed all wavelengths of light on its surface its L value would be 0 and it would appear black.

**"a” value** is a measure of the red or green colour component present in the sample. The larger the positive a value the more red is present in the sample. The larger the negative a value the more green is present in the sample.

**“b” value** is a measure of the yellow or blue colour component present in the sample. The larger the positive a value the more yellow is present in the sample. The larger the negative a value the more blue is present in the sample.

***Fishtube***

An instrument called the FishTube has been developed at the Norwegian Institute of Fisheries and Aquaculture (Fiskeriforskning) in Tromsø, Norway which records spectra in reflection, transmission and transflection (diffuse reflection) modes in the visible spectral range approximately 500 to 650 nm (Heia et al.. 2003). Fillets are placed on a diffuse glass plate, over a metal plate and incident light is shone through a pinhole. The whole is covered by a metal housing on which the FishTube is mounted and the light transmitted through the fillet is received and the spectrum collected. The spectral data, analysed by PLS provided a good fit to a predictive model of storage period for both cod and hake.




**(Fig 3) Picture of the assembled Spextube IV Imager at Fiskeriforskning in Tromsø, Norway.** (1) is detector (CCD), (2) camera lens, (3) instrument house containing grating and fixed front surface mirror, (4) grating stepper motor, (5) Stamp II microcomputer, (6) adjustable iris, (7) variable length tube / barrel with collector lens, fixed slit and camera bayonet adapter, (8) field lens, (9) front surface mirror, (10) gear box, (11) stepper motor, (12) aluminium mount bars, and (13) steel rods

***Computer vision, digital cameras and RGB values.***

Computer vision has successfully been applied to many foodstuffs and the simplest version of this equipment is found in digital cameras. Most commercial digital cameras mimic the human colour vision system. Digital cameras scan images with a matrix of hundreds of thousands of microscopic photocells, creating pixels where colour is recorded as brightness values of between 0 to 255 for the primary colours red, green, and blue (RGB) (Villafuerte & Negro,1998).

***Image analysis with the CCD Camera***

A charge coupled device (CCD) camera is an apparatus which is designed to convert optical brightness into electrical amplitude signals using arrays of semiconductor gates formed on a substrate of an integrated circuit or chip. The gates of the CCD are operative to individually collect, store and transfer charge. The use of different a number of different wavelengths can transform an image into a spectrum of coherencies which can then be analysed. The SEQUID project (Kent et al.. 2005) (see below) used a CCD camera sensitive in the 400 to 1000nm range using multiple wavelength combinations. They concluded that their results should be repeated using light emitting diodes in the Ultraviolet range 350 to 400 µm.

***Imaging spectroscopy to detect parasites***

Imaging spectroscopy (Heia 2007) has been developed for the detection of nematode and other parasites in white fleshed fish. Fluorescence detection has been used but a common practice is for process inspectors to candle the fillets in visible light on the process line.

## *Research Applications*

Sashimi tuna flesh is judged on both fat and colour by buyers in the market and research in the Aquafin CRC has shown that using a digital camera to measure the RGB ratios provides an index related to quality and price as judged on the market floor (www.seafoodservices.jp). This is an extremely promising technique and is worth exploring in all other instances where flesh colour is

important. Digital cameras are commonplace, portable and inexpensive in comparison with most scientific equipment. Standard operating procedures for measurement are required such as operation in sample box at a set distance from the object, and a constant luminescent light source but this is trial and error and not complicated.

Computer vision of RGB values and CIE Lab values has been adapted for sorting of salmon fillets with performance at least equal to the manual use of the Roche SalmoFan$^{TM}$ commonly used for this purpose thus lending itself to automation (Misimi, Mathiassen and Erikson 2007).

Imaging spectroscopy with the SpexTube IV which uses a rotating mirror in front of the spectrograph to obtain full spatial resolution has been developed for use in detection of nematode parasites in cod fillets (Heia et al.. 2007).

Quatity index methodology has been complemented with Minolta colour determination for the establishment of a quality index scheme for

*Curtin University of Technology - Hannah Williams*

The Seafood CRC has received an application that utilises sensory scale techniques, laboratory instrumentation (HPLC) and tristimulus colour determinations to establish rapid measurement of polyphenol oxidase degradation metabolites and oxidative degradation products in raw and preserved prawns and Western rock lobster. The establishment of a quality scale measurement for these species provides a mechanism for quality improvement through alteration of handling or storage conditions and an assessment of the effectiveness of preservative actions that have implications in domestic and international export acceptability.

Association between sensory assessment instrumental analysis and colour scale determination enables the establishment of an instrumental quality index parameter for these products that is rapid, non destructive and able to be conducted on site with limited staff training and allows refinement of this aspect of sensory assessment.

## *Recommendations*

Colour is a particularly important characteristic for several species of fish and crustacean. Prices for both tunas and salmonids rely on the flesh colour and for crustacean carapace colour can be an indicator of key quality parameters. Australian and international research clearly indicates that monitoring of visible spectrum attributes can provide a means of differentiation of value based product attributes.

Visible spectrum characteristics of reflectance, transmission and imaging remain a cheap almost universally available research tool. As a rapid, portable, non destructive, low cost methodology that is highly adaptable to production line requirements this is a technology that is already proving its worth in industry applications internationally, particularly in inline prawn quality differentiation.

Development of species or production specific models that utilise rapid diagnostic colour values should remain as a co-developed target for any shelflife and product development research.

There is an ongoing need for understanding of international buying standards and the utilisation domestically of these standards and methodology in product quality assessment and segregation prior to export to ensure capture of optimal product value.

QIM species predictive storage model development should include a non-sensory colour measure that complements the visual ratings adopted.

**Investment in research and application in the utilisation of visible spectroscopic instrumentation is highly recommended.**

## Near Infrared Spectroscopy (NIR*)*

NIR has been widely used to measure properties of foodstuffs and commercial instrumentation is well developed (Jørgensen 2000). It measures the complex spectral bonds in components of foods such as those of carbon to hydrogen (C-H) common in fat and protein, oxygen to hydogen (O-H) common in water and carbohydrates and, nitrogen to hydrogen (N-H) common in protein. The resulting spectra are complex and in seafoods dominated by that of water. It is often necessary to transform them and always necessary to use multivariate data analysis to tease out the desired results. Large sets of data for calibration are necessary and the technique can be used in both transmission and in reflectance modes depending on requirements.

NIR is quite versatile as it measures surface properties and can thus be used directly onto a sample without need for extraction or other preparation. In addition, changes in the state of some of the bonds due to other factors, such as presence of salt, mean that the other factors can be estimated indirectly.

NIR technology provides a scanning spectroscopic fingerprint based on the near infrared spectrum and the use of chemometric software enables the establishment of relationships between the reflectance spectrum and reference values derived from traditional reference methodology for key quality indicators of the product of interest.

The establishment of a library of sufficient size and dynamic range of response enables a correlation to be established between the spectrum and reference values that provides a quantitation method.

Traditional laboratory based NIR have been utilised for feed quality and nutritional value quantification for many years. NIR have also been utilised in product quality assessments with direct calibrations developed for sensory and quality index physical and chemical attributes in many food products, and is well established in the aquaculture feed sector.

Advantages are it is a rapid non destructive methodology with low operational costs once calibrations are established.

Disadvantages are need to establish a calibration database, the database is quite specific for a particular matrix database and establishment costs are high relative to instrument cost.

## *Rapid Diagnostic Techniques*

### *Hand held NIR*

Modular optimal subsystems developer Polychromix has released what it claims is the first low cost hand held, data analysing all-in-one spectroscopic unit.

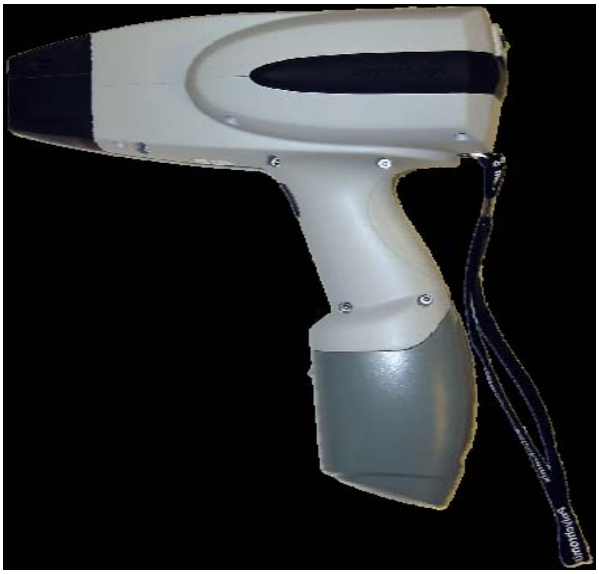
The handheld Near Infrared Digital Transform Spectrometer (DTS) analyzer, called Phazir (Fig 4 ), can be used to collect application specific spectra in field to support chemometric model development, thus offering applications from ingredient and feed value database compilation, material identification, quantitative analysis, purity analysis, quality control and material inspection.

The Phazir combines a Digital Transform Spectrometer engine, its MobiLight lightsource, reflectance probe, rechargeable batteries, integrated computer and LCD display and software into one unit that can be used remotely, such as in field applications.

Polychromix's DTST technology uses an innovative MEMS spatial light modulator in a portable form factor, featuring a single InGaAs detector and no moving parts. The device features low power consumption, utilising a standard USB connection for both communications and power interfaces and comes fully integrated with software that can be configured to specific applications.

The DTST devices are powered by Polychromix's telecommunication MEMS technology and are currently targeted at general purpose spectroscopy applications such as feed quality determinations that can incorporate database development for true protein and oil content in aquaculture feeds and lysine availability if calibrations are performed**.**

**(Fig 4 ) Phazir handheld NIR**



### *Research Applications*

NIR has been applied to measuring fat, protein and dry matter (Jørgensen 2000), quality measures in cooked shrimp (Brodersen and Bremner 2001), 'freshness' estimation (Nilsen 2001 & Nilsen et al.. 2002), sensory quality (Warm, Martens and Nielsen 2001), frozen minced hake (Pink, Naczk and Pink 1999), quality attributes of frozen cod (Bechmann and Jørgensen 1998) for fat and moisture in Atlantic Salmon (Wold and Isaksen 1997), separating fresh from frozen fish (Uddin et al.. 2005), Iodine and saponification values in fish oils (Endo, Endo & Kimura 2005), fat content in frozen skipjack (Shimamoto et al.. 2003), chloride in cured salmon (Huang et al.. 2003), spoilage of Rainbow Trout (Lin et al.. 2006), fat in frozen and thawed mackerel (Shimamoto et al.. 2004) and fat in big-eye tuna with a portable instrument (Shimamoto 2003). This is by no means an exhaustive list but it demonstrates a range of applications of NIR to seafood.

Feed ingredient quality measures such as melamine incorporation as a protein substitute, ingredient and nutritional composition analysis and nutritional value factors such as lysine availability are all areas that have demonstrated value from NIR calibration development.
Correlations between sensory, chemical and physical attribute monitoring in seafood products represents the next generation of methodologies that could be tested for NIR correlation. Stress indicators in capture and production have been established for the meat industry and testing of model development for fish glycogen levels that effect flesh pH and therefore shelflife and texture characteristics also have potential.

### *Recommendations*

The low ongoing operational cost and the non invasive and high speed of modern NIR assessment make this methodology attractive. Provision of handheld NIR enables field and aquaculture applications that can complement inline NIR sensor application in a production line environment.

Funding of NIR calibration development should be seen as a long term investment in lowering quality attribute identification costs, frequently reference methodology are prohibitive for industry in a regular assessment. Establishment of licensed NIR calibrations that are updated and maintained by research providores on the production site at negligible cost provide a mechanism for more frequent utilisation and product value addition or quality control.

Drawbacks are that calibration establishment is typically expensive because it requires individual database acquisition in conjunction with reference method calibration over a wide dynamic range that is relatively matrix specific.

**That NIR spectroscopy be applied to product and ingredient control applications and that handheld instruments be explored for field or processing applications**.

## Fluorescence Spectroscopy

Only a few specific compounds in foods fluoresce and those that do generally emit an intense fluorescence. This makes the technique suitable for some specific compounds measured at specific wavelengths of both stimulation and emittance. However the whole spectral profile of a food can be analysed to yield further information as a 'fluorescence landscape' that can be used to mathematically separate different components if emission spectra in the 250-600nm band are scanned at a number of

incident wavelengths (250nm-400nm). Three way multivariate analysis of the results using programs like PARAFAC is then generally done.

Use of florescence stimulation and emmittance instrumentation is typically as a laboratory based diagnostic tool, instrument cost is in the medium price range for benchtop instruments, production line implementation or portable/handheld instrumentation is currently available but limited in application.
The high specificity of fluorescence wavelengths allows advantages over other spectral absorption or transmission techniques in detection limits and signal to noise ratios.

Disadvantages are excitation light source degradation is an issue in maintenance of calibration and application for some cheaper instrumentation. Between instrument agreement is rare so calibrations tend to be instrument specific however use of standard or control material corrections may see between instrument differences lowered but is not routinely available.

The susceptibility of fluorescent molecules to changes in pH and temperature suggest that individual matrix calibrations will be required for each species or application. Sample temperature control has a critical influence over emittance intensity as collision frequency is enhanced as temperatures increase. As many organic species fluoresce contamination is frequently an issue especially in the case of oil disposition from human handling or during production.

### *Research Applications*

Fluorescence has been applied to lipid-protein interactions in mackerel (Saeed et al.. 1999), changes in stored sardines (Auborg et al.. 1998), changes in canned sardine (Auborg and Medina 1997), stability of cod and bovine myosin (Amin Amiza and Owusu Apenten 1992), oxidation in dried fish (Hasegawa, Endo and Fujimoto 1992). Fluorescence has often been used as a technique to indicate oxidation as products of oxidation invariably fluoresce at characteristic emmittance wavelengths.

### *Recommendations*

As a research instrument fluorescence spectroscopy represents a tool that can aid in understanding degradation or shelflife processes however its utilisation as an effective screening tool is still to be demonstrated.

**Recommended as a research tool for specific attribute or process effect elucidation, not recommended for major investment for routine production applications at this stage. Detection limit advantages may present long term applications not currently highlighted.**

## Nuclear Magnetic Resonance (NMR)

This method has been applied to foods in two modes (a) high resolution methods in which compounds containing carbon-13 or phosphorus-31 are found and (b) low resolution mode for protons.

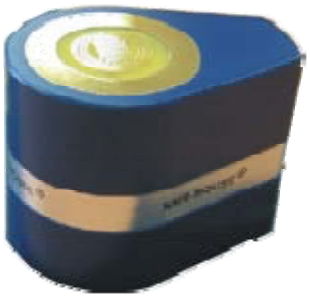
Mode (a) has been used to follow time domain changes of ATP and other metabolites. This is no rapid method and forms the basis of CAT scans. Indeed animal metabolism and fish metabolism have been examined after hours in hospital scanners. There is a vast literature on the use of NMR applied to foods in this high-resolution mode (Hills 1998).

Mode (b) has been explored more recently as bench top stable instruments have been developed. Since foods are comprised mostly of water the proton signal is dominated by that from water and consequently the application has been looked at to examine water structures in fish and meats in an endeavour to relate this to the real practical problem of drip loss and structural change (Jepsen, Pedersen and Engelsen 1999) and simultaneous measurement of both fat and water (Renou et al.. 1987). Although the measurement time is fairly rapid sample preparation and equilibration can be time-consuming as are establishment of calibration correlations.

## *Rapid Diagnostic Instrumentation*

### *Handheld instrumentation*

A small handheld low field NMR device the *M*obile *U*niversal **S**urface **E**xplorer (MOUSE) (Fig 5) has recently been developed that can be applied directly to surfaces (Eidman et al.. 1996). Its advantage is that it is unrestricted by sample geometry but has the disadvantage of a reduction in homogeneity of magnetic field. Although it's use is being explored more in materials science (www. nmr-mouse.de) it has application to foods and has the potential to complement current laboratory based low power NMR benchtop instruments with field transportable application.



**(Fig 5) Mobile Universal Surface Explorer (MOUSE)**

## *Research Capability*

The oilseed industry currently utilises NMR calibrations for the assessment of oil levels and quality in canola rapeseed mustard and other oilseeds.

The Chemistry Centre (WA) and many university participants including University of Queensland have NMR development capability.

NMR spectrometry and imaging is a specialist field and the multivariate analyses required for different applications are constantly being developed. It is not immediately obvious if there are matters of importance in the CRC that need the application of NMR to solve. If some are identified, many universities have magnetic resonance research centres (University of QLD has one that has worked on meats) and cooperative arrangements for research using their expertise can be made. Investigations into drip loss have been discussed as an issue for producers within the CRC due to the loss in value of produce sold by weight over time. Understanding of species or production characteristics that accelerate or minimise drip loss is of immediate and demonstrable research value. Handheld device penetrations of approx 3mm indicate that exploration of surface changes are possible in a field environment however this would need demonstrated application not currently available.

### *Recommendation*

**That NMR spectroscopy techniques be maintained as a research tool with calibration and correlation development supported where seafood applications are demonstrated and that the use of first generation handheld instrumentation be monitored for useful production line or field applications.**

## Time domain reflectometry of microwave dielectric properties (TDR)

The SEQUID project focussed on development of a new instrument to measure the reflectance of incident microwaves Kent et al. (2005). The measurement depends on the structure of water molecules because the centre of mass of the negatively charged electrons are off-centre to the mass of positively charged nuclei. This results in a permanently charged dipole with dielectric properties that change when stimulated with micowaves or other electromagnetic energy. The time taken to return to the original condition is an arbitrary measure of the state of the water and is affected by all matters such as proteins, solutes, salts etc., that are around it. The instrument bombards the sample with pulses of microwave energy in various patterns eg 10 pico-second pulses rising to 50 pico-second pulses in 1 nano-second and measures the time domain for it to recover (relax) from each. A selection of the pulse data is subject to analysis by PLS and by artificial neural networks (ANN) if a database exists for comparison.
Results on chilled stored Salmon, Cod and Herring indicated good agreement with the reference QIM results with equivalent standard errors of prediction around ± 1.7 days varying with species and circumstance.

Good results were achieved with frozen fish in the relationship between predicted and actual storage periods at -10$^{o}$C, -20$^{o}$C and -30$^{o}$C and predicted storage temperature and actual temperature. In addition the results gave reasonable correlations with other measured variables QIM, and the sensory properties of tough, dry, fibrous, age, fishy, unpleasant and cold store flavour.

***Recomendation***
This TDR technology is worth keeping an eye on, but, as for many technologies, it is in the development phase, the range of species and storage conditions investigated to date is small and for the CRC the need for it must be clearly identified.

## Texture Measurements

Hyldig and Nielsen (2007) have recently reviewed texture of fish and shellfish.

The complex structure of seafoods, its' very short fibre length in the muscle in comparison to meat and poultry, the nature of post mortem changes and their relative rapidity has meant that texture measurements are difficult. In addition, most seafoods are cooked and there is no correlation between the texture of cooked flesh and raw flesh (on which most estimations are made in the field). This is because in the raw state it is the collagen of the connective tissue that is tough and the muscle fibres themselves are soft. Upon cooking this situation is reversed, collagen softens and fibres become firm. Further, human estimates of texture are made by eating hot to warm samples but this is impractical for

instrumental measures. The compromise has been to cook the samples, cool them, then take texture measurements.

The other complicating factor is that the structure and integrity of raw fish flesh is not merely due to the major fibrillar components and the connective tissue structure but to very minor, but critical, components of the cell structure - the cytoskeleton. These can be equated to nuts and bolts in an engineering structure, which represent only a small amount of the whole but, which interlock the major components such that if they are removed, collapse results. These cytoskeletal elements degrade post mortem, some within hours, others over a few days. They are also denatured when flesh is cooked, as they are mainly proteins, but their contribution to texture e.g., force required to shear or deform a sample, is unknown. They may act as anything from an adhesive to a point of weakness.

Since the presence, location and function of the spectrum of cytoskeletal elements in most live fish flesh has never been established (some have, Bremner 1999) and only the stability of a few have been investigated in post-mortem fish, and since cooked flesh has never been examined for them (indeed most techniques would no longer work on cooked material) their role, if any, is a total unknown.

Consequently, many empirical methods have arisen whose results cannot be analysed or related to specific structures; the exception being for surimi where torsion tests on specifically cast gel structures have been developed. Thus no real advances in measurement techniques have been made – for all the above reasons and the common method is to use a shear cell in which blades are pulled through or pushed through a sample at constant speed by a machine e.g., an Instron or a TA- TXT2 Texturometer. Breakpoints and force deformation curves result which can be used to differentiate between treatments in experiments. Various shaped blades or plungers have been used, but often on an empirical basis. The results are not easy to interpret for their meaning in structural and diagnostic terms or in physics terms to develop useful equations.

The simplest form of penetrometer is the ‘calibrated finger’ in which the resistance of samples, generally whole, is estimated by prodding it and by noting the speed with which the deformation is corrected. This parameter is used in the QIM system.

Penetrometers are used to give a measure of hardness or firmness of a sample, they typically comprise the measurement of two forces, shear and compression which will vary dependant on the probe type, temperature, angle, penetration time, and type of material. The measurement can be of depth of penetration with a constant load (desk mounted needle penetrometer) or constant depth penetration measuring the force required (hand held penetrometer).

Firmness and texture form intrinsic components to mouthfeel and the sensory experience but subtle changes induced during post harvest handling and storage effect these qualities and influence product value, Storage condition that induce cell rupture or gaping accelerate enzymic decomposition and allow external surface bacteria access to internal fish flesh, Physical changes to tissue strength may compromise the ease of fillet production, unsightly gaping in fillets induced by poor freezer application reduce product value.

Texture and penetrometer measures allow differentiation between storage conditions, variations in freezer types and species differences combined with lifecycle and environmental influences on whole

fish and fillet freezing characteristics make this measure a useful parameter when establishing a storage profile for a particular species or product.

Mechanical penetrometers such as single pin punches have been used successfully to map the texture of cooked abalone as this has a more uniform structure and a greater range of resistance which provides finer differentiation between samples. The ball penetrometer is used widely in Japan to give empirical results of surimi texture, but it does not measure known physical properties nor is it not relevant in the context of the Seafood CRC. It is an example of a routine quality control test. There are many other simple empirical tests used in-house for textural changes that occur in frozen and post mortem seafoods. Sensory testing of product on both category and unstructured scales is common and provides an estimate directly related to how the product is used.

### *Recommendation*

That international texture measures be monitored for market compliance measurement and adopted where required.
The diverse range of test beds and sensors utilised suggest that this area needs a more standardised approach or ability to cross validate results to gain across species or between company comparative value.
Individual company investment in texture analysers that complement their sensory assessments may have value for local product control or regulation however their use as a product value estimator in a market place is clouded by cross instrument variability.

No technique can be given a particular recommendation.

## Smell – Volatile Constituent Indicators

Customer smell is a highly defined selection tool established through learned associations, as one of the four senses utilised by customers in the selection of produce, identification of characteristic aromas can also prove beneficial in the production process as these aromas can be early indicators of environmental or process influence on product attributes.
Training and maintenance of trained aroma specialists in a shift environment that may contribute to quality index measures is time consuming and complicated by routine day to day influences on human sensitivities or perceptions.
The human nose is incredibly sensitive to odorous compounds - often detecting concentrations of a few parts per billion. Few analytical instruments are as sensitive even in controlled conditions such as a laboratory however indicator aromas of deleterious or degradation products such as formaldehyde, ethanol, short chain fatty acids, aldehydes and ketones or sulphur containing organic compounds can often be present in concentrations orders of magnitude higher than that detected by the human nose before product quality is affected. These compounds can therefore provide rapid and suitable targets for instrumental quantification.,

### *Seafood CRC research capability*

The Seafood CRC has identified industry partners interested in expanding quality index applications strong capability exists at the Chemistry centre (WA) for primary quantitative analysis of volatile components as well as a diverse range of e-nose array portable sensors and a Inficon portable GCMS. The analytical group has a strong history of identification of food taints or adulterants and shelflife

studies for industry. The team would welcome working with other research providors in elucidating predictive shelflife models.

## *Research Examples*

An example of an exploration of an electronic nose occurs in the EC project FishNose (QLK1-Ct-2002-71304) ran from 2002 to 2005 to develop a sensor to discriminate between different sources and different storage characteristics of smoked Atlantic Salmon. A commercial instrument the Gemini electronic nose (Alpha M.O.S., Toulouse, France) and a sample device made by OTOTEK (Slovenia) was employed. This had a 10mL sample loop, an inlet tube heated to 55$^{o}$C and a 200ml/min pump drawing on a bell shaped sampler 10ml diameter placed over the fish. Purging between samples (held at 5$^{o}$C) was unnecessary. Four sensors were based on tin oxide, one on tungsten oxide and the sixth on a chromium and titanium oxide mixture.

The data from the FishNose was compared with a battery of usual tests including total volatile base, total viable count, lactobacilli count, yeast and mould counts, enetrobacteriaceae counts, Listeria, Salmonella and coliform counts, thiobarbituric acid values (estimate of oxidation), identification of volatiles by gas chromatography and mass spectrometry, colour, sensory analysis and chemical analysis – fat, moisture, protein.
Correlations between analytical results, discriminant analysis between samples (SIMCA), and discriminant regression was used to aid selected of parameters to be measured.
The device responded mainly to the most volatile products of bacterial metabolism rather than on deposited smoke compound (such as guaiacol). Correlations between statistical models based results of Principal Component Analysis (PCA) and Partial Least Squares Regression (PLSR) were developed for newly made and stored samples from a number of suppliers. The global models could not differentiate between the global set of samples from different sources but the local models were more successful with different batches from the same producer. Consequently the technique could not provide an indicator of 'quality' on an overall scale – an aim of the project.
Although the local models could often pick differences between samples stored for 10 and 28 days, experiments on one set of standard material, measured at regular intervals, stored at 3 temperatures were not done and consequently the FishNose was not fully put to the test as a rapid diagnostic technology for use in quality control.
The project is a good example of the amount of technical work which surrounds, and is essential to, experimental trials. It is not a matter of getting an instrument off the shelf and applying it to a variety of circumstances and products.
An edited version of the highlights from this report has recently been published (Ólafsdøttir et al. 2006)

In a separate project, SEQUID, funded as part of the EC 5$^{th}$ framework (project QLK1-2001-01643) the electronic nose could distinguish between spoiled and fresh Baltic cod stored in ice with a calibration coefficient $R^2$ 93.5% and a standard error of prediction of 1.3 days. However, it was unable to detect any reliable differences in several species frozen stored at temperatures of -10$^{o}$, -20$^{o}$ or -30$^{o}$C either between the temperatures or between periods of storage. Unless sensors responsive to changes in volatiles in frozen storage are developed then the E nose is unsuited to frozen product (Kent et al.. 2005).

Haugen and Undeland (2003) used a commercial hybrid gas sensor array to measure volatiles of a batch of herring (*Clupeus harengus*) stored in ice for 15 days and compared results from the sensors

with results of chemical analysis for oxidation products and antioxidants. Eight of the 16 sensors gave results correlated to chemical measures ranging from 0.9 to 0.98. The prediction errors of the sensors were similar to those of the chemical methods indicating that the instrument was at optimum performance under the circumstances.

Chantarachoti et al. (2006) stored Alaska Pink Salmon (*Onchorhynchus gorbuscha*) in both slush ice and at 14$^{o}$C and compared the results from a handheld electronic nose with sensory and microbial counts using stepwise discriminate analysis. The classification rate for the electronic nose results on volatiles from the belly cavity samples held at 14$^{o}$C and 0$^{o}$C was respectively 85% and 92%. This indicates a promising method for following spoilage of these salmon.

Evaluation by electronic nose was included in evaluation of chilled and super-chilled Cod (*Gadus morhua*) fillets (Ólafsdøttir et al. 2007) and higher responses indicating increasing levels of alcohols and aldehydes were noted at the time of sensory rejection.
Sarnoski JP (2007) utilised a cyranose 320 microarray instrument, draeger tube and SPME GCMS in combination with Instrumental Methods for Determining Quality of Blue Crab (Callinectes sapidus) Meat.

Potential and suggested uses in Australia are for

1. Product quality taints - Measuring the algal taints in fresh and brackish water fish due to compounds such as methyl iso borneol and geosmine. These occur in freshwater aquaculture of barramundi, rainbow trout and silver perch. These compounds are detected by humans in the part per billion range with bacterial decomposition or alteration effecting the ability to quantitate in remote laboratories. Development of handheld onsite monitoring is preferable and is more likely with use of the new pre-concentration adaptation technology.

2. Tracking changes related to shelflife such as ethanol, formaldehyde production, biogenic amines, amino acid degradation products. The references above from the European work indicate that this is not straightforward and careful consideration of species, storage conditions and instrumentation are needed, correlations between sensory assessments are suggested. Other water quality contaminates - petroleum products, volatile organic compound pollutants

3. Feed quality, baits and attractants – monitoring rancidity, development of short chain fatty acids, and oxidative and enzymatic monitoring, identification of preferential feeding compounds and attractants in baits and feeds and monitoring of levels or stability attributes of these compounds.

## *Rapid Diagnostic Techniques*

### *Draeger-Tubes*

Volatile amines are a traditional indicator of spoilage in seafood, total volatile base (TVB) analysis is often used to determine spoilage. For this reason it is also important to have a rapid test for detecting volatile amines. In that regard, Draeger-Tubes®, which have been mostly used for the detection of ammonia gas leaks, may work as a rapid analytical test to determine spoilage. These tubes can be used to detect low levels of ammonia, in the ppm range. These tubes also show response to other amines, not only ammonia. The tubes have cross sensitivities with other basic substances such as

organic amines that are likewise indicated, but have differing sensitivities. Sarnoski JP (2007) demonstrated that draeger tubes showed considerable promise in their ability to predict shelflife for

***Electronic Nose Instrumentation***

The artificial, or electronic, nose is not a nasal prosthesis but an instrument that combines output from an array of physical sensors, made either of metal oxides e.g., of tin, chromium, tungsten, titanium singly or in admixtures oxide, or of plastic polymers, or quartz crystal micro-balances that change in electrical resistance when volatile constituents are passed over them. In many models the gas headspace over a product held in a sample chamber passes over the sensors which are allowed to stabilise. The sensors were originally developed for inorganic gases but they respond to organic volatiles as well and a variety of applications are being tried out (http://www.aaai.org/AITopics/html/nose.html).

Several instruments are available commercially and a first step in development of a new application is to establish which sensors are useful in the array to detect specific compounds or classes of compounds present. Additionally, a reliable method for sample gas headspace including gas volume, duration of sampling and sampling temperature must be established. All these factors depend on the nature of the material and what the results are to be used for.

**Table 1. Summary of Commonly used Electronic Nose Sensors (Sarnoski JP 2007)**

| Active material | Sensor type | Measure | Advantages | Disadvantages |
|---|---|---|---|---|
| Conducting polymer | Chemoresistor | conductivity | Operate at ambient, inexpensive, diverse range of coatings | Sensitive to temperature and humidity |
| Metal Oxide | Chemoresistor | conductivity | Fast response and recovery time, inexpensive | High operating temperatures, sulfur poisoning problem, limited range of coatings |
| Lithium niobate, polymeric, liquid crystal, lipid layer, etc. | surface acoustic wave device (SAW) | piezoelectricity | Diverse range of coatings, high sensitivity, good response times | Complex surface circuitry |
| Quartz crystal with membrane coating (usually a type of polymer) | quartz crystal microbalance (QCM) | piezoelectricity | Diverse range of coatings, good reproducibility | Poor signal to nose ratio, complex circuitry |
| Catalytic gate (usually a catalytic metal) | MOSFET | threshold voltage change | Small, inexpensive sensors | Baseline drift, need controlled environment |

***Cyranose 320***

**(Fig 5) Cyranose 320 with EDU 3 preconcentrator from airsence analytical**

Using technology licensed from Caltech, Cyrano Sciences has developed a portable electronic nose, It operates by imprinting a "smellprint" on its 32-sensor NoseChip, the Cyranose 320 (fig 5) can be used for monitoring of odours specific for product quality.
The human nose is incredibly sensitive to odorous compounds - often detecting concentrations of a few parts per billion. Few analytical instruments are as sensitive even in controlled conditions such as a laboratory, Cyranose 320 is not as sensitive as the human nose frequently however useful indicator limits may be several magnitudes higher than this. Where this is not the case reconcentration may be attempted, in order to detect odours at low concentrations, an odorous sample needs to be pre-concentrated before it is sampled by the e-nose. The preconcentration system indicated is the EDU 3 by Airsense Analytics. This system was chosen as it is small, light weight and can be powered by a battery.

This technology was suggested as it may be used in a variety of harsh environments, the device is provided with a rubber boot to protect it from falls or liquids. The removable snout is long and tapered, with an adjustable tip for sampling in difficult-to-reach places. The snout is locked on with a quarter turn, for easy replacement. A complex pneumatic manifold routes the air past an array of devices, including bellows, filter, sampler, etc. The backlit LCD screen has large easy to read characters, and the buttons are large for use with gloved hands. It fits comfortably in the hand, with a finger grip recess on the back, and a button configuration that enables one-handed use. Complex software features and on-screen information are simplified and organized to facilitate quick navigation.

Previously, electronic noses were large, stationary, expensive machines that required significant training. The Cyranose 320 is a portable handheld e-nose that allows the user to characterise odours on-site anywhere.

Cyranose 320 may be used where the dynamic range of characteristic value measures influenced by volatile constituents are already mapped. The use of an instrument such as this may alow greater resolution precision within the range and provide improvement in reliability and accuracy over sensory applications.

***Inficon Hapsite Portable GCMS***

Portable GCMS technology is based on the principle of quadrupole GC/MS for compound identification and quantitation. The sample components are separated by a GC column and passed into the mass spectrometer (MS) through a membrane interface. The interface between the GC and the MS is a 70% dimethyl silicone/30% polycarbonate membrane that provides the permeability for volatile organic compounds to the MS, but excludes inorganic constituents, such as nitrogen carrier gas, from the MS. As each compound emerges from the GC column, it passes through the selected membrane into the MS where the sample is fragmented by high-energy electron impact ionization. The mass fragments are then detected through quadruple filter. Compound identifications may be achieved by matching ion spectra in the National Institute of Standards and Technology (NIST) library. The HAPSITE is capable of measuring volatile organic compounds with molecular weight typically 45 to 300 amu, boiling point approximately from -50ºC to +180ºC. The internal standard gas is used as mass calibrator for compound identification and quantitation.

California EPA's Department of Toxic Substances Control (DTSC) has certified the analytical capabilities of the HAPSITE portable gas chromatograph-mass spectrometer (GC-MS) system as a field-based analytical method as well as a laboratory instrument for measuring volatile organic compounds (VOCs)

## *Recommendations*

Rapid and low cost Draeger tube analysis of volatile amines shows some potential for examination of shelflife, the relatively non discriminating nature of the tubes actually provides an interesting concept for measurement of organic compound breakdown products however application is limited to one study in Australia at this stage.

The approach using the electronic nose arrays in seafood science shows great promise, but its strength in being non-specific is also its weakness as a diagnostic tool alternative portable rapid diagnostic methodology such as portable GCMS available at the Chemistry Centre (WA) that allows identification and direct quantification is significantly more expensive but allows onsite cross comparisons and secondary status calibration to be achieved where required.

It is acknowledged that the factors that affect volatile characteristics are many and are not likely to be formed linearly with storage period or equally at comparable rates especially when environmental or product diversity is introduced. It remains however a potential tool for the tightening and maintaining of trained panel sensory analysis of smell within Quality index methodology applications.

Establishment of working databases that allow predictive diagnosis is expensive due to the need for primary analysis calibration such as sensory or GCMS calibration however once established operation is rapid, cheap, simple and functional. Maintenance of calibrations can be achieved through licence arrangements and periodic update.

Specific uses have not been defined yet for most circumstances and certainly not in Australian contexts. The development of specific applications to particular products and circumstances is not simple and straightforward and requires careful planning and control over trials.

Equating the E nose to, and discussing it as, an instrument that can measure global concepts like ‘quality’ and ‘freshness’ confuses the issue. Its role is likely to be one of quality control in defined circumstances and any wider role will need to wait for corroborative data development however its speed of application and relatively low cost suggest that incorporation in model development is warranted.

Its use should be considered as a serious technique that may be of value in the CRC if it provides results more rapidly, cheaply and reliably, that otherwise are not obtainable by other means, or that are a new requirement for some purpose.

Availability of portable GCMS within the CRC allows onsite rapid examination, identification and quantification of taints and degradation characteristic volatile constituents strengthening enose and sensory capabilities.

Onsite application removes time delay decomposition questions in analysis from an elucidation model therefore this instrumentation should be incorporated into research projects where production impacts on shelflife estimations are an outcome.

**PRODUCTION MONITORING**

## *Traceability*

Traceability including barcoding, radio frequency identification (RFID) and mixed tags have been covered in a parallel review, Review of Traceability and Product Sensor Technologies, available from the Seafood CRC Secretariat.
Use of combined RFID and electronic sensor interactions with food provide real time monitoring of seafood environment that can significantly improve prediction model effectiveness.

Some additional information is given here.

## *Recent Demonstrations in Australia*

Example 1 (1)
A two-month Australian pilot of the use of radio frequency identification (RFID) tags where they were attached to pallets was managed by GS1 Australia a branch of the international standards-setting organization GS1, in a consortium including RMIT University in Melbourne and Telstra

During the pilot, which ran from March to May 2007, tags were accurately read 100 percent of the time, demonstrating successful electronic proof of deliveries (ePODs), the program provided proof the technology can raise productivity and efficiency in the supply chain.

The National EPC Network Demonstrator Project (NDP) Extension was managed by GS1 Australia, with RMIT University in Melbourne as a partner and included other consortium members such as Chep, an equipment pooling firm, who provided the 3,300 wooden pallets that were fitted with Impinj-supplied electronic product code (EPC) Gen 2 RFID tags, Telstra who provided the adaptive asset management (AAM) software that was used to manage and share the RFID information.

During the pilot, participants were able to check the results online, while data was sent to handheld personal digital assistants (PDA) used by Chep's truck drivers, the tags were read by the drivers, when they picked up an order, which could be accessed by their PDA. The loads on the pallets were then delivered to participating companies, including Franklins supermarket and Masterfoods.

As each tag passed a fixed RFID reader, the information was sent to the AAM, which was then relayed using GPRS back to the drivers' PDA.

The return process of retrieving empty pallet was also tested during the pilot. Logistics provider, Westgate, collected the empty wooden pallets from Franklins, and read the tags at its facility before delivering them back to Chep.

Some customers in the pilot reported productivity gains of 14.3 and 22.2 per cent due to reduced process times, and by the use ePODs rather than paper-based processes, while Chep estimated productivity gains of 28 per cent for the entire delivery process.

Reference
1 Reynolds, G (2007) Australian RFID pilot achieves 100 per cent read rate. *AP-Food technology (Nov 2007)*
*:*

**Software supplier not noted in previous review**
Example 2
InSync Software (www.Insyncinfo.com) has its Edgeware 3.2 software that forms an application platform that leverages RFID and sensor technologies, including GPS, barcode and environmental measurement devices. It deals with all aspects of automated shipping and receiving.

## *Advanced nanotechnology sensors*

The EC GoodFood project has explored the possibility of using gas sensing metal oxide sensors (MOX) embedded with a battery powered RFID tag so that gas atmospheres or volatile metabolites can be monitored simultaneously with identity (Zampolli et al. 2005 on the GoodFood site www.goodfood-project.org)). The layout of the tag is shown below.

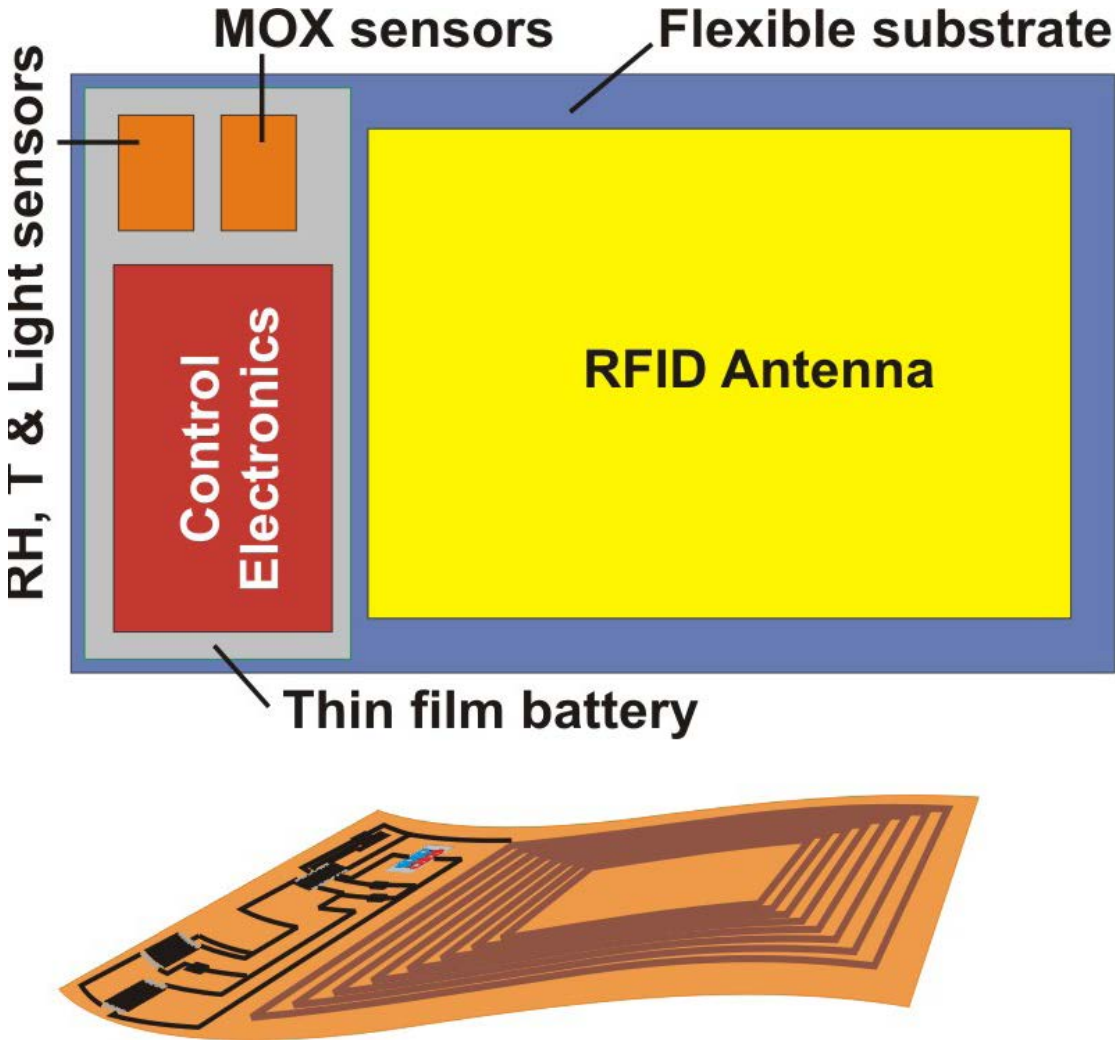



***Fig Layout of the Flexible Tag Microlab (FTM) from www.goodfood.org***

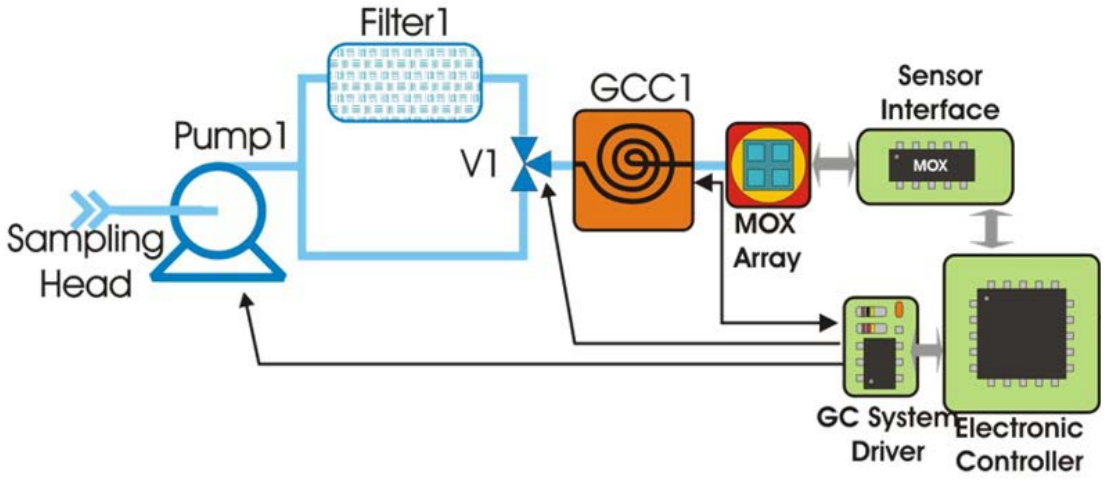
The MOX sensors are ultra-low power hotplates developed from the type used in E nose devices. They are arrays of circular and rectangular suspended
Si3N4/SiO2 hotplates smaller than 100 μm with geometrically optimized Pt heaters and electrodes and new technologies to incorporate the MOX sensors and to build the complex chip had to be developed.

This system is probably indicative of how things will proceed in this field as the ability to build low power chips and nano gas sensors improves. However, cost would prohibit their use to valuable products for some time.

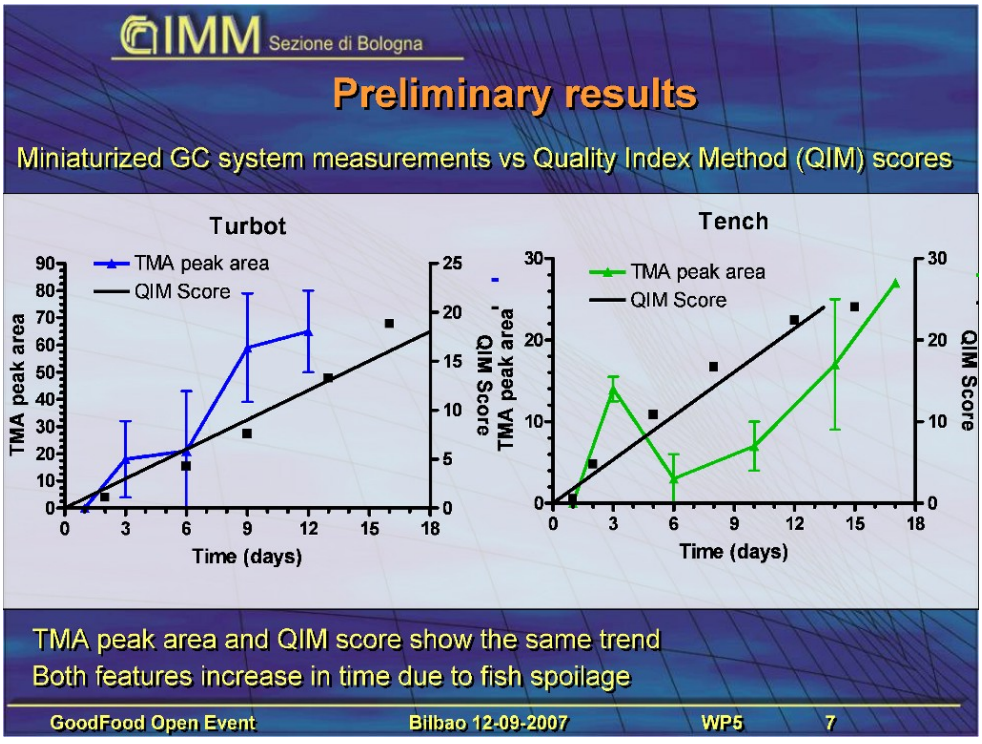
In another development from GoodFood the selectivity of the gas chromatograph columns coupled with E nose as sensors using micro-machined components has been designed into a mini –detector system that is said to be capable of analysing TMA and DMA down to levels of 1ppm (presumably in a headspace). This represents a novel combination of sensitivity, selectivity and miniaturisation. It

may suffer from the usual restrictions of interpretation of TMA but would revolutionise measurement of DMA.

The layout is shown in the figure taken from Elmi et al. 2006 (www.goodfood-project.org)




Trials on at least three species of fish Cod, Tench and Turbot indicate the use of the prototype instrument and a reasonable relationship of TMA levels with QIM resulted. The figure below is taken from the presentation of the experiment which is available on the website http://www.bo.imm.cnr.it/~zampolli/GoodFood/




.

Other units in which the gas sample is split and passed through two columns, one with a MOX array and one with a cantilever array have also been developed. Microcantilever arrays are MEMS (micro-

electromechanical systems) structures with the beam surface coated with chemically sensitive films, often porphyrin derivatives.. When a gas is absorbed on the film the bend in the cantilever is measured by integrated piezo-electric resistors. The micro-machining, design and microcantilever surfaces etched to a thickness of about 10µm is a feat of nano-engineering!

Contact has been made with the developers as to the state of progress in these devices. As yet they are prototypes but their website shows a variety of applications ranging from explosives, olive oil, tainted corks, milk, coffee through to detection of metabolite volatiles in human breath resulting from infection of the ulcer-causing bacteria *Helicobacter pylorae* .These applications appear to be the way of the future.

If these approaches become standard and columns and films can be developed or coated with suitably specific receptors then analysis for specific volatiles will be revolutionised.

## *Indicator Packaging*

Utilisation of indicator packaging is growing, internationally incorporation of identifier compounds that react visually to detrimental compound or spoilage indicator compounds activity or environmental measures are of interest and provide rapid easily identifiable mechanisms for the monitoring of seafood quality once quality attribute modelling is completed.

A wide range of temperature indicating packaging or adhesive labelling is available to the market, the products typically monitor temperature and time exposure and must be modelled to individual product characteristics to provide a mechanism for product management.

Active packaging provides a simple, visual reassurance of quality at the marketplace and to customers it can provide a point of differentiation between competitive products.

A range of products are available including

***Industrial product***

3M™ MonitorMark™ Time Temperature Indicators provide an affordable solution for monitoring products that are sensitive to time temperature abuse, they provide an easy-to-read signal when threshold temperature has been exceeded.

Features:
Easy-to-read blue irreversible signal estimates the time the threshold temperature was exceeded
A pressure-sensitive adhesive backing allows convenient attachment to most clean, dry surfaces
Primarily used within secondary packaging such as the case or box.
Sold in quantities of 100 indicator per box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# PART D - PREDICTIVE MODELLING AS A TOOL TO IMPROVE SEAFOOD QUALITY AND LOWER MONITORING COST.

## MODEL DEVELOPMENT

Properly functioning HACCP systems ensure product safety but do not necessarily deal with all matters that are considered as quality. The main inherent quality attributes of post-mortem seafoods are conserved by maintaining low temperature and expediting handling, processing and distribution to minimise the total time-temperature profile of the product. Appropriate handling and general work protocols that ensure that the product is not physically damaged are also necessary. Organisation and selection of capture methods to ensure minimum damage to the catch e.g., shorter trawl times consistent with good catches, correct gear selection, frequent checking of drop lines or traps are measures to decrease catch damage. In aquaculture, minimisation of harvest stress and humane slaughter conserve the inherent flesh properties and provide more processing opportunities to place top grade product on the market.
The usefulness of a diagnostic result depends on the overall model in which it is to be applied. Some results are simply pass/fail based on presence or absolute absence of a compound. Chemical residues or toxic compounds tend to be in this category. Other models desire to predictive outcomes that require extensive historical analysis and multiple variable monitoring for successful outcomes.

### SINGLE COMPOUND QUALITY INDICATORS (SCQI)

These relate 'quality' to a single attribute generally some metabolite such as TMA, a property such as saline extractable protein, or a chemical index such as k-value the value of these indices is variable and frequently differs between species and can be confounded by environmental or other exogenous effects.
Use of single compound quality indicators is highly valuable where critical safety attributes are directly related to specific compound exposure, these often feature in export certification and food safety guidelines with maximium permissible levels (MPLs) or zero tolerance characterisation.

### MULTIPLE COMPOUND QUALITY INDICES (MCQI)

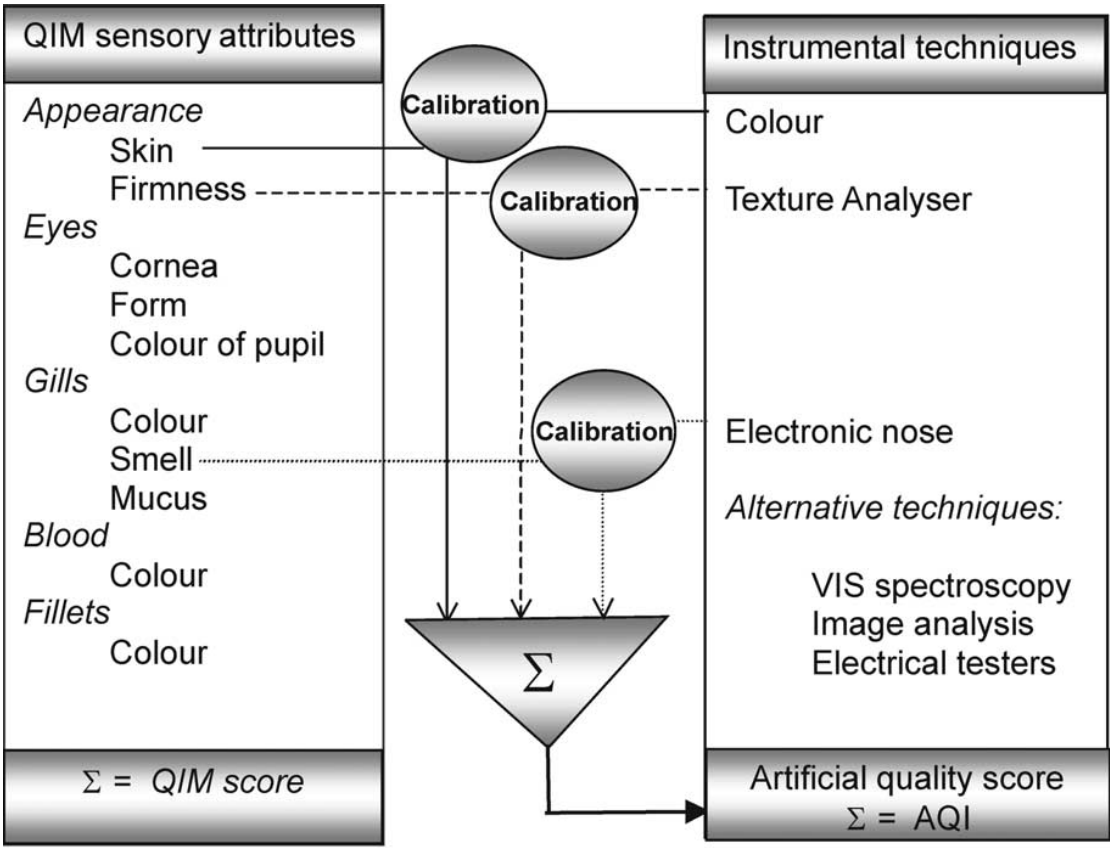
Jørgensen and Dalgaard (2000) examined bacterial relationships and metabolites in smoked salmon and used the results from multivariate regression analysis and partial least squares regression to reduce the number of variables from 44 to 4 that corresponded with sensory scores and shelf-life. The called this the multiple chemical quality index (MCQI) and equated it to pH, and the levels of tyramine, cadaverine, putrescine and histamine

### ARTIFICIAL QUALITY INDEX (AQI)

The MUSTEC project 'Development of multisensor techniques for monitoring the quality of fish '(EC FAIR grant CT98-4076) (Ólafsdøttir et al. 2004) brought together a working group from seven countries that applied a variety of techniques to the same 3 batches of cod in the same laboratory. They related instrumental measurements to specific attribute measurements in the QIM. The results from the electronic nose (LibraNose , http://www.technobiochip.com/www_en/a2nasoBiol.asp), texture analysis on the TA.XT2i instrument and colour measurements using the colourmeter Spectro-

pen - a grating colorimeter that gives results in the CIE colour space were combined to form the AQI. The instrumental measures were then calibrated against the relevant QIM parameters of colour, texture and smell by PLSR and the values for each storage period were combined to give the AQI (Figure ). These AQI values were plotted along with the QIM versus storage period. Although the slopes were different both plots were linear but the correlation coefficients were the same (0.96).

## *Diagnostic Methodology*




The appropriate measures from the QIM results for skin colour, firmness and smell were combined to form a value called a subQIM. Although the subQIM had a much lower slope (naturally because it is only the sum 3 measures) it had a similar correlation to that of the total QIM.

The instrumental measures tended to overestimate the sensory values in the early storage period for each attribute and this is reflected in the AQI and the overall slope is about half that for QIM. This implies that the instrumental responses are less sensitive and less likely to show small changes during the storage period or between samples of different storage age.

However, the approach has shown that a possibility exists to use trained sensory panels to re-calibrate, or re-design instrument responses to obtain results closer to those provided by the senses. This would allow the use of instruments in the field where sensory panels were not available.

## Quality Index Methodology

Quality index methodology provides a framework for the measure of attributes of a product at a particular time in its post harvest process using sensory characteristic measures. The establishment of key sensory criteria and the establishment of scale measures that enable a total score to be established can be used to determine what stage a product is presenting during its post harvest degradation process. Monitoring of these scores during storage, transport or processing allows decisions on remaining shelf life and value of a seafood product to be made with confidence against standard operating conditions.

Establishment of characteristics that accelerate degradation process individually or synergistically can be elucidated from frequent assessments association with monitoring of influential environmental characteristics surrounding the product can establish key drivers that effect shelf life, once historical consistency in product performance is established under standard conditions.

A Quality Index Scheme developed from quality index monitoring methodology is a quick, easy to use and consistent method for assessing qualities of seafood throughout the entire supply chain.

The sophistication of a quality index scheme and its applicability can be varied according to the needs of the producer and can be upgraded with measures that improve the ruggedness, precision of measure and accuracy in prediction according to the outcomes required.

Utilisation of quality index schemes currently relies on trained sensory assessment and reasonable consistency in source product characteristics. Widening in application will occur as data base accumulation of response variability is tracked.

Introduction of instrumental or rapid diagnostic tests such as initial microbiological load or other key chemical attribute measures can widen the applicability of sensory test panel or trained technical operator QIM sensory assessment. Other rapid diagnostic tests can reset key predictive model characteristics based on a QIM developed degradation pathway model. This can improve the speed and accuracy of identification of production problems and aid in establishing consistency in prediction for any product.

Use of other complementary rapid diagnostic tests or instrument references for sensory assessments can be particularly effective in initial sensory training, and can improve management speed and flexibility in inline product handling decisions and result in the establishment of real time decision making. This combined with increased precision in monitoring of key attributes in a product suggest that instrumentation and non sensory assessment has a place in Quality index methodology and should be considered when constructing a quality index predictive scheme.

The need for such quality control systems is critical as the industry moves towards remote selling, electronic marketing and trading, and increased exports to discriminating markets.

## *Diagnostic Methodology*

The QIM evaluation is made by rapid visual and sensory inspection with scoring of a sufficient number of common major characteristics on simple 0 to 3 point scales then totalling the scores to provide an overall measure score that may be utilised in and index evaluation. Index establishment is represented as a linear relationship against days of storage of the fish at $0^{o}$C, this is conducted under representative physical or environmental conditions and where impacts in this relationship such as handling are standardised and constant. An evaluation under these circumstances can provide information on the current state of a fish and with the establishment of a normal index and understanding of time elapsed since capture enable estimates of rate of change comparable to the normal index and therefore remaining shelf-life of the product.

Logically establishment of an index using optimal product attributes and conditions can provide a production benchmark to industry for comparison of initial product state, transport effects or processing procedure effects.

## *Australian Development*

Developed by the Tasmanian Food Research Unit of CSIRO in the early 1980s (Bremner 1985) it was adopted in Denmark, Iceland and Holland by both research groups and industry and rechristened as the Quality Index Method (QIM) it is now the reference method for assessing chilled seafoods throughout Europe (www.QIM-Eurofish.com). It is not only used in electronic auctions and by buyers seeking high quality products, but is also the preferred sensory assessment reference method in all European fish research laboratories and is becoming the approved EC official reference method for seafood quality scores.
Continued Australian development should maintain these international synergies.

As an already established program in Australia for fresh or fresh chilled seafood, groundwork for the project commenced in 2001, with initial funding from Seafood Services Australia sourced through the Seafood Industry Development Fund. In September 2002, a workshop was held in Hobart to discuss the development of a quality index for Australian seafood.
The working group (comprised of a wide range of seafood stakeholders from industry, service providers, Seafood Services Australia and government organisations) appointed a steering committee, outlined a strategic plan, developed an action plan, identified key stakeholders, listed key contacts and considered potential funding sources.
The agreed overall objective was to develop a method for all major Australian seafood and have a quality measure that has wide market acceptance within both domestic and export markets by 2010.

The Quality Index method measure would be in a structure that is readily understood by industry members and will hopefully gain wide acceptance and give advantages to industry in meeting consumer demands through:

- Grading;
- Shelf life prediction;
- Improving buyer certainty
- Supply chain management;

- Conflict resolution; and
- Education and training.

The Australian QI project, funded by FRDC began in July 2003 and the QI schemes for 6 species have been developed. Food Science Australia, based in Victoria, developed QI schemes for snapper and silver warehou. Cooked tiger prawns, gold band snapper and Spanish mackerel schemes were developed by the Centre for Food Technology in Brisbane and the Australian Maritime College in Tasmania took the lead role for their farmed Atlantic salmon scheme.

The QIM Manual has now been published, with the six schemes included. As part of the FRDC funding, training in the use of the scheme was developed and piloted at several industry locations.

FRDC funding has now been allocated for the development of QI schemes for 8 other species. The target species are barramundi, yellowtail kingfish, eastern king prawns, Spanish mackerel, silver warehou, pink ling, Australian sardines, and Saddlerail snapper, and the work is being undertaken in Queensland and Tasmania. As well, A QI scheme for Blue spot emperor was developed at Curtin University, Perth. In addition, a WA FRDC supply chain project will result in the development of schemes for blue mussels, blue swimmer crabs and Australian salmon. All these additional QI schemes will eventually be incorporated into the manual. Just to note that at present, copies of the manual and relevant training around the implementation of QI for interested businesses incurs a cost.

## *Future Directions*

Further work is planned through the Seafood CRC, it is planned to develop the scoring schemes onto handheld PDAs so the results can be electronically stored and transmitted.
Predictive model development requires constant appraisal for functionality, standardisation of predictive model characteristics can allow across species identification of critical drivers of shelflife extension.
Base line establishment can allow benefit analysis of modified practice to be assessed with greater surety.

A suggested list of topics for consideration, some of which are already underway, includes:-

- Ongoing revisions of available technology that may assist or improve the sensory assessment of
    - a particular quality attribute measures that may be market driven or related to at a particular location or within a particular process.
    - the identification of any environmental, microbiological or processing measure that changes the character or rate of degradation of a QI species measure and storage relationship.
- Look at developing methodology to facilitate rapid (cross over) development of QI schemes for similar fish species (eg modification of Atlantic salmon scheme to ocean trout).
- Identification of locational or site specific processing applications where QI assessment can facilitate better handling or quality outcomes.

- Circulation to all CRC participants of information on the current use of QI methods, benefits etc so individual participants may become aware of the method and identify possible benefits to their business/industry.
- From feedback and other consultative processes, identify a list of additional priority species or locations for QI development or utilisation.
- Develop an agreed funding mechanism for QI development of priority species (eg interested businesses may be able to input by providing fish etc).
- Look at training mechanisms eg inclusion of QI training in modules such as Seafood Industry Training Packages.
- CRC adopt a policy where any projects that focus on quality, spoilage or supply chain management of species, must develop and use QI parameters to measure and report variations and outcomes. This should result in the QI scheme being recognised as the endorsed scheme for measuring quality in Australian seafood.
- The approach of QI used to establish rapid and effective sensory quality parameters particularly relating to shelf-life extension, modified atmosphere packaging use of preservatives or antimicrobials or the creation of altered product such as ready to eat portions, could be attempted.
- Consideration be given to particular cases where addition of other non-sensory measures could result in a combined, sensory, chemical and microbiological result could provide additional benefit to current QI methodology efforts.

### *Recommendation*

**That the Sensory Quality Index be the first developed and standard reference method used for species of interest within the CRC.**

**It should be a strong indicator and highly valued tool to evaluate shelf life benefits of dietary, husbandry, harvesting, handling and processing methods.**

**That uniformity in assessment be progressed through consistency in training and continued standardisation in application.**

**That complementary source data, rapid diagnostic and instrumental method information, production environment monitoring and inline production system assessments be incorporated and electronically compiled into overall predictive model development for shelflife and product quality assessments and this information be registered for each production batch or sourced product via RFID or suitable traceabilty technology .**

**That sensory or instrumental analysis utilisation in predictive model development be guided by safety, cost benefit and desired accuracy of outcomes required and considerations to production line implementation and most rapid, least invasive techniques adopted.**

*Time-temperature model and Icedays*

The main changes in temperate water fish all have similar kinetics and are well described by the relationship:-

$r = (1+0.1t)^2$ or,
alternatively in square root form as $\sqrt{r} = 1+0.1t$

where r is the rate of change and t is the temperature in degrees Celsius (Bremner, Olley and Vail 1987).

Effectively this means that the rate of change at 4°C is twice that at 0°C which shows how absolutely critical is temperature control in the chill region.

Any temperature history can be expressed in Icedays – equivalent days in ice. Any known time-temperature history can be re-expressed as Icedays. As example, a fish that has been stored at 6°C for 4 days clocktime has actually been 10.2 Icedays in storage! If the agreed shelf-life for this species is 14 days, then it has 3.8 days left if chilled to 0°C but about 1.4 days if left at 6°C. Thus product with a potential shelf-life of 14 days has been ruined in 5.4 days of neglect.

## *QI and Icedays*

The theory behind the QI schemes is that they are consistent with the square root relationship (above) and hence can be used to estimate Icedays (equivalent days in ice) thus bringing a sensory score to a useful time equivalent. Conversely, a known time-temperature history can be converted to a QI score that can then be confirmed by examining the product. One can be predicted from the other.

## *Environmental modelling*

Environmental management systems (EMS) are out of the scope of this review but have extensively been developed and promoted through Seafood Services Australia (www.seafoodnet.com.au) from whom information is readily available.

## *Bacterial modelling*

### *Introduction*

Use of predictive modelling of microbiological activity or function can prove a powerful tool in the management of risk in harvest, production process, delivery and consumption. Changes in microbiological populations on seafood can be estimated from changes in product parameters such as temperature, storage atmosphere pH salt level, water activity, chemical preservation etc).
Such models have been developed from the initial square root relation to incorporate terms dealing with pH, water activity and the like.
The use of predictive models for microbiology is tempered by the dynamics of the model. Control or prediction is only possible for conditions addressed in the model generated and only if those conditions do not have synergistic effects outside or in collaboration with external unexplored factors.

Predictive microbiology involves the development of mathematical models to describe the effects of the most important environmental factors controlling the response of micro-organisms in food.

Predictive microbiology has immediate practical applications that improve microbial food safety and quality. Model establishment can lead if properly established to physical, chemical and biological understanding of food response and the quantitative understanding of the microbial ecology of foods.

***Example 1***

The University of Tasmania has an international reputation for expertise in the area of modelling of the microbial ecology of foods, a discipline that has been termed '*predictive microbiology'*. Products of this research, primarily in the form or predictive tools and risk assessment, have been used by various businesses and government organisations to enhance access of food products in domestic and international markets.

Examples include the *Refrigeration Index* (RI) and *E. coli Inactivation Model*. The RI underpins food safety management practices for beef products that have high access to USA markets, and resulted in the Australian Quarantine Inspection Service (AQIS) revising the Export Control (Meat and Meat Products) Orders in *AQIS Meat Notice: 2001/19 - Assessment of deterioration of refrigerated meat affected by refrigeration breakdown, incidents and accidents* to require that the RI be used to ensure the safety of chilled meat products (AQIS, 2005). As a result, these tools have been independently assessed to return a net industry benefit of $44 million. The industry benefit-cost ratio from predictive microbiology is $260 million of additional social benefits are projected over 30 years.

The *E. coli Inactivation Model* has supported the development of domestic markets for fermented meat products because it was developed in conjunction with industry and Food Standards Australia New Zealand. MLA funded the UTas group to provide a review and analysis of relevant published literature and unpublished data to identify key process parameters affecting inactivation of *E. coli* during fermentation and maturation of fermented meat products. The analysis of available data combined with data from earlier strategic-basic research by the group provided new insights enabling the development of a predictive model, the utility of which was endorsed by FSANZ and other regulatory bodies, and accepted for use as a tool to judge process safety.
The group's modelling expertise has been applied in other projects related to market access, including the Pork-to-Singapore project, as well as the integration of models with traceability technologies. Trials have included SmartTrace® data loggers coupled with the *E. coli* RI model.

New opportunities exist to extend models to predict other relevant microbiological characteristics. Such models can predict the growth of spoilage bacteria and their impact on product spoilage, shelf-life, drip-loss and other quality attributes.

**Information systems in Food Safety Management**

This is the title of a recent paper by McMeekin et al. (2006) which describes the many uses for the combination of large US and UK databases into the web-based searchable *ComBase* (*Com*bined or *Com*mon i.e., joint data*Base*) by Baranyi and Tamplin (2004) and detailed on the website (www.combase.cc). This merged two major databases the US Pathogen Modelling Program (PMP) and the UK Food MicroModel which represent a colossal gathering of information on the growth and death of bacteria and pooled available predictive microbiology data.

This review paper is so detailed, knowledgeable and of such complexity that it is impractical to précis it for this current review and the reader is referred to the reference for details, the journal for doi:10.1016/j.ijfoodmicro.2006.04.048 or to contact the author (tom.mcmeekin@utas.edu.au.

Nevertheless, the theme lies in the philosophy of linking or combining databases and how rapid electronic transfer of information can speed up diagnosis, identification or prediction. It does this under the headings of;-

- Food borne pathogens: enumeration and identification
- Philosophy underlying databases
- Information systems and microbial systematics
- Information systems and investigation of food-borne diseases
    - Epidemiological surveillance of foodborne diseases
    - Outbreak detection
    - Response to outbreaks
- Predictive microbiology application software
- Quantitative microbial risk assessment software
- Use of decision-support systems in food safety management
    - Decision support systems and expert systems
    - Food safety control
    - Hazard identification
    - Critical control points and chain analysis
    - Quantitative risk assessment
- HACCP software and traceability systems
- RFID technology, standards and traceability
- Conclusions

The paper thus outlines many matters of relevance to seafood

**Seafood Safety and Spoilage Predictor (SSSP)**

The Seafood Safety and Spoilage Predictor (SSSP) is a predictive modelling freeware developed for seafoods by Paw Dalgaard of the microbiology section and the IT section of in the Danish Institute for Fisheries Research and downloadable from http://www.dfu.min.dk/micro/sssp/Home/Home.aspx . Dalgaard is a co-author in the above paper by McMeekin et al. 2006 and the SSSP fits in as Predictive microbiology application software. However, it includes quality, shelf-life and other characteristics as well as safety.

The model is based on numerous investigations published in the literature for fresh fish, fish previously frozen - then chilled, MAP fillets, vacuum packed product, smoked product, temperate, cold and warm water species, and for factors such as water activity, effects of preservatives, $CO_2$ concentration and covers spoilage organisms, specific spoilage organisms, food safety organisms and biogenic amines. It is continually being updated as new information is published and new results come to hand in the world literature (Dalgaard 2000). For some cases results are backed by validation studies in commercial products and challenge studies in laboratories.

It is by far the most useful and comprehensive database for seafood of its kind and is highly recommended as the first stop for work in this area. It is available in 10 different languages and has been used in over 90 countries As most of the work that is incorporated in the SSSP has come from

the Northern Hemisphere and unfortunately, very little investigative work on the microbiology of Australian seafoods has ever been done and there is little option but to use the current models as a guide. The range of specific spoilage organisms in Australian waters and on Australian seafoods and the effects of various conditions (pH, water activity, $CO_2$ concentration etc) has never been systematically investigated.

Any investigative or routine microbiology done within the CRC should be designed with the aim of providing information that can be added to the database. In this way more solid evidence can be built up to provide predictive tools that can be applied directly to product development.

As example using the SSSP the effects of different $CO_2$ concentrations on microbial growth can be modelled for different temperatures of storage and for different starting concentrations of organisms. This obviates having to do much developmental experimentation and means that a narrow range of conditions can be selected for pilot trials.

**Fish shelf-life prediction program (FSLP)**

This is a similar, program to the SSSP developed by the Basque group Azti –Tecnalia and is downloadable from the website www.azti.es in English or Spanish version. It is less comprehensive than SSSP in that it covers fewer factors.

**Frozen product**

There are no good predictive models for frozen seafood products. The reason that SSSP and FSLP work is that they are based on microbial growth and since other reactions like nucleotide changes and bulk properties follow similar kinetics they can reasonably follow changes that can be related to acceptability or an overall concept of ‘quality’.

However, in frozen storage the changes that occur are not dominated by one factor such as bacterial growth and a multiplicity of deteriorative reactions occur that have different rates. There are mixtures of first and second order rates of reaction so that it is possible for one mode of deterioration to become more important than another partway through storage as the second reaches an asymptote. Any result on shelf-life depends entirely on the method and the criteria used.

Two mechanisms that often occur are oxidation and protein denaturation (Hedges 2000). The first leads to off-odours and off-flavours that start as being described as cardboardy, straw-like and nutty and move through to rancid. The second reveals itself in textural changes with progressive toughness, fibrousness and loss of moisture holding capacity such that the cooked flesh ends up like wet cotton wool or blotting paper.

These two mechanisms may proceed at similar rates in some species and circumstances and not in others. A third factor is formation of free fatty acids mainly from phospholipids and these lead from a bland taste through to unpleasant mouth feel and to soapy then bitter flavours. For these and other reasons simple general relationships between storage period and temperature do not occur and either rules of thumb or experience with particular product are applied.

The Time Domain Reflectometry (TDR) of microwaves (see above) may turn out useful for whitefish in which lipid is low and oxidation is not an important factor.

## PART D DISCUSSION

### Preamble

This section is arranged with recommendations in dot point format.

This review was hampered by the fact that the range and depth of the technologies and current methods used by CRC participants and industry partners was not known by the reviewers: nor were their aspirations known. The reviewers recommend that some form of survey to establish this be conducted and that this current review be presented to participants as it may stimulate ideas and questions from which opportunities may arise.

- Present this review to participants and survey spokespeople in person to obtain responses, ideas and survey opportunities.

### General

This review has covered the major approaches to predictive and rapid diagnostic technologies that are used or are in process of development with seafood. Most of the topics could not be done justice even in an individual review of the same length as this so in many instances websites, textbooks and papers have been cited for the interested reader to find more detail.

This present review has not covered rapid microbiological methods themselves individually but has provided an APPENDIX 1 that lists trials conducted by Sue Pool and Steve Slattery (with their kind permission) of rapid diagnostic kits trialed in Australia and has indicated recent directions that this type of analysis has undertaken.

- If required conduct a separate review on rapid microbiological methods to make recommendations on techniques

### *Prediction*

Databases and their predictive uses are well developed in microbiology and it is anticipated this will proceed at an even greater pace as there are now widely recognised structures that give a lead to researchers as to the form to collect data. Data should be gathered with the aim of placing it into the available structured systems.

For seafoods the Seafood Shelf-life and Safety Predictor (SSSP) database is the most versatile and useful. Any microbial work in the CRC should be aimed at adding to this database in order to fill in the obvious gaps that relate to tropical, sub-tropical and temperate species that are found in the Southern Hemisphere. There is an absolute dearth of information on the Specific Spoilage Organisms (SSO) that occur around Australian, North to South, East to West. Specific models cannot be constructed for local use if the relevant information is missing. Until then we must use data on

Northern Species that is dominated by whitefish from the North Sea. Only recently is Spanish, Portuguese and Mediterranean data being made available.

Without knowledge of the SSO there is no target at which to aim the technologies to impede spoilage and to increase shelf-life. Technologies are currently applied blind. Nothing is optimised and it is time to mature from the near enough is good enough approach of the pioneering days.

The adoption of the standard approach using Icedays as the basis for all calculations to do with chilled product can provide a uniform means of comparing different situations.

Similarly, the QI is a *lingua franca* that conveys volumes of information in a number. Just note that it has a solid basis in theory despite its deceptive simplicity. Its' use should be adopted throughout the CRC.

Note as well that the first attempt at developing an artificial QI by summing up instrument responses showed that the response slopes of the three types of instruments used were so much less sensitive than those of the QIM panel. Thus differentiation between samples of different ages was far less sure.

- Seek to obtain results that can be added to the models in SSSP so it covers Australian seafoods with greater certainty
- Plan microbial activities in CoolFish so that SSO are established
- Promote the concept of Icedays through training and information dissemination

***Multivariate statistics***

The use of databases for predictive purposes and of the generation of large datasets by instrumental analyses is an issue in its own right. It is extremely important to note that, particularly for research and exploratory purposes, a range of statistical software and expert advice is generally needed as the programs supplied with most instruments fall short of user needs. In Europe, some Food Science Departments in Universities deliberately have chosen to specialise in chemometric and multivariate analyses as it is the process of manipulating, calculating and modelling and understanding data where the problems, and the intellectual challenges lie. They are equipped to write their own algorithms and to get different programs to communicate. This aspect seems neglected in Australia and individual researchers often have to battle on their own, either with data or, with access to, or acquisition of, appropriate software. They have to make do with inadequate resources. The Program Leaders should make provision for adequate support in multivariate statistics, firstly by having it listed as an item on each project, secondly by taking advice and, thirdly by ensuring support mechanisms are considered e.g., CRC licensed shared softwares,.cooperative arrangements with an expert group.

- Establish mechanisms to encourage use of and ready availability to multivariate methods

***Rapid Technologies***

There are so many rapid technologies being explored that it is difficult to discuss and compare them without specific knowledge of industry and project requirements.

They therefore can be thought of in two categories, those techniques that are ready for use now, but may not yet be standard practice and those that require more development.

Many are still at the research stages while others such as NIR and electronic noses have moved through the development path into second and third generation models so that handheld instruments are now available.

***Ready or near-ready technologies***

*Heavy metal analyses*

Development of speciation techniques for heavy metal analysis enables better qualification of toxicity previously only reflected by total heavy metal analysis. Emergence of 4$^{th}$ generation handheld XRF instrumentation each with lower detection limits indicates this is a technology worth watching as it could be significantly useful in application. Instrument manufacturer negotiations could deliver a prototype next generation device ideal for seafood applications.

*NIR*

In many laboratories NIR has replaced some of the more tedious methods of analysis, sometimes even for the simple fact that several parameters can be estimated on the one sample at the same time with little need for elaborate sample preparation e.g., fat, moisture, protein. Salt levels in brined products could be monitored (indirectly) by NIR.

There are options to use it in process control or QA to measure changes or some characteristics that relate to specific quality attributes. However, it should never be thought of as a quality meter.

- Establish what NIR is used for at present in seafood analysis

*Electronic Nose*

The electronic nose is likely have advantages in response time in processing or in monitoring materials in storage. It has a future in being calibrated to measure specific attributes.

- Liaise with users of electronic noses e.g., FSA Chemistry Centre WA to identify specific areas where it may meet a need

*Cameras, computer vision, colour, imaging*

Colour of many seafoods is important and low cost approaches using RGB values from digital cameras – perhaps even mobile phones- should be looked at.
Industries like the salmon industry in which selection of batches or of individual carcasses or fillets for different ranges of products are required could use colour vision in an automated or semi-automated way.
Imaging for parasites may be attractive to some industry sectors.

- Explore opportunities in which digital cameras and RGB values would be of use in selection and control
- Seek industry sectors that could benefit from imaging to detect parasites

*Histamine*

At least one kit for histamine has been test proven and awareness of it should be kept to the fore.

- Inform CRC participants of the Alert kit for histamine, I would recommend laboratory testing of values close to safety cutoffs

*Lab on a chip technologies*

The Agilent BioAnalyser can lend itself to a large variety of laboratory analyses. When these are routine each analysis is relatively cheap. For the seafood industry it is more a matter of identifying what needs to be analysed.

- Canvas industry and contract analysts for opportunities to increase efficiencies with Lab-on-a-chip technologies

*Realtime PCR for Hepatitis A and Norovirus*

These techniques have been developed in the SEAFOODplus project and will be available soon.

- Monitor progress of real time PCR techniques for hepatitis and noroviruses being developed in SEAFOODplus and inform participants and analysts.

**Techniques less ready and those that should be watched**

*Nanotechnologies*

*A) Coupled with RFID*

None of the proposed technologies to be coupled with RFID are yet of interest as the metabolite TMA is itself a poor indicator of spoilage.

Those technologies that seek to couple RFID with temperature measurement do not appear to offer any advantage over the local Ceebron Smart Trace technology. It has the advantage of being local and has now the experience of several trial shipments.

*B)Specific sensors*

The GoodFood Project has several great ideas in it. The researchers work with the developers of LibraNose and have the capacity to develop new gadgetry. The idea of having a specific GC column attached to a headspace sampler and an E nose as a sensor is a brilliant combination of technologies. The prototype is too large yet but the concept is great.

More specific sensors to volatiles

*NMR and NMR Mouse*

These are probably still research tools although NMR has been applied to foods for over 20 years.

*Time Domain Reflectometry*

Physicists have been trying to develop instruments to measure fish properties for thirty years. None have gained acceptance in industry and this latest attempt using a new principle may have some success with frozen material. Much more work is needed.

*Fish Tube and similar devices*

These devices are in exploratory stage and any further progress should be monitored to see if it has use in the CRC.

***Overall recommendation for this section***

- Establish a Diagnostic Working Party to share information, evaluate techniques, conduct comparative trials on methodology and to help plan analytical aspects of project work
- Diagnostic Working Party to be clearing house for information on new technologies as they become ready for trial
- Hold a one-day satellite workshop on rapid diagnostic technologies at either Seafood Directions or the Inaugural CRC Conference

**The place for rapid diagnostics in the CRC**

Many diagnostic procedures are not done in-house in the seafood industry but are outsourced to specialist laboratories, both for control and quality purposes as well as for regulatory compliance. The CRC needs to consider whether it has a stated aim to encourage the development of rapid diagnostics so, where possible, they can be done at source to arrive at answers for decision making sooner than if samples were sent away.

There is a history of methods being developed, ostensibly in response to an industry need, but they are never taken up by industry. This points to the need for involvement and commitment by industry at an early stage and throughout the development phases. This generally requires the originators of an idea or approach to relinquish part of their IP so that industry has some ownership, will make an investment in it and seek to use it to make the investment pay off.

Questions arise with rapid diagnostics:-

1. Do the participants agree it is a useful aim to devolve diagnostics from contract laboratories, wherever practical (and NATA status or suchlike is not required), to the boat, factory or farm at least for QC purposes and,
2. Is it likely they intend to take up developments that would allow this or,
3. Do they wish to support development that may only be used in laboratories and if so under what mechanisms of transfer or licensing would this occur?

    - Develop policy of industry involvement in development of rapid diagnostics
    - Consider whether an aim is to devolve rapid diagnostics to parts of the chain and to consider the role of contract laboratories

Consideration also needs to be given to the following issues.

***Implications of outsourcing***

Outsourcing depends on the balance between the need for the information, the timeliness of it, the cost and the benefit obtained from it. Although cost of analysis can be an issue the effect of outsourcing means that industry is less keenly aware about advances in analysis and rapid diagnostics, as these matters are normally the prerogative of the service provider doing the analyses. For the CRC this means that it is essential that some participating company, or companies, recognises the potential value in investing in any rapid diagnostic.

- Estimate the costs involved in outsourcing and the potential savings in either reducing the overall cost or in gaining much more information needs exploring.

***Implications for chain management***

Both predictive and rapid diagnostic techniques can be applied at any step along the chain and although there is collective responsibility the burden of cost often falls on one step only – generally the processor.

Chain management models try to spread the costs, profits and responsibilities with some equality throughout the chain. The practice of chain management in the seafood industry in Australia is embryonic and cost spreading is rarely planned.

Industry or government bodies e.g., agencies with a development brief, often underwrite common causes where environmental matters or those of market access in the State or National interest are concerned. However, the cost generally falls to the most active organisation in the chain and the rest obtain the benefit. Processors and marketers are generally the most active rather than fishers and farmers, unless they are in integrated structures.

Thought is required on chain management models that can deal with these matters and where responsibility for diagnostics enters the chain. Note these are issues, broader than just rapid diagnostics and predictive technologies, that apply across the CRC activities.

- Develop ideas of quality chain management models suited to the CRC

**RECOMMENDATIONS**

- Present this review to participants and survey spokespeople in person to obtain responses, ideas and survey opportunitites.
- If required conduct a separate review on rapid microbiological methods to make recommendations on techniques
- Seek to obtain results that can be added to the models in SSSP so it covers Australian seafoods with greater certainty
- Plan microbial activities in CoolFish so that SSO are established
- Promote the concept of Icedays through training and information dissemination
- Establish mechanisms to encourage use of and ready availability to multivariate methods
- Establish what NIR is used for at present in seafood analysis

- Liaise with users of electronic noses e.g., FSA to identify specific areas where it may meet a need
- Explore opportunities in which digital cameras and RGB values would be of use in selection and control
- Seek industry sectors that could benefit from imaging to detect parasites
- Inform CRC participants of the Alert kit for histamine
- Canvas industry and contract analysts for opportunities to increase efficiencies with Lab-on-a-chip technologies
- Monitor progress of real time PCR techniques for hepatitis and noroviruses being developed in SEAFOODplus and inform participants and analysts
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- Estimate the costs involved in outsourcing and the potential savings in either reducing the overall cost or in gaining much more information needs exploring
- Develop ideas of quality chain management models suited to the CRC
- Establish mechanisms to educate and encourage use of and ready availability to utilise multivariate methods and secondary analysis techniques.
- Educate and promote understanding of analytical regulatory compliance and encourage establishment of inter-laboratory or between industry analytical quality control to ISO 1725 and NATA specifications.
- Ensure analysis in research is conducted to international standard and encourage and support development of international interactions that can enhance Australian analytical quality control and between agency continuity.

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