Molecular tools for detection of marine pests: *Musculista senhousia, Corbula gibba* and *Perna canaliculus* quantitative PCR assays.

Nathan J. Bott and Danièle Giblot-Ducray

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Report prepared for Biosecurity SA
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Executive Summary

The National System for the Prevention and Management of Marine Pest Incursions requires tools for the detection and monitoring of marine pests. Specific, robust molecular assays for the identification and quantification of marine pests (including eggs and larval stages) from environmental samples facilitate rapid, low-cost surveillance to be undertaken and effective control strategies to be implemented where marine pest incursions are detected. The use of quantitative Polymerase Chain Reaction (qPCR) techniques will be suitable for this purpose. The polymerase chain reaction (PCR) is an enzymatic technique used for the amplification of nucleic acids (e.g. DNA), and qPCR is a PCR technique monitored in real-time through changes in fluorescence.

Currently, detection of marine pests is based primarily on traditional survey techniques such as observational walks, snorkel and dive surveys, plankton trawls, traps and netting, with manual sorting and identification. The use of qPCR offers the ability to conduct testing of very large numbers of samples to rapidly identify the genetic material of the targeted organisms (referred to as high-throughput screening). The successful development and implementation of these methods will allow for the testing of plankton samples to rapidly verify the presence or absence of potential pest species in marine waters. This study details the development and assessment of qPCR assays for the Asian Date Mussel, *Musculista senhousia*, the New Zealand Greenlip Mussel, *Perna canaliculus* and the European clam, *Corbula gibba*.

We have designed a qPCR assay that is, based on available controls, specific to *M. senhousia*. We have adapted a specific PCR assay for *P. canaliculus* to a qPCR assay that is, based on available controls, specific to *P. canaliculus*. We have further tested the specificity of the *C. gibba* qPCR assay, developed by Ophel-Keller et al. (2007), with closely related corbulid species, and based on available specimens this assay is specific to *C. gibba*.

In the future, these qPCR assays, along with other marine pest qPCR assays, are expected to be used to monitor ports for marine pests, using DNA extracted from plankton samples. Specific diagnosis of marine pests is central to rapidly establishing the distribution and prevalence of marine pest species to manage and restrict their spread, and monitoring changes in marine pest distribution spatially and temporally facilitates targeted eradication and control programmes where feasible.
Acknowledgements

Assistance from the following individuals is greatly acknowledged:

- Dr Paul Fisher and Bradley Pease (Queensland Department of Employment, Economic Development and Innovation) for the provision of mytilid bivalve samples.
- Dr Richard Willan (NT Museum) for identifying *Musculista senhousia* samples and Dr Jawahar Patil (CSIRO Marine Research) for providing *M. senhousia* DNA.
- Mr Anders Hallan (University of Wollongong) for providing corbulid specimens.
- Dr Alan McKay, Dr Kathy Ophel-Keller, Dr Herdina and Ms Teresa Mammone (SARDI Diagnostics) for assistance and advice in the laboratory.
- Dr Maylene Loo (SARDI Aquatic Sciences) for assistance in obtaining *M. senhousia* samples from Singapore.
- Dr Marty Deveney and Ms Kathryn Wiltshire (SARDI Aquatic Sciences)
- Dr Michael Sierp, Mr John Gilliland and Mr Vic Neverauskas (Biosecurity SA).
Introduction

The National System for the Prevention and Management of Marine Pest Incursions requires tools for the detection and monitoring of marine pests. Specific, robust molecular assays for the identification and quantification of marine pests (including eggs and larval stages) from environmental samples facilitate rapid, low-cost surveillance, and inform effective control strategies where marine pest incursions are detected.

Marine pests have the potential to cause significant harm to endemic biodiversity and habitats (Galil, 2007; Wallentinus and Nyberg, 2007). Marine pests can be translocated and introduced by numerous vectors including ship ballast, hull fouling, floating debris and man-made structures such as drilling platforms and canals (Bax et al., 2003). Marine pest introductions continue to occur and threaten the marine environment and associated industries (Hayes and Sliwa, 2003). With increasing globalisation comes faster and more frequent shipping and air transport of live seafood. Propagule pressure is only likely to increase unless effective strategies are employed for early detection, prevention and control. Central to such strategies is the ability to rapidly identify the presence of a particular pest species.

The development and implementation of rapid, sensitive and accurate diagnostic techniques for the identification and surveillance of marine pests from environmental samples (e.g. sea water, sediments, and ship ballast), is an essential step in early detection and control of marine pests, to maintain the status of pest-free areas and to limit the economic impacts of management in areas where pest are established and cannot be eradicated.

Current marine pest diagnostics research at SARDI includes the development and refinement of specific, sensitive, quantitative Polymerase Chain Reaction (qPCR) assays for the detection of several marine pest species. PCR is an enzymatic technique used for the amplification of nucleic acids (e.g. DNA) and qPCR is a PCR technique monitored in real-time through changes in fluorescence. Through consultation between SARDI Aquatic Sciences and Biosecurity SA it was decided to undertake a study to implement and/or develop qPCR assays for three marine pest species of significance to Australia, two of which: European clam, Corbula gibba; and Asian Bag Mussel, Musculista senhousia, are part of the Consultative Committee on Introduced Marine Pest Emergencies (CCIMPE) Trigger List, which is endorsed by the National Introduced Marine Pest Coordinating Group (NIMPCG). The third is the New Zealand Greenlip Mussel, Perna canaliculus, which has high potential as an invasive species and has been previously reported but eradicated from Outer Harbor and a hopper barge docked in Port Adelaide (Wiltshire et al., 2010).
**Musculista senhousia**

*Musculista senhousia* (Figure 1) is commonly known as the Asian date mussel and is native to the Pacific Ocean, inhabiting coastal areas from Siberia and the Kuril Islands south to Singapore (Slack-Smith and Brearley, 1987). Aggregations can attain very high densities in intertidal and subtidal soft sediments, typically 5 000-10 000/m², and up to 15 000/m² (Crooks and Soule, 1999; Dexter and Crooks, 2000; Reush and Williams, 1998). When in high densities, *M. senhousia* can cause significant habitat alteration, which can have profound effects on native infaunal communities, and native seagrasses (Crooks, 1998). Based on seawater temperature, *M. senhousia* has significant potential to become established across Australian waters (http://marinepests.gov.au/__data/assets/pdf_file/0005/952529/Musculista-ncp-08.pdf).

![Figure 1: Asian date mussel, Musculista senhousia](image)

**Corbula gibba**

The European clam, *C. gibba* (see Figure 2), is commonly found in subtidal environments, in coastal and estuarine silts and muddy gravels, in its native Europe (Holmes and Miller, 2006). Very high densities of *C. gibba* have been recorded in Port Phillip Bay (up to 2600/m²), and have been linked to changes in benthic community structure (Currie and Parry, 1999). In Europe, high abundances of *C. gibba* tend to be associated with habitat degradation, although this has not been adequately studied in Australia.
**Figure 2: European clam, *Corbula gibba***

*Perna canaliculus*  
*Perna canaliculus* (Figure 3) is commonly known as the New Zealand greenlip mussel and is endemic to New Zealand waters, it has been previously reported from Outer Harbor and from a vessel in Port Adelaide, but it was successfully eradicated (Wiltshire et al., 2010). *Perna canaliculus* is commercially harvested as seafood in New Zealand. Potential impacts of *P. canaliculus* on native populations are unknown but it would likely include competition with native bivalve species, fouling structures and potential introduction of diseases to the Australian mollusc aquaculture industry.
Molecular testing methods for marine pests

Development of rapid testing methods for marine pests has recently focussed on molecular techniques. A broad range of these techniques have been developed for marine pests (see Bott et al. 2010 and references therein). Polymerase Chain Reaction (PCR) has revolutionised many areas of biological research including species and strain delineation. PCR can amplify minute amounts of template DNA, and its high specificity makes it highly effective for species and strain identification for a wide range of organisms. The relatively low cost of equipment and reagents makes PCR accessible to a wide range of laboratories. Quantitative PCR (qPCR) allows the amplification of a target DNA to be monitored in real-time as amplification occurs. qPCR offers a relatively rapid analysis (< 2 hours), the potential for high-throughput, allows linear quantification over a wide dynamic range (>6 orders of magnitude), and has the benefit of not requiring post-PCR handling (“closed-tube” format), decreasing the likelihood of sample contamination. It is now routinely used in numerous clinical applications for the detection of a wide range of bacterial, fungal, parasitic and viral diseases of humans (Espy et al., 2006). Recent advances have seen a
number of studies utilising qPCR-based techniques for the identification of marine pests (see Galluzi et al., 2004; Pan et al., 2008).

The development of these tests requires that the target organism is taxonomically unambiguous. Testing of a number of species closely related to the target organism is required as well as testing of environmental samples containing unknown taxa. Most test development achieves the first criterion but for implementation, it is important to validate tests on samples exhibiting higher complexity such as natural water and sediment samples.

Many PCR-based tests are developed based on nuclear ribosomal and mitochondrial gene sequences (including tests from this study). Genes evolve at different rates and a suitable DNA region should vary in sequence sufficiently to allow the identification of an individual to the taxonomic level required. For specific identification, the DNA marker should exhibit little or no genetic variation within a species but differ sufficiently between species so as to allow unequivocal delineation.

In nuclear ribosomal genes and spacers, there is typically little variation amongst individuals of a species within and between populations (Larsen et al., 2005; Livi et al., 2006). The ribosomal DNA (rDNA) genes, Internal Transcribed Spacers (ITS) and Intergenic Spacer (IGS)/ Non-transcribed spacer (NTS) regions have been shown to be particularly useful in defining species specific markers for marine pest assay development. The mitochondrial genome is also utilised for diagnostic purposes; mitochondria are generally inherited maternally making them particularly useful as a species-specific marker for the delineation of closely related species (e.g. Blair et al., 2006, Kamikawa et al., 2008).

In this report we detail the development of qPCR assays for *M. senhousia* and *P. canaliculus*, and further specificity testing of the *C. gibba* qPCR assay developed by Ophel-Keller et al. (2007).
Methods

Quantitative PCR (qPCR) assay design
Assays were developed in the SARDI Diagnostics laboratory (see Figure 4) as qPCR, using TaqMan® minor groove binder (TaqMan MGB) chemistry. DNA sequences of the desired genetic marker of target and related organisms were imported into the sequence manipulation software Bioedit (available from http://www.mbio.ncsu.edu/RNaseP/info/programs/BIOEDIT/bioedit.html.), and aligned using Clustal W. The genetic marker of choice is defined by the ability for that marker to delineate the target from heterologous species and also by the availability of sequences from publicly available databases. A range of DNA sequences was obtained from the public domain database GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The National Centre for Biotechnology Information (NCBI), as a division of the National Library of Medicine (NLM) at the National Institutes of Health (NIH), has developed databases to deal with molecular data, and facilitates the use of molecular databases by the research and medical community, Genbank is one of these databases and is an annotated collection of all publicly available nucleotide and amino acid sequences.

Sequences of target and related taxa were aligned to infer sequence regions that appeared to be useful diagnostic regions. DNA sequences were identified which were common to the target taxa but where there were enough differences to distinguish target from related taxa. Specific PCR primers and TaqMan MGB probes were developed for target taxa using the assay design software Primer Express v2.0 (Applied Biosystems), an application that designs primers and TaqMan MGB probes that display suitable thermodynamic properties and nucleotide content for efficient amplification.
Samples

*Musculista senhousia* samples (preserved in ethanol) were obtained from Singapore (thanks to Dr Maylene Loo) and *M. senhousia* DNA was kindly donated by Dr Jawahar Patil (CSIRO). *Perna canaliculus* samples were obtained frozen and purchased from an Adelaide fish monger. Other mytilid material was obtained through Dr Richard Willan, Northern Territory Museum and Dr Paul Fisher, Primary Industries and Fisheries, Queensland. Specimens of *Corbula gibba* were collected from Port Philip Bay for Ophel-Keller et al. (2007). Specimens of *Lentidium dalyfluvialis*, *L. mediterraneum* and *Potamocorbula amurensis* (Corbulidae) preserved in RNAlater (Ambion), were obtained from Mr Anders Hallan, University of Wollongong. Other samples utilised for specificity testing were collected whole (as part of other SARDI marine pest qPCR projects), and immediately stored frozen, or preserved in ethanol, for genomic DNA (gDNA) extraction.

DNA extractions

gDNA was extracted from target and non-target samples by one of two methods. The first method was the Root Disease Testing Service (RDTS) commercial DNA extraction method, a service provided by SARDI Diagnostics, while the second method was the QIAGEN DNeasy Blood and
Tissue kit following the manufacturer's instructions. DNA concentration was estimated by fluorometry (Wallac 1420 multilabel counter) using Quant-iT™ PicoGreen® (Invitrogen). gDNA for qPCR specificity experiments was typically diluted to 200 pg/μl.

**Quantitative PCR**

qPCR reactions were carried out in 384 well plates for analysis on an ABI HT 7900 sequence detection system (Applied Biosystems, Foster City, CA) using QuantiTect™ qPCR mastermix (QIAGEN). Each qPCR assay was run with plate controls (no DNA control and positive control for each assay) and was analysed with ABI SDS 2.3 software (Applied Biosystems). The PCR cycling conditions were: 15 minutes at 95°C (activation) plus 40 cycles of 15 secs at 95°C (denaturation) and 60°C at 1 minute (annealing). qPCR results are given as cycle threshold (Ct) values. The Ct value represents the PCR cycle number at which the fluorescence signal passes a fixed threshold, displayed as a horizontal green line in plots showing number of qPCR cycles vs magnitude of the fluorescence signal intensity (∆Rn) (see Figures 5-7).

**Results**

**Primers and probes**

We designed a range of potential qPCR assays for the detection and enumeration of *M. senhousia* and *P. canaliculus*. Primers and TaqMan MGB probes that have exhibited the highest level of specificity to date are shown in Table 1. Other primer and probe combinations that did not offer appropriate specificity or amplification efficiency were not considered further (data available on request). Table 1 lists the genetic marker that the qPCR assay targets, the nucleotide content of the primers and probes, and the melting temperature (Tm) of the primers and probes, which is important for determining the reaction conditions of qPCR experiments. The primers and probe for the *C. gibba* qPCR assay (developed by Ophel-Keller et al., 2007) are also shown in Table 1.
### Table 1: Primers and TaqMan MGB probes

<table>
<thead>
<tr>
<th>Assay</th>
<th>Genetic Marker</th>
<th>Forward Primer (5'-3')</th>
<th>Tm (°C)</th>
<th>Reverse Primer (5'-3')</th>
<th>Tm (°C)</th>
<th>Taqman MGB probe</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Musculista senhousia</em></td>
<td>28S</td>
<td>CGGCCGTCAGAAGCCTGT</td>
<td>61</td>
<td>CCAGCTATAAACTCCCCGACG</td>
<td>60</td>
<td>6FAM-CCGGAAGGTGACCCG-MGB</td>
<td>68.1</td>
</tr>
<tr>
<td><em>Corbula gibba</em></td>
<td>28S</td>
<td>GGCCAGCCTCGCTTGTG</td>
<td>61</td>
<td>CTATCGGACTCGTCGCTAGTATTTAG</td>
<td>58</td>
<td>6FAM-ATTCCCCAAACACCCG-MGB</td>
<td>68</td>
</tr>
<tr>
<td><em>Perna canaliculus</em></td>
<td>IGS</td>
<td>CGTAATCCTCAGTACTGGCTA</td>
<td>60</td>
<td>CTCTAACATAGGGGCTCCTA</td>
<td>61</td>
<td>6FAM-ATAGAGTAGAGCTATTAGGG-MGB</td>
<td>69.8</td>
</tr>
</tbody>
</table>

Key: 28S - 28S ribosomal DNA. IGS- Intergenic spacer of mitochondrial DNA. Tm-Melting temperature of primer/probe. 6FAM- 6 Carboxyfluorescein (fluorophore), MGB-Minor Groove Binder non fluorescent quencher.
Musculista senhousia
A TaqMan MGB PCR assay was designed for *M. senhousia* by Ophel-Keller et al., (2007). Subsequent analysis of environmental samples collected from Queensland showed that the assay lacked specificity, so it has been re-designed and re-evaluated as part of this project. New primer and TaqMan MGB probe combinations were designed in silico, based on alignments of 28S rDNA sequences, with the intention of developing an assay specific to *M. senhousia*, based on available controls (see Table 1). The assay was screened against a wide range of heterologous controls, including mytilids, other bivalves and a range of other invertebrate species (see Table 2 and Figure 5). We note that with one gDNA sample of *P. viridis*, late amplification (i.e. ~ Ct of 40) was exhibited (not shown). However this result is not consistent across all our *P. viridis* gDNA samples, and may reflect a low level contamination of this sample with *M. senhousia* gDNA.
### Table 2: Results of specificity testing for *M. senhousia* qPCR assay

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Genus</th>
<th>Species</th>
<th>DNA (pg/ul)</th>
<th>Ct values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mollusca</td>
<td>Bivalvia</td>
<td>Musculista</td>
<td>senhousia</td>
<td>200</td>
<td>19.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corbula</td>
<td>gibba</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Musculus</td>
<td>cummingianus</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Musculus</td>
<td>miranda</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perna</td>
<td>viridis</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Limnoperna</td>
<td>securlis</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Modiolus</td>
<td>micropterus</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichomya</td>
<td>hirsutus</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perna</td>
<td>canaliculus</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td>Echinodermata</td>
<td></td>
<td>Asterias</td>
<td>amurensis</td>
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</tr>
<tr>
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<td>Ciona</td>
<td>intestinalis</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascidiecola</td>
<td>sp.</td>
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<td>UD</td>
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<tr>
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<td>Crustacea</td>
<td>Carcinus</td>
<td>maenas</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
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<td>Sabella</td>
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<tr>
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<td></td>
<td>Undaria</td>
<td>pinnatifida</td>
<td>200</td>
<td>UD</td>
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<tr>
<td></td>
<td></td>
<td>NTC</td>
<td></td>
<td></td>
<td>UD</td>
</tr>
</tbody>
</table>

**Figure 5:** Amplification plot for *Musculista senhousia* qPCR assay specificity testing.
**Corbula gibba**

A TaqMan MGB qPCR assay was designed for *C. gibba* by Ophel-Keller et al. (2007). While there have been no reported issues with the specificity of the *C. gibba* qPCR assay, it has not previously been adequately tested on DNA from other corbulid species.

The *C. gibba* assay was further tested with the corbulid species: *Lentidium dalyfluvialis, L. mediterraneum* and *Potamocorbula amurensis* and found not to cross-react with any of these species (Table 3 and Figure 6). Lack of specificity was not encountered with the *C. gibba* qPCR assay during this study.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Genus</th>
<th>Species</th>
<th>DNA (pg/ul)</th>
<th>Ct values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mollusca</td>
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<td><em>Corbula</em></td>
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<td><em>Ciona</em></td>
<td><em>intestinalis</em></td>
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<td><em>sp.</em></td>
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Table 3: Results of specificity testing for *C. gibba* qPCR assay
Figure 6: Amplification plot for *Corbula gibba* qPCR assay specificity testing.
**Perna canaliculus**

Blair et al. (2006) designed an end-point PCR assay for the specific detection of *Perna canaliculus* based on mitochondrial gene sequences. The assay was modified from Blair et al. (2006), utilising their specific reverse primer and designing a new forward primer and Taqman MGB probe suitable for use as a qPCR assay. We have tested the *P. canaliculus* qPCR assay with a wide range of heterologous taxa, including *Perna viridis*. We attempted to obtain sample of *P. perna* from South Africa but were unsuccessful. The *P. canaliculus* qPCR assay did not cross react with the range of invertebrate taxa shown in Table 4 and Figure 7.

**Table 4: Results of specificity testing for *P. canaliculus* qPCR assay**

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Genus</th>
<th>Species</th>
<th>DNA (pg/ul)</th>
<th>Ct values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mollusca</td>
<td>Bivalvia</td>
<td>Perna</td>
<td>canaliculus</td>
<td>200</td>
<td>22.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corbula</td>
<td>gibba</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Musculus</td>
<td>cummingianus</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Musculus</td>
<td>miranda</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perna</td>
<td>viridis</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Limnoperna</td>
<td>securis</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Modiolus</td>
<td>micropterus</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichomya</td>
<td>hirsutus</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Musculista</td>
<td>senhouisia</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td>Echinodermata</td>
<td></td>
<td>Asterias</td>
<td>amurensis</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td>Chordata</td>
<td></td>
<td>Ciona</td>
<td>intestinalis</td>
<td>200</td>
<td>UD</td>
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<tr>
<td></td>
<td></td>
<td>Ascidieella</td>
<td>sp.</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td>Arthropoda</td>
<td>Crustacea</td>
<td>Carcinus</td>
<td>maenas</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sabella</td>
<td>spallanzani</td>
<td>200</td>
<td>UD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Undaria</td>
<td>pinnatifida</td>
<td>200</td>
<td>UD</td>
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<td>NTC</td>
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</table>
Discussion
Quantitative PCR (qPCR) assays have been designed for *M. senhousia*, *C. gibba* and *P. canaliculus*. These assays have been designed in TaqMan MGB format and have been tested against a range of target and heterologous taxa.

Ophel-Keller et al. (2007) developed a TaqMan MGB qPCR assay for *M. senhousia*, but subsequent testing of plankton samples from Queensland indicated that there were specificity issues with it. This study set out to design a new *M. senhousia* qPCR assay; once we had designed a putative assay the first step was sourcing new target material from Singapore and gDNA from CSIRO Marine and Atmospheric Research. Closely related native mytilids were also sourced for specificity testing. Based on available specimens it appears that the qPCR assay described here is specific to *M. senhousia* gDNA.
We adapted an end-point PCR assay for *P. canaliculus* (see Blair et al., 2006) to a qPCR assay suitable for routine use in the SARDI Diagnostics laboratory. The *P. canaliculus* qPCR assay is specific, based on available heterologous specimens, including a range of mytilids. Testing the *P. canaliculus* qPCR assay with *Perna perna* would be advantageous and efforts to source material will continue. It should be noted that the end-point PCR developed by Blair et al. (2006) was specific to *P. canaliculus* and we have utilised the specific element (reverse primer) of that assay and developed the forward primer and TaqMan MGB probe to be specific to *P. canaliculus*.

The *C. gibba* qPCR assay was developed by Ophel-Keller et al. (2007). While this assay did not exhibit any specificity issues when first developed, there was a need to test the assay with other corbulid species. Specimens were obtained from Anders Hallan, University of Wollongong and the *C. gibba* qPCR assay did not cross react with any of these specimens, or a range of other heterologous specimens.

**Conclusions and future approaches**

With continued development of these qPCR assays, in conjunction with the development of assays for other significant marine pest species, it is feasible that comprehensive surveillance for marine pests in South Australia can be achieved using DNA based assays. Molecular-based testing of environmental samples (water and sediments) offers the potential for more rapid and cost effective testing than more resource and time intensive traditional sampling methods. The Australian Testing Centre for Marine Pests (ATCMP) is proposed for establishment in partnership with Biosecurity SA at SARDI’s Diagnostic laboratories; qPCR assays developed for marine pests (including for this study) will be utilised for routine use at ATCMP.

In a parallel project (also funded by Biosecurity SA), SARDI has been developing a plankton collection method, in which samples are filtered and preserved for later molecular analyses. It is anticipated that this method can be used, in conjunction with qPCR, to detect a broad range of pest species for surveillance purposes. The *M. senhousia*, *P. canaliculus* and *C. gibba* qPCR assays will be utilised for the detection of DNA of target larval stages using this plankton collection strategy.
Specific diagnosis of marine pests is central to: (a) rapidly establishing the prevalence and distribution of marine pest species in the environment in conjunction with traditional sampling and taxonomic techniques; (b) monitoring changes in marine pest distribution spatially and temporally; and (c) conducting targeted eradication and control programmes if economics and logistics permit.
References


Slack-Smith, S.M., A. Brearley. (1987) Musculista senhousia (Benson, 1842); a mussel recently introduced into the Swan River estuary, Western Australia. Records of the Western Australian Museum 13, 225-230.


Glossary of Terms

ATCMP- Australian Testing Centre for Marine Pests.

CCIMPE- Consultative Committee on Introduced Marine Pest Emergencies.

Ct-Cycle threshold: qPCR cycle where fluorescence is observed above a threshold level indicating a positive result.

DNA- Deoxyribonucleic Acid: genetic information responsible for the development and function of all organisms, with the exception of some viruses.

gDNA-genomic Deoxyribonucleic Acid: the total DNA of an organism, or the genome of an organism.

IGS- Intergenic Spacer of mitochondrial DNA.

ITS-2: second Internal Transcribed Spacer: a region of ribosomal DNA that does not code for any genes.

mtDNA- Mitochondrial DNA: the genome of the intracellular organelles called mitochondria. Considered an informative diagnostic region.

NIMPCG- National Introduced Marine Pest Coordinating Group

NTC- No Template Control: a PCR reaction with no DNA template added, is used to ensure that PCR is not previously contaminated i.e. NTC should not be a positive result.

Nucleotide: Molecules, that when joined together make up the functional units of DNA.

PCR- Polymerase Chain Reaction: enzymatic technique used for the amplification of nucleic acids (e.g. DNA).

qPCR- Quantitative Polymerase Chain Reaction: PCR reaction whereby amplification is monitored in real time through the use of fluorescent dyes or probe based chemistry.

TaqMan MGB probe-TaqMan Minor Groove Binder probe: hybridises to specific fragment of DNA and emits fluorescence; used to quantify target DNA in a sample.

rDNA- ribosomal Deoxyribonucleic Acid: codes for vital cellular components in Eukaryotes; an informative diagnostic marker.

RDTS- Root Disease Testing Service: a commercial diagnostic service at SARDI.