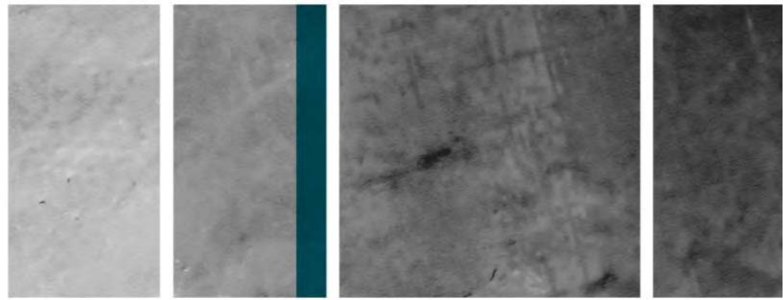

Forum Proceedings: Tilapia in Australia - state of knowledge

Brisbane, 15-16 May 2012

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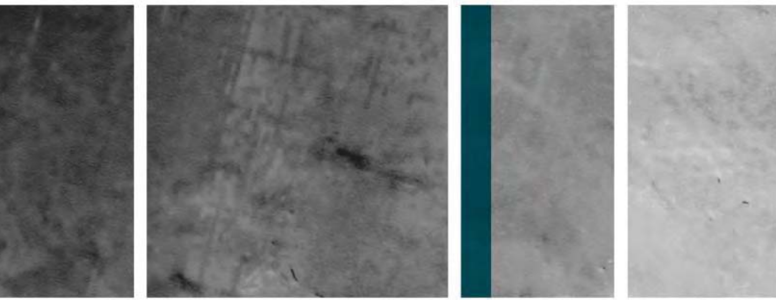
Hosted by the Invasive Animals Cooperative Research Centre
and the Murray-Darling Basin Authority

An IA CRC Project



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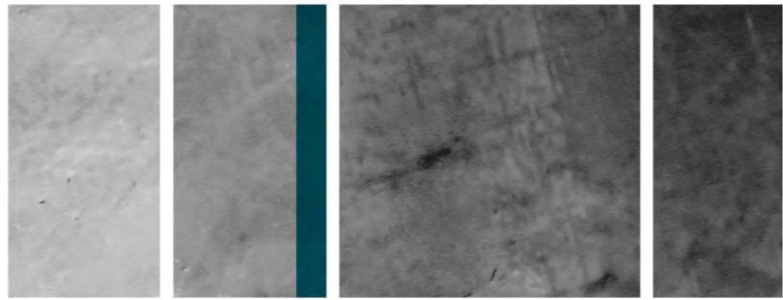

eDNA surveillance for high risk invasive fish

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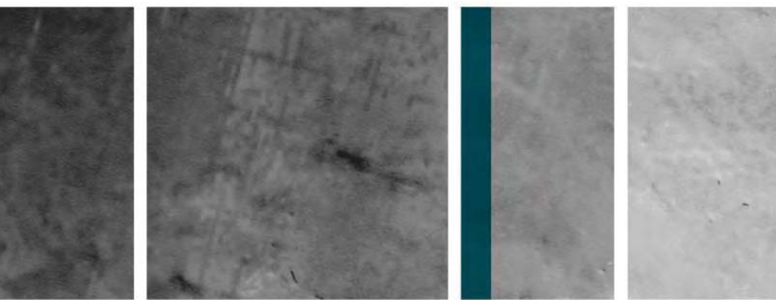
Introduction

The early 'acclimatisation phase' of human introduction of sport fish (such as trout, tench and redfin perch) has passed (Clements 1988) and ornamental fish now are the main source of new introductions of exotic fish to Australia. The ornamental fish industry in Australia is estimated to be worth $350 million a year. There are around 2,000 species in the ornamental fish trade nationally, and many of the species favoured by aquarists are exotic. The volumes are huge — Australian Quarantine and Inspection Service (AQIS) data show that approximately 19 million ornamental fish were imported into Australia in 2008 alone (Biosecurity Australia 2009).

Of the estimated 12.5 million freshwater ornamental fish imported into Australia in 2003–2004 by one major importer, approximately 57% were poeciliids, 25% goldfish, 8% catfish, 8% gouramis and 2% cichlids (Biosecurity Australia 2009). Approximately 569 fish species are on the national noxious list. A further 778 species which are potentially noxious are on a gray list on a grey and require further investigation and risk assessment (NRMMC 2006). Some 130 of these are considered high risk. Australia has established populations of at least 37 alien fish species, and the number continues to grow (Lintermans 2004, Corfield et al 2008). Many of these species have been shown to, or are suspected of, having a significant impact on native biodiversity through predation, aggression, competition for resources, habitat change, spreading disease and parasites or through hybridization and gene introgression (Lintermans 2004, Corfield et al 2008).

Many fish species in the ornamental fish trade are not on the current national permitted species lists of the *Environment Protection and Biodiversity Conservation (EPBC) Act 1999* or covered by quarantine regulations. It may be that such species have been permitted under previous statutory arrangements, some have been and will continue to be smuggled in, but many are also likely to have been imported undetected as part of legitimate shipments. As prevention is better than cure, one obvious point of control is at our borders. Distinguishing fish species is a matter for experts, especially if they are juveniles. Reliable identification is difficult or impossible for those charged with border inspections, and so there is a need for a rapid and reliable method of screening imported fish. A second key area for control is early detection and intervention before a new invasive species is able to establish and spread. Once established, exotic fish can be very difficult and expensive, if not impossible to eradicate (Simberloff 2003).

Fish surveillance approaches such as nets or electrofishing have low capture probabilities and are only reliable indicators of occurrence for species present at moderate to high abundance (Magnuson et al 1994). These methods are also resource intensive meaning that widespread and effective programs cannot be supported. As a consequence, the low detection probability for rare species, such as those likely following a recent incursion event, may lead a species to be considered absent when it is actually present (Gu and Swihart 2004). The cost of a false negative for highly invasive species may be catastrophic if, as a result, eradication measures



are not implemented when eradication is possible. Reducing the risk of a false negative for rare species requires increased sampling effort or improvement of the detection technique (McDonald 2004). DNA technologies promise a rapid and reliable method for early detection of invasive species to complement traditional approaches.

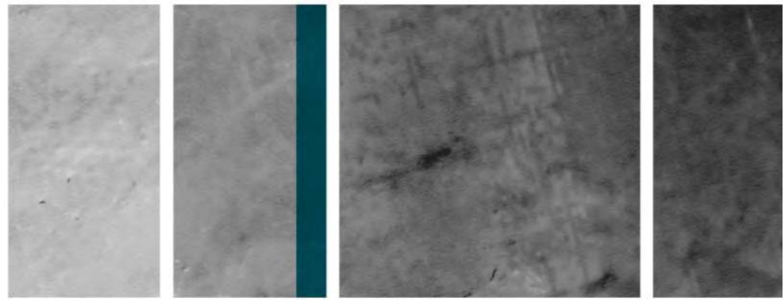
These technologies rely upon the observation that aquatic organisms, including fish, discharge cells containing DNA with faeces or urine, as skin sloughs or in mucoidal secretions. These cells and extracellular DNA become adsorbed to particles and persist and accumulate in water (Poté et al 2009, Dejean et al 2011) to the point where individualised sequences are detectable using PCR amplification of species-specific DNA markers. This approach, together with the new wave of DNA technologies (Glenn 2011), promises to provide tools for detecting illegal shipments at the time they cross our borders and for early detection of invasions in our rivers and wetlands. These technologies are being used in the detection of biota in ballast water (Deagle et al 2003), in benthic community composition in estuaries (Purdy et al 2002), estimating fish composition (Minamoto et al 2011), in early detection of fish invasions in freshwaters (Jerde et al 2011), estimation of fish biomass (Takahara et al 2012), and to determine the presence of species of interest that are rare or in low abundance (Ficetola et al 2008, Goldberg et al 2011, Jerde et al 2011, Thomsen et al 2012).

Our project, funded by the Invasive Animals Cooperative Research Centre, will develop a generalised system of detection for fish of high risk of establishment and invasion, using environmental (water-borne) DNA (eDNA). Our approach will deliver both quality diagnostic tests for species of high invasive risk and a framework for developing tests for additional species. This general approach has been used successfully to detect Asian carp in the Great Lakes of North America (Jerde et al 2011, Mahon et al 2011), will be trialled for the detection of Tilapia in Australia, and expanded to include a range of high profile invasive species. Our project includes a method for producing multiple diagnostic markers simultaneously for non-model species and potential approaches for the rapid processing of water samples. Success in this project will pave the way for broadscale eDNA surveillance of invasive fish with complementary applications in the survey of rare and endangered species.

The changing landscape of environmental genomics

The field of genomics is moving with astonishing rapidity. Tasks that were challenging or prohibitively expensive only a few years ago are becoming routine. The most commonly cited example of this progress is that of generating a full genome sequence, which cost approximately $95 million a little over a decade ago, only to decrease in cost for comparable sequencing progressively and precipitously to around $7,000 at last estimate (2011 data from the NHGRI Large-Scale Genome Sequencing Program presented by the National Human Genome Research Institute), and likely to drop to around $1,000 within the next five years. A de novo sequence from an organism with little or no prior genomic information now costs only $220,000 in sequencing and bioinformatics costs of assembly and gene annotation (as of June 2012, Guojie Zhang, BGI-Shenzhen, personal communication).

Next generation (NextGen) sequencing (Glenn 2011) has opened the door to genomic analysis of non-model organisms — those without the prior and solid foundation of genomic sequence information available for model species such as mouse, human, chicken and zebrafish. The value of NextGen sequencing in the study of human genetics and disease are unquestioned (Mardis 2008), and it promises to revolutionize agriculture (Varshney et al 2009). However, NextGen sequencing also promises to accelerate the rate of discoveries in population genetics, ecology, evolution and environmental genomics of non-model organisms, as well as presenting opportunities to answer questions that were intractable using earlier technologies.



Without trying to be comprehensive regarding the value of these technologies, specific attention is drawn to the dramatically improved capacity to identify microsatellite markers for use in population genetics of non-model organisms (Malausa et al 2011) — this is now becoming routine, where previously the availability and quantity of microsatellite markers was a serious constraint. It required a painstaking process of enrichment of sequence containing microsatellites, cloning and Sanger sequencing. There are new avenues for genome-wide identification, characterization and screening of SNP markers (single nucleotide variants that, in combination, can be diagnostic at almost any taxonomic level, in demonstrating introgression (Hohenlohe et al 2011), or establishing familial relationships). They have proven extremely valuable in studies of human genetics and disease. Even in the absence of a reference genome, by appropriate filtering and definition of allelic sequences, techniques such as Restriction site Associated DNA sequencing (RADSeq) can deliver huge numbers of SNPs for analysis (Baird et al 2008, Davey and Blaxter 2011). The information generated by several hundred diagnostic SNPs will be superior to that generated by the current standard of about a dozen microsatellites for most applications in population genetics (Seeb et al 2011). NextGen technology will soon be sufficiently inexpensive for feasible large scale screening of samples in addition to marker generation.

NextGen sequencing is clearly a transformative technology, which brings a much wider range of species, disciplinary areas, and problems within reach of genomics. It is changing the face of the field, where the physical infrastructure and technology is not the limiting factor in data generation, and greater emphasis is placed on having the necessary samples to address novel questions of substance or novel applications. Of direct interest to us is the potential of NextGen technologies in environmental genomics, where one can screen for diversity and compositional change across sites in taxa that are indicators of pollution, such as chironomids and mayflies, or screen for 'ring-ins' in importation of ornamental fish, for early detection of invasive species in our waterways or for detection of endangered species which may be present in very low abundances.

Approach

Target species

The large number of exotic fish species with the potential to invade Australian inland waters demands a targeted approach to marker development. Priority invasive species will be selected for marker development through consultation with federal and state biosecurity agencies — species on the surveillance alert or national noxious fish lists; those identified as having a high risk of establishment or spread through risk assessment (Bomford 2008); and species already well established, but which pose significant risks to threatened native species in catchments where the exotic species is currently absent (Table 1).

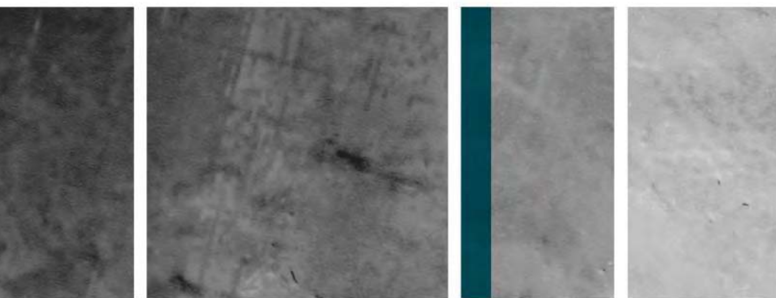


Table 1: Preliminary list of target species for which the diagnostic test will be developed for eDNA detection.

Species common name	*Scientific name*	*eDNA sample source*	*Invasion status*
Oriental weatherloach	*Misgurnus anguillicaudatus*	Molonglo, Murrumbidgee, Lower Cotter catchment	Established
Redfin perch	*Perca fluviatilis*	Murrumbidgee, Molonglo, Paddys	Established
Brown trout	*Salmo trutta*	Lower Cotter catchment, Queanbeyan, Goodradigbee	Established
Pearl eartheater	*Geophagus brasiliensis*	Perth (WA) including Swan River and Bennett Brook	Established
Speckled mosquitofish	*Phalloceros caudimaculatus*	Perth (WA) including Canning River, Bull Creek, Churchmans Brook	Established
Walking catfish	*Clarias batrachus*	From frozen food trade	Established in PNG
Snakehead	*Channa spp.*	From frozen food trade	Established in PNG
Climbing perch	*Anabas testudineus*	From frozen food trade	Established in PNG

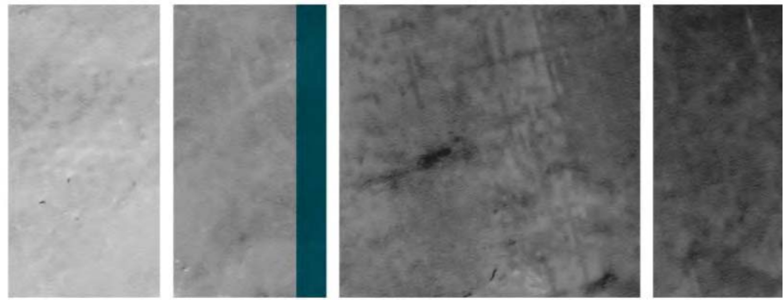
Selection of markers

Specific genes or genomic regions that are diagnostic at the level of species, such as 18S rRNA, 12S rRNA and mitochondrial DNA (Hardy et al 2011), will yield a suite of markers of considerable utility for species identification from water samples. In a second approach, drawing upon new generation sequencing technologies, we will use RADSeq tags (Baird et al 2008) to develop a series of diagnostic single nucleotide markers (SNPs) for our target species. The SNP approach will be more sensitive at the population level than will be markers obtained from 18S rRNA, 12S rRNA and mtDNA, and so may be useful in establishing provenance of source populations, and will be more amenable to automation.

Protocols for water sampling

A key ingredient in the success of this project will be the development of protocols for water sampling and DNA extraction. Using Tilapia as a trial species, water samples will be collected from replicate tanks containing low fish densities and DNA extracted using published protocols developed for eDNA extraction (Ficetola et al 2008). The fish will be removed once DNA concentrations have reached equilibrium, and DNA monitoring will continue until DNA can no longer be detected. These data will provide baseline information on the persistence of our target DNA over time in a simple aquatic system and will form the base-line for detection trials in natural waterways.

To determine the effectiveness of our markers in natural systems, we will take replicate water samples across a range of Australian sites known to contain populations of Tilapia. We will focus particularly on sites where the species is known to occur and will use site occupancy models to evaluate the covariates that lead to increased detection (Jerde and Lewis 2007, Jerde and Bossenbroek 2009). Standard environment covariates (eg flow, pH, turbidity, conductivity, temperature, habitat, substrate, depth and width) will be measured at each site. Turbidity can present a formidable challenge to eDNA detection.



Genotyping samples

Under our current proposal, a custom microarray using species-specific short sequence identified in rRNA and mtDNA genes will be generated to include all fish species of interest, not just invasive species. A custom Fluidigm Dynamic Array (Wang et al 2009) will be developed for SNPs that are collectively diagnostic at and below the species level. Fluidigm Dynamic Array Integrated Fluidic Circuits are designed for high sample throughput and low to mid-multiplex SNP genotyping (for up to 96 SNPs) which enables fast and accurate low-cost genotyping in plants and animals compared to the traditional microarray approach (Wang et al 2009).

However, options available for high-throughput genotyping of gene makers and SNPs are rapidly changing, both in availability and cost (Table 2). The sequencing technologies announced by Oxford Nanopore (Eisenstein 2012) promise to generate up to 1Gb of genomic data on the desktop with a device little larger than a thumb drive and permits real time analysis of sequences as they are generated. This, at a cost of $900 per run, promises to meet the primary challenge faced in monitoring the passage of unwanted fish across our borders, that of real time detection by customs authorities. For larger scale screening projects, direct sequencing of samples, enriched for markers of interest, is available through the Illumina sequencing platforms, and likely soon to become sufficiently affordable to challenge existing approaches using custom microarray chips or Fluidigm dynamic arrays. The rapid development of the technologies demands a flexible and adaptive approach to generating solutions to the problems of invasive fish detection.

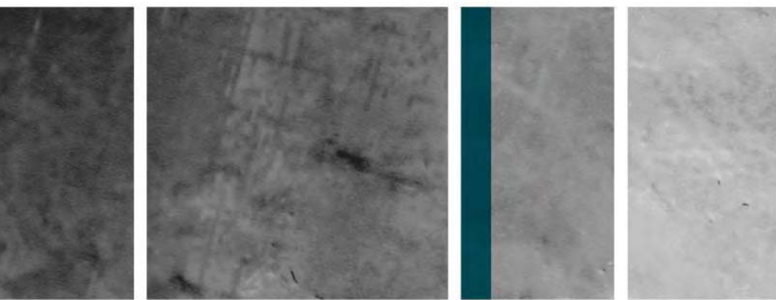
Table 2: Comparison of chemistry and detection of various currently popular SNP genotyping (Peatman 2011).

Platform	*Company*	*Chemistry*	*Detection*
iSelect HD Custom	Illumina	Single-base extension	Fluorescence
GoldenGate	Illumina	Allele-specific primer extension	Fluorescence
MyGeneChip Custom Array	Affymetrix	Differential hybridization	Fluorescence
MassArray	Sequenom	Single-base extension	Mass Spectrometry
SNPstream	Beckman Coulter	Single-base extension	Fluorescence
Taqman Open Array	Applied Biosystems	TaqMan-5′ nuclease	Fluorescence
Dynamic Array	Fluidigm	TaqMan-5′ nuclease	Fluorescence

Conclusions and future directions

Completion of this work will see the development and testing of a low cost and robust high throughput technology for the detection of specific invasive fish species. We will have estimates of the sensitivity of these approaches in relation to fish presence and abundance and therefore estimates of the detectability provided by the technology. In addition, we will have a clear methodology for producing markers for additional species of concern. Given that it is likely that there are more than 1,100 potentially invasive fish species in the Australian aquarium trade, the ability to rapidly develop diagnostic markers for new species will be of paramount importance.

By targeting the detection of minute amounts of DNA from complex eDNA samples, these technologies will provide the ability to detect multiple invasive species simultaneously from the same water samples. This in turn, will provide the ability to detect introductions of key invasive species threatening range extension earlier. These same technologies, including the



promise of technologies from Oxford Nanopore (the MinIon; Eisenstein 2012), will provide the ability to screen effectively live aquarium fish at the point of importation and before their dissemination into the wider Australian community. Interception at the border will greatly slow down the rate at which new invasive species will be added to Australian waterways with the commensurate reduction in the cost of eradication or control and in the damage done to Australian aquatic ecosystems.

When combined with a coordinated approach to the systematic collection of water samples across states and territories, these technologies will provide the means to provide early warnings of introductions. They also enable the establishment of early management strategies such as intervention to eradicate or contain the spread, and thereby facilitate more cost effective management of nationally or regionally listed threatened species.

References

Baird N, Etter P, Atwood T, Currey M, Shiver A, Lewis Z, Selker E, Cresko W and Johnson E (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. ***PLoS One*** 3:e3376

Biosecurity Australia (2009). ***Importation of Freshwater Ornamental Fish: Review of the Biosecurity Risks Associated with Gourami Iridovirus and Related Viruses***. Draft import risk analysis report. Biosecurity Australia, Canberra.

Bomford M (2008). ***Risk Assessment Models for Establishment of Exotic Vertebrates in Australia and New Zealand***. Invasive Animals Cooperative Research Centre, Canberra.

Clements J (1988). ***Salmon at the Antipodes: A History and Review of Trout, Salmon and Char and Introduced Coarse Fish in Australasia***. Privately published, available from the author, 22 Mimosa Avenue, Alfredton, Ballarat, Victoria 3350, Australia, Ballarat.

Corfield J, Diggles B, Jubb C, McDowall R, Moore A, Richards A and Rowe D (2008). ***Review of the Impacts of Introduced Ornamental Fish Species that have Established Wild Populations in Australia***. Unpublished report to the Australian Government Department of the Environment, Water, Heritage and the Arts, Canberra.

Davey J and Blaxter M (2011). RADSeq: next-generation population genetics. ***Briefings in Functional Genomics*** 9:416-423.

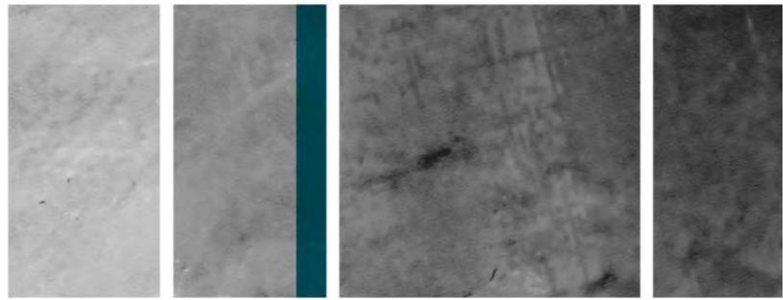
Deagle BE, Bax N, Hewitt CL and Patil JG (2003). Development and evaluation of a PCR-based test for detection of ***Asterias*** (Echinodermata: Asteroidea) larvae in Australian plankton samples from ballast water. ***Marine and Freshwater Research*** 54:709–719.

Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P and Miaud C (2011). Persistence of environmental DNA in freshwater ecosystems ***PLoS One*** 6:e23398.

Eisenstein M (2012). Oxford Nanopore announcement sets sequencing sector abuzz. ***Nature Biotechnology*** 30:295–296.

Ficetola GF, Miaud C, Pompanon F and Taberlet P (2008). Species detection using environmental DNA from water samples. ***Biology Letters*** 4:423–425.

Glenn T (2011). Field guide to next-generation DNA sequencers. ***Molecular Ecology Resources*** 11:759–769.



Goldberg C, Pilliod D, Arkle R and Waits L (2011). Molecular detection of vertebrates in stream water: a demonstration using rocky mountain tailed frogs and Idaho giant salamanders. PLoS One 6:e22746.

Gu WD and Swihart RK (2004). Absent or undetected? Effects of non-detection of species occurrence on wildlife-habitat models. *Biological Conservation* 116:195–203.

Hardy CM, Adams M, Jerry DR, Court LN, Morgan MJ and Hartley DM (2011). DNA barcoding to support conservation: species identification, genetic structure and biogeography of fishes in the Murray–Darling River Basin. *Australia Marine and Freshwater Research* 62:887–901.

Hohenlohe P, Amish S, Catchen J, Allendorf F and Luikart G (2011). Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. *Molecular Ecology Resources* 11:117–122.

Jerde CL and Lewis MA (2007). Waiting for invasions: a framework for the arrival of nonindigenous species. *The American Naturalist* 170:1–9.

Jerde CL and Bossenbroek JM (2009). Uncertain invasions: a biological perspective. In: RP Keller, DM Lodge, MA Lewis and JF Shogren (Eds), *Bioeconomics of Invasive Species: Integrating Ecology, Economics, Policy and Management*. Oxford University Press, New York. Pp 126–150.

Jerde CL, Mahon AR, Chadderton WL and Lodge DM (2011). 'Sight-unseen' detection of rare aquatic species using environmental DNA. *Conservation Letters* 4:150–157.

Lintermans M (2004). Human-assisted dispersal of alien freshwater fish in Australia. *New Zealand Journal of Marine and Freshwater Research* 38:481–501.

Magnuson JJ, Benson BJ and McLain AS (1994). Insights on species *richness and turnover from long-term ecological research: fishes in north temperate lakes. American Zoologist* 34:437–451.

Mahon AR, Barnes MA, Senapati S, Feder JL, Darling JA, Chang HC and Lodge DM (2011). Molecular detection of invasive species in heterogeneous mixtures using a microfluidic carbon nanotube platform. *Plos One* 6:5.

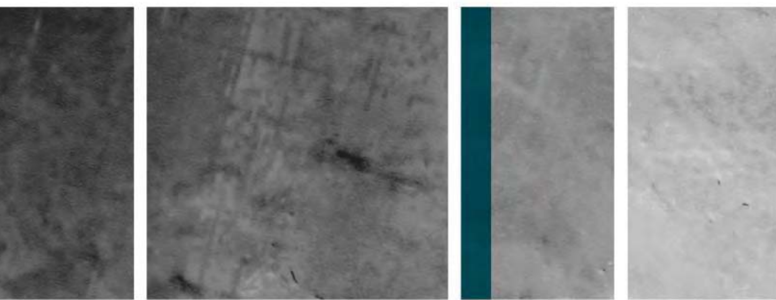
Malausa T, Gilles A, Meglécz E, Blanquart H, Duthoy S, Costedoat C, Dubut V, Pech N, Castagnone-Sereno P, Délye C, Feau N, Frey P, Gauthier P, Guillemaud T, Hazard L, Le Corre V, Lung-Escarmant B, Malé P-J, Ferreira S and Martin J-F (2011). High throughput microsatellites isolation with 454 GS-FLX Titanium pyrosequencing. *Molecular Ecology Resources* 11:638–644.

Mardis E (2008). Next-generation DNA sequencing methods. *Annual Review of Genomics and Human Genetics* 9:387–402.

McDonald LL (2004). Sampling rare populations. In: WL Thompson (Ed), Sampling Rare or Elusive Species. Island Press, New York. Pp 11–42.

Minamoto T, Yamanaka H, Takahara T, Honjo MN and Kawabata Z (2011). Surveillance of fish species composition using environmental DNA. *Limnology* DOI 101007/s10201-011-0362-4

NRMMC (2006). *A Strategic Approach to the Management of Ornamental Fish in Australia*. Natural Resource Management Ministerial Council and the Department of Agriculture, Fisheries and Forestry, Canberra.



Peatman E (2011). Next generation sequencing and whole genome selection in aquaculture. In: Z Lui (Ed), *Next Generation Sequencing and Whole Genome Selection in Aquaculture*. Blackwell Publishing Ltd, New York. Pp 125–132.

Poté J, Ackermann R and Wildi W (2009). Plant leaf mass loss and DNA release in freshwater sediments. *Ecotoxicology and Environmental Safety* 72:1378–1383.

Purdy K, Munson M, Nedwell D and Embley T (2002). Comparison of the molecular diversity of the methanogenic community at the brackish and marine ends of a UK estuary. *FEMS Microbiology Ecology* 39:17–21.

Seeb J, Carvalho G, Hauser L, Naish K, Roberts S and Seeb L (2011). Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. *Molecular Ecology Resources* 11:1–8.

Simberloff D (2003). How much information on population biology is needed to manage introduced species? *Conservation Biology* 17:83–92.

Takahara T, Minamoto T, Yamanaka H, Doi H and Kawabata Z (2012). Estimation of fish biomass using environmental DNA. *PLoS One* 7:e35868.

Thomsen P, Kielgast J, Iversen L, Wiuf C, Rasmussen M, Gilbert M, Orlando L and Willerslev E (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology* 21:2565–2573.

Varshney R, Nayak S, May G and Jackson S (2009). Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends in Biotechnology* 27:522–530.

Wang J, Lin M, Crenshaw A, Hutchinson A, Hicks B, Yeager M, Berndt S, Huang W-Y, Hayes R, Chanock S, Jones R and Ramakrishnan R (2009). High-throughput single nucleotide polymorphism genotyping using nanofluidic dynamic arrays. *BioMedCentral Genomics* 10:561.

Questions

Q: A lot of the threats are going to come from Northern Australia, can you tell me about the longevity of eDNA in warm water?

Tariq: Depends on how the DNA is in the water. If it is a free DNA it degrades very quickly at 18-20 degrees C. If it is deposited in the sludge then it can stay non-degraded for quite a while. It will degrade, but more slowly, so it can be used for this sort of detection. These markers are really small so you do not need a large chunk of DNA for this method.

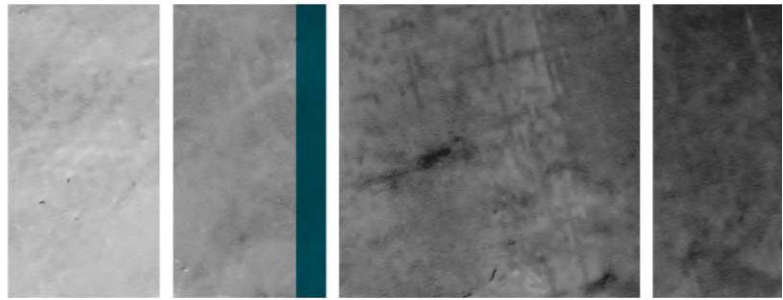
Q: At 30 degree water you wouldn't expect it to last very long at all?

Tariq: If it is free DNA it will be broken down but if it is deposited in the sludge then it will be protected to some degree for a lot longer

Q: Are waters with high levels of organics or high turbidity an issue?

Tariq: No, because when you isolate the DNA you can optimise that option

Q: At CSIRO in Hobart we do a lot of work with ballast water but we have to filter 1000's of litres to get a detection event and we are still concerned about false negatives. There is a concern about how far you can push the limits…are you going to have to filter 1000's of litres of sludge to get a detection event and do you have any feel from the literature as to what the real sensitivity is?



Tariq: For the ballast water technique they needed quite a bit of DNA for that. Recent papers have looked at this problem and we will use a different method where we can amplify the amount of DNA using a bacterial replication method.

Q. A question about the accuracy of your system, if some person wants to bring in a fish species?

Tariq: The technique will be very powerful because the markers that we will use are very specific

Q. Is there potential for cyptic species to come into Australia if they are not already on your target list?

Tariq: Yes there will be if they are not on the list.