Metagenomic Amplicon Sequencing as a Rapid and High-throughput Tool for Aquatic Biodiversity Surveys

PhD Thesis

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Metagenomic Amplicon Sequencing as a Rapid and High-throughput Tool for Aquatic Biodiversity Surveys

A Thesis Submitted By

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For Mum, Dad, and Stephen.
(Your love and support made this possible)
xxx

Summary

Healthy aquatic systems are essential for life on this planet, and provide a variety of goods and services for humans such as drinking water, food production, waste disposal, and climate regulation. Anthropogenic impacts such as over exploitation of aquatic resources, introductions of invasive species, pollution, and climate change pose great risks for the health and sustainability of these ecosystems. Because of these risks, extensive and detailed biological surveys are regularly required to monitor and manage aquatic ecosystem health. Traditional survey approaches, including morphological-based identification and counting of organisms, are time consuming, costly, and dependent upon highly skilled taxonomic experts. Recently developed molecular methods, where DNA mixtures present in environmental samples are sequenced and taxonomically identified using genomic markers, are rapid and cost-effective, and may substantially improve biological surveys and ultimately aquatic system management. In particular, metagenomic amplicon sequencing of environmental DNA (eDNA) can characterize hundreds to thousands of species within a single sample in a timely and cost effective manner, and allow hundreds of samples to be sequenced in a single reaction.

This PhD thesis aims to develop and refine eDNA amplicon sequencing approaches in order to examine current global problems in aquatic ecosystems. Specifically, this thesis includes a review of eDNA amplicon sequencing protocols and provides recommendations for sampling aquatic environments, laboratory procedures, and bioinformatics processes (Chapter 2). Following this, chapter three utilized eDNA sequencing to monitor invasive and threatened fish species in a sensitive and ecologically important river system, comparing results from fyke net- and eDNA-based surveys to analyze the accuracy and effectiveness of eDNA amplicon sequencing approaches (Chapter 3). In Chapter four, I

examine the introduction and distribution of harmful algal taxa in high-risk marine locations across the entire continent of Australia, characterising a variety of harmful algal species associated with international and domestic shipping activities to potentially manage and mitigate the spread of these species. The use of historical port sediment samples within this study provides valuable temporal information to establish a baseline for the biodiversity distribution of harmful algae, essential for several international conventions focused on limiting the transmission of these harmful microorganisms. In chapters five and six, eDNA sequencing was utilised to identify bacterial taxa within drinking water distribution systems (DWDSs). Chapter five focuses on comparing current commonly used compliance measures with metagenomic approaches while screening for key indicator and pathogenic species throughout two full-scale DWDSs. Chapter six utilized this novel sequencing approach to analyze the efficiency of different water treatment procedures, while focusing on limiting biofilm formation in DWDSs. Overall, the thesis develops and demonstrates the practical applications of metagenomic eDNA sequencing on three distinctive taxonomic groups: vertebrates, eukaryotic microbes, and bacteria. The methods developed throughout provide critical advances for environmental monitoring organisations, including governmental departments, shipping, fishing and aquaculture industries, and water quality corporations. Further, this thesis and the future use of these molecular approaches greatly extends the knowledge of both bacterial and eukaryotic microbial communities in natural and man-made aquatic environments, improving industry efficiency and mitigating public health crises.

Declaration

- This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been made in the text.
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Jennifer L. A. Shaw

Date 25th Feb 2015

Preface

This PhD thesis is a result of a three-year project conducted at the University of Adelaide, South Australia. Laboratory work was carried out in both the South Australia Water Corporation's microbiology laboratories in Victoria Square and dedicated low-contamination DNA facilities at the Australian Centre for Ancient DNA (ACAD) within the University of Adelaide. Both laboratories contain up to date appliances and technologies, which enabled this project to be carried out effectively with minimal contamination risks. All material enclosed in this document is my own work except where there is clear acknowledgement and reference to the work of others. This PhD study was funded by the Australian Research Council (LP0991985) and is part of a collaboration between ACAD and SA Water. SA Water has shown significant interest in the development of this technology due to a high cost currently associated with environmental and water quality monitoring, which they are aiming to reduce. This thesis also resulted in collaborations with other environmental organisations and universities; for example, the Department of Environment, Water and Natural Resources (DEWNR), the Murray-Darling Basin Authority, and the Institute for Marine and Antarctic Studies (IMARS) at the University of Tasmania (UTAS). The methods developed and discussed within this thesis will enable greater sampling effort and increase the amount of data available to decision makers, allowing more informed water management decisions at a time when water and food security, and general aquatic system health is critical.

‘Only when the last tree has died, the last river been poisoned, and the last fish been caught, will we realize we cannot eat money.’

~ Cree Indian Proverb ~