Forest & Wood Products Australia

Project Report

DEVELOPING A METHOD TO SURVEY FOR GIANT FRESHWATER CRAYFISH using eDNA

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Developing a method to survey for giant freshwater crayfish using eDNA

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Amelia Koch, Leon Barmuta and Alejandro Trujillo Gonzalez

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Researcher/s:

Amelia Koch, Perpetua Turner
Forest Practices Authority, 30 Patrick St, Hobart 7000

Leon Barmuta, Anne Watson
University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia

Sam Godwin, Dianne Gleeson, Rheyda Hinlo, Alejandro González, Elise Furlan
University of Canberra, ACT 2617

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Forest & Wood Products Australia Limited
Level 11, 10-16 Queen St, Melbourne, Victoria, 3000
T +61 3 9927 3200 F +61 3 9927 3288
E info@fwpa.com.au
W www.fwpa.com.au

Executive Summary

The giant freshwater crayfish (*Astacopsis gouldi*) is listed as threatened on both the State and National legislation, and the Recovery Plan for the species lists forestry as potentially negatively impacting the species. The aim of the current study was to develop a genetic assay to enable detection of the species from environmental DNA (eDNA) water samples.
We designed primers and probes specific to *A. gouldi* and tested their specificity and sensitivity in the lab. We then applied the method to water samples collected from aquaria and natural freshwater streams in Tasmania where *A. gouldi* is known to occur. In addition, we sampled from a field site with no known *A. gouldi* presence to serve as a negative control. We demonstrate that eDNA-based detection can enhance field monitoring of *A. gouldi*, potentially leading to a more cost-effective survey method. eDNA sampling for this species has the potential to improve planning tools such as habitat suitability maps as well as facilitating monitoring to demonstrate effectiveness of management.

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Introduction

The giant freshwater crayfish (*Astacopsis gouldi*) is listed as vulnerable on both the State and National Threatened Species Protection Acts. It is the largest freshwater invertebrate in the world (Walsh and Haller 2012) and found across the north of Tasmania in a wide range of streams. Adults typically occur in slower flowing sections of stream and pools, often sheltering under decaying logs and undercut banks. Juveniles are more likely to shelter under cobbles and boulders in flowing sections of stream (Walsh and Walsh, 2013).

Forestry operations (e.g. harvesting, roading, plantation establishment, etc) have the potential to create adverse impacts on *A. gouldi* through the loss of riparian canopy cover, increased runoff, sedimentation, changes in hydrology and chemical spraying. A habitat suitability map has been developed for this species which is used routinely by forest planners for prioritising management actions. However the coarse nature of the map means that in some cases surveys of habitat characteristics known to be important for the species are also required. This survey method is time consuming and subjective and further testing of the habitat suitability map is required to improve its usefulness in coupe scale planning.

Environmental DNA (eDNA) is genetic material obtained from environmental samples such as water. eDNA-based detection is becoming widely used for a range of taxa, including several crayfish species (Harper et al., 2018; Ikeda et al., 2016; Mauvisseau et al., 2018; Tréguier et al., 2014; Cowart et al., 2018). eDNA sampling has the potential to be a very efficient technique for determining the presence or absence of crayfish species, including *A. gouldi*. The aim of the current study is to develop a highly sensitive, highly specific genetic assay to enable detection of the Tasmanian giant freshwater crayfish from environmental DNA (eDNA) water samples. This will enable testing and refinement of the habitat suitability map used by forest planners operating in both native forest and plantation landscapes, but also facilitate other research on the species.

The full process undertaken for this project is published Trujillo-González et al (2021), but a summary is provided below.

Methodology

Primer development and testing

The first stage of the process was to identify a genetic primer that was found consistently, but exclusively, in *A. gouldi* across their range. There are two other *Astacopsis* species found in Tasmania (*A. franklinii* and *A. tricornis*), and several species from a different genus of crayfish (*Engaeus*) so it was particularly important to ensure the primer could differentiate *A. gouldi* from these other species.

A total of 37 tissue samples, representing 13 different species, were collected from the Tasmanian Museum and Art Gallery (TMAG), the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Todd Walsh Collection, and wild specimens (IFS collection permit no. 2019-41, DPIPWE Permit No. TFA 19084). All tissue samples were sent to the University of Canberra for DNA extraction (Table 1).

Sequenced amplicons from this study were compared to other registered *Astacopsis* sequences. Regions where differences in the genetic signature existed between species were identified. A primer pair and probe combination was selected which appeared during the primer testing to be a sensitive test, efficient in terms of processing, and specific to the species.

The specificity of the primer developed was evaluated using published (National Centre for Biotechnology Information (NCBI) website) and tissue-derived genomic DNA. The assay was tested on six *A. gouldi* tissue samples and 12 other crayfish species known to occur in Tasmania and south eastern Australia (Table 1).

The qPCR efficiency and sensitivity of the assay was assessed by obtaining the limit of quantification (LOQ) and limit of detection (LOD) using tissue-derived DNA and synthetic standards. During the testing, triplicate positive and negative control samples were included in each run.

Species	Number of samples
Astacopsis gouldi	6
Astacopsis franklinii	7
Astacopsis tricornis	5
Engaeus mairener	4
Engaeus granulatus	1
Engaeus fossor	4
Engaeus yabbimunna	1
Engaeus cunicularius	1
Engaeus lengana	1
Engaeus nulloporius	1
Geocharax tasmanicus	1
Ombrastacoides huonensis	1
Ombrastacoides sp.	1
Cherax destructor	2

Table 1. A summary of the genetic samples considered when developing the primer.

eDNA sampling and field validation

Environmental DNA samples were obtained in November 2019 from eight sites in northern Tasmania, seven with known presence of *A. gouldi* (two aquaria/troughs and five streams) and one site with no known *A. gouldi* populations (Fig. 1).

At each stream site, water samples were collected in eight 1 L bottles, with one 1 L field control. Samples were collected along a 100 m length of stream where possible. The aquaria had four tanks containing adult *A. gouldi* and a flow-through trough containing ~30 juvenile *A. gouldi.* A single 1 L sample was taken from each tank and three 1 L samples were collected from the trough. A single field control was taken for the aquaria samples. All water samples were pumped through 42 mm, 1.2 µm pore size filter papers.

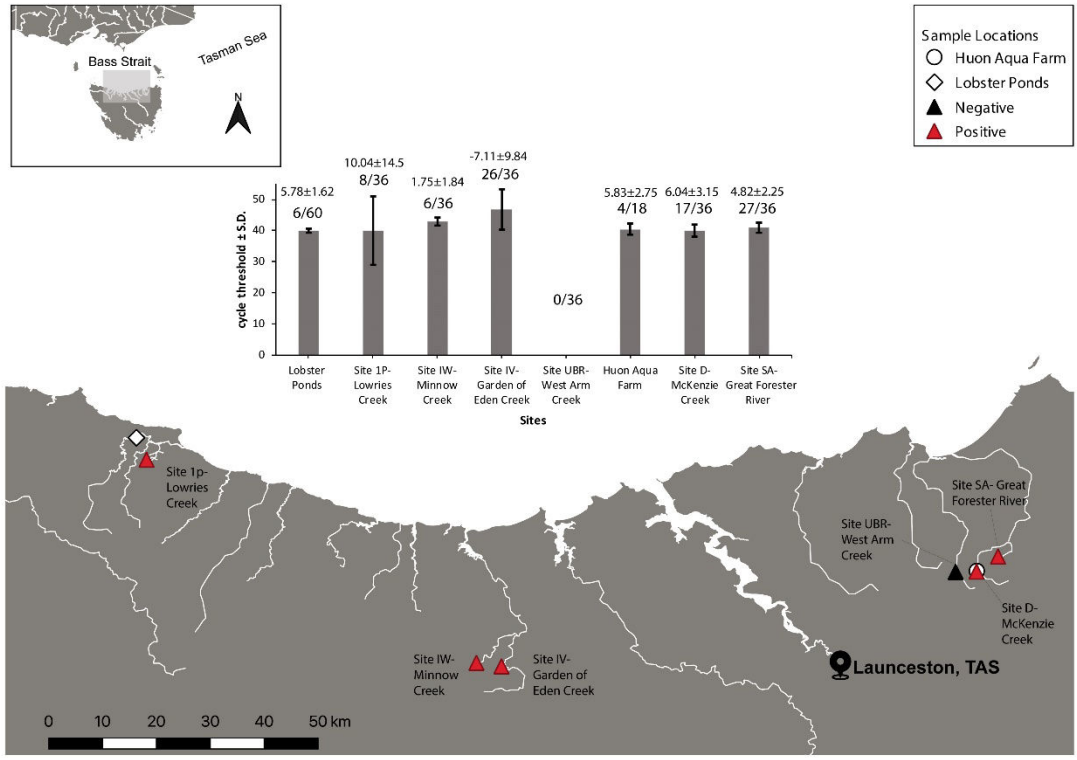

Figure 1. Location of the field sampling sites in northern Tasmania. Reprinted from Trujillo-Gonzalez et al (2021).

DNA extraction of eDNA field samples

DNA from the filter papers were extracted using standard procedures. A negative extraction control was included in each batch to monitor potential contamination in procedures.

All samples were initially amplified in duplicate to check for the presence of PCR inhibitors. If no inhibitors were detected, the samples were used for further testing. Samples were then run using the identified *A. gouldi* assay. If all replicates from a sample showed no amplification, the samples were amplified again. If after three repeats there was still no amplification, the samples were deemed negative. All positive replicates were purified and sequenced.

Results

Developing the primer

The qPCR primer and probe developed in this study successfully amplified the target genetic region in all five *A. gouldi* tissue samples. There was no amplification with the other crayfish species tested in this study. Amplification efficiencies ranged from 89–93% for plates assayed in this study. Positive controls amplified in all plates and no amplification occurred in the negative template controls.

Field validation

Astacopsis gouldi eDNA was successfully detected in all sites where the presence of the species had been previously confirmed. Minimal detection was observed in one of four tanks with live *A. gouldi* specimens, with only 2/24 positive technical qPCR replicates. The only site with no detection was the negative control site. Inhibition was detected in all samples collected during this study. All positive eDNA detections were confirmed by Sanger sequencing and found to have 99–100% similarity with *A. gouldi* sequences from this study and NCBI.

Discussion

The giant freshwater crayfish is listed as threatened, and monitoring of the species is one of the requirements in the *A. gouldi* Recovery Plan (Commonwealth of Australia, 2017). Monitoring is needed to help inform population trajectories, as well as determining if current management actions are effective. Traditional monitoring methods (hand searching) are time consuming, invasive, and can vary in efficiency between searchers. This study has been successful in identifying a sensitive new technique for determining the presence or absence of *A. gouldi* that can be used to develop a monitoring program or update management tools.

This study adds to the literature showing that eDNA sampling has considerable potential for monitoring crayfish species (Harper et al., 2018; Ikeda et al., 2016; Mauvisseau et al., 2018; Tréguier et al., 2014). The results from this study indicate that minute traces of eDNA were detected in water samples from all study sites known to have *A. gouldi*. “Testing water samples using real time PCR could inform future monitoring efforts on the presence of *A. gouldi* in low densities, thus making formal occupancy modelling (MacKenzie et al., 2018) feasible for this species across landscapes with varying practices in catchment management” (Trujillo-Gonzalez et al, 2021).

However, it should be noted that only one of the four aquaria sampled with adult *A.gouldi* had positive detection, indicating that false negatives can occur in these enclosed systems. However, inhibition in water samples was detected in all tanks, and it is known that inhibitors can compromise detection probability and increase the likelihood of false negatives (Hunter et al., 2019). Partial or full inhibition of eDNA amplification decreases assay sensitivity and increases the potential for false negatives (McKee et al., 2015). The imperfect detection that can occur using eDNA surveys is widely acknowledged and methods/models are used to develop sampling regimens with high detective probability (Furlan et al., 2015; Song et al., 2020).

Marginal detections were also found from streams previously reported as having *A. gouldi* populations (Lowries Creek = 4/24 and Minnow Creek=3/24 positive qPCR replicates). Levels of eDNA in the water can be low when target animals are small, at low densities, or when they shed eDNA at low rates (Furlan et al., 2015; Hunter et al., 2019). Little is known about the rate at which *A. gouldi* shed DNA, or DNA degradation rates in the water. Further research is needed to better understand these matters in order to facilitate interpretation of any monitoring results. Research is also needed to determine the most efficient time of year to survey *A. gouldi* using eDNA-based methods.

Conclusions

This study validates the use of a newly developed probe-based qPCR assay used to detect eDNA from endangered *A. gouldi* in Tasmania. This technique can facilitate the development of a population monitoring program, be used to conduct research on habitat associations of the species or the effectiveness of management actions, or to update management tools such as the habitat availability map used by the Tasmanian forest industry.

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A more detailed account of this research is published in

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