Vulnerability of an iconic Australian finfish (barramundi – *Lates calcarifer*) and aligned industries to climate change across tropical Australia

Dean R. Jerry, Carolyn Smith-Keune, Lauren Hodgson, Igor Pirozzi, A. Guy Carton, Kate S. Hutson, Alexander K. Brazenor, Alejandro Trujillo Gonzalez, Stephen Gamble, Geoff Collins and Jeremy VanDerWal

Centre for Sustainable Tropical Fisheries & Aquaculture

Australian Government
Department of Climate Change and Energy Efficiency

Australian Government
Fisheries Research and Development Corporation

Project No. 2010/521

ISBN 978-0-9875922-9-3

Inquiries should be directed to:

James Cook University

Douglas,

Qld 4810

Disclaimer

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a readers particular circumstances. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the FRDC.

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the Federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

NON TECHNICAL SUMMARY

2010/521. Vulnerability of an iconic Australian finfish (barramundi – *Lates calcarifer*) and aligned industries to climate change across tropical Australia

PRINCIPAL INVESTIGATOR: Dr D.R. Jerry

ADDRESS: Centre for Sustainable Tropical Fisheries and Aquaculture
James Cook University
Douglas
QLD 4810
Telephone: 07 4781 5586 Fax: 07 4871 4585

OBJECTIVES:

1. Define current genetic stock structure of barramundi using microsatellite markers and overlay genetic structure with environmental data to identify climatic scenarios stocks may be exposed to in the future.
2. Examine current thermal tolerances and associated physiological/energetic consequences of thermal adaptation in genetically divergent barramundi stocks across tropical Australia.
3. Quantify parasite impacts on sea-cage aquaculture of barramundi under different temperature and salinity conditions leading to the development of adaptive management strategies to minimize impacts under altered climate change scenarios.
4. Develop predictive models incorporating new physiological and genetic data with available population genetic, environmental and fisheries data, to identify potentially vulnerable wild stocks and associated stakeholders under realistic climate change predictions. Opportunities for expansion of fisheries and aquaculture will be determined.

OUTCOMES ACHIEVED TO DATE

Barramundi, *Lates calcarifer*, is an iconic, tropical, finfish species on which important commercial, aquaculture and recreational fisheries are based across the breadth of northern Australia. Due to this diversity in stakeholder interest, the barramundi fishery is important socio-economically for regional northern communities. As such, understanding how climate change will impact on fishery productivity and connectivity and the identification of adaptation options if the fishery is adversely affected will be of importance to the prosperity and resilience of many tropical communities.

The primary outcome of this project was to provide various stakeholders in the multifaceted barramundi fishery (including both the commercial/recreational wild fishery and aquaculture industry) with targeted scientific data and models assessing the vulnerability of this iconic species to future impacts of climate change. Access to this data will enable stakeholders to identify adaptation strategies and put in place informed planning processes ensuring the future viability of commercial and recreational activities dependent on the species.

To assess the vulnerability of barramundi to future climate impacts the project was divided up into four components, each of which addressed a separate scientific question. Firstly, a comprehensive genetic audit with high resolution DNA markers was undertaken to describe the current stock structure of wild Australian barramundi. This audit was required as there had been no previous studies that had genotyped fish from the entire Australian distribution in the one study. Additionally, other large-scale genetic audits conducted on this species were completed 25 years ago. It was possible that over this period anthropogenic impacts such as fishing and restocking, as well as the possibility of altered environment, may have disrupted historical genetic structure. Using 16 microsatellite DNA markers we were first able to demonstrate temporal stability of population genetic structure in Australian barramundi over 25 year time scales. We were then able to confirm the existance of 21 genetically distinguishable subpopulations (largely interbreeding management units) spread across the species' distribution. These management units can be grouped into six broader genetic stocks, with some stocks showing a strong signature of mixed ancestry from neighbouring stocks, while others form more genetically discrete units (potential Evolutionary Significant Units). Some evidence suggestive of natural selection on parts of the barramundi genome across the

species range were detected and warrants further investigation, as it implies that some genetically divergent stocks (or subpopulations) may have become locally adapted to varying environmental conditions.

Parasites present major economic and environmental concerns for barramundi aquaculture. As well as possible impacts on barramundi itself, climate change is expected to also affect the frequency and intensity of parasite epizootics in aquaculture by enabling parasites to complete their life-cycles faster. These changes may be primarily driven by warmer water temperatures and variation in salinity. To understand the role parasites may have in future barramundi aquaculture production we first conducted a survey of parasites that can affect barramundi and performed a risk assessment to identify parasites posing high risk to the marine barramundi aquaculture industry. Experiments were also conducted on two important marine parasites, *Neobenedenia* and *Lernanthropus latis*, to predict their life-cycle response to water temperature and salinity. These studies confirmed that the life cycle of these two important species will speed with warming temperatures. Barramundi from different genetic stocks were also shown to have the same susceptibility to *Neobenedenia*.

The current research project also provided baseline data on aerobic metabolism and hypoxia tolerance across five sub-populations of barramundi from the different genetic stocks. Experiments did not detect any significant changes in energy metabolism or tolerance to low oxygen amongst fish from the various stocks when exposed to cool, warm and hot temperatures. Barramundi therefore appear to cope with wide ranges in environmental temperature well above that expected to occur under climate change, as well as to possess a high-degree of tolerance to temperature-induced hypoxic episodes.

Finally, models were developed incorporating both biological characteristics and environmental projections from 18 “business as usual” climate scenarios to predict changes in the future species’ distribution and catch per unit effort (CPUE) of the wild fishery, and productivity and geographical range suitable for aquaculture. All projected climate scenarios were consistant and predicted an expansion in the distribution of barramundi in a southward fashion towards the year 2085, as well as thermal regimes suitable for barramundi pond aquaculture. Habitat for barramundi in northern Australia was predicted to expand inland, particularly in the Northern Territory and Gulf of Carpentaria. CPUE is predicted to increase, indicating that fisheries in most regions may become more productive. Aquaculture

productivity linked to barramundi growth models was also predicted to increase with warming conditions, primarily due to higher minimum winter temperatures and an increase in temperatures around the optimum for growth in the species.

Overall, the results from this project highlight a degree of flexibility and resilience of barramundi to cope with temperatures and environmental regimes predicted by future climate scenarios. Physiological tests demonstrate that the species performs robustly under a wide range of thermal conditions even when challenged with temperature rises expected to occur by 2085 (~3.6 °C), whilst models predict increased productivity and expansion of the fishery. Adaptation strategies and future planning processes need to consider and account for the real possibility of a southward expansion of thermal habitat suitable for the wild fishery and aquaculture production.

KEYWORDS: Barramundi, aquaculture, fisheries, genetic structure, hypoxia, parasites, climate simulations.

ACKNOWLEDGEMENTS

The genetic audit of wild barramundi stocks was only possible due to a large number of sample donations. Sample donations were kindly received from numerous recreational and commercial fishers and fisheries monitoring personnel. A large number of historical tissue samples were also made available from earlier genetic audits by Prof. Jane Hughes, Griffith University. A large number of recreationally fished samples from the remote Kimberley Region of Western Australia were provided by Ray Lanaghan. Numerous other samples were provided by the following people; Alexander Brazenor, Bill Palmer, Glen Bowry, Paul Bowry, Jay Arnold, Tim Freebody, Daneil Godfrey, Matt Brook, Graham Minion, Greg Smith, Tansyn Noble, Dick Pasfield, Danny Grassel/Alex Julius and fishing guides from the Arnhemland Barramundi Nature Lodge; Bill Sawynok and members of the King Ash Fishing Club; Russel Bowman (The Lure Shop) and members of the Endeavour Sportfishing Club; Warren Dewich (Katherine Rod'n'Rifle); Blake Benbow, Damien Bode, Jay Wheelock, Scott Medling and Ken Macfarlane from the Nikol Bay Sport Fishing Club; Jane McNeil from Broome Aquaculture Centre; Kevin Bochow and other participants of the 2008 Daly River Barra Classic; Fisheries personnel including Brenda Foley (QLD), Jonathan Staunton-Smith (QLD), Quentin Allsop (NT fisheries) and Steve Willemore (WA fisheries). Assistance in the laboratory extracting genomic DNA and amplifying genetic samples was provided by Shannon Loughnon (Flinders University) and by Tansyn Noble (JCU). Students Jose Domingos and Mackenzie Hansler helped with the dissociated cell analyses. Bluewater Barramundi, Clear Water Marine Farms, Good Fortune Bay and Marine Produce Australia are thanked for provision of fish specimens for parasite analyses. Professor Ian Beveridge (University of Melbourne), Professor Geoff Boxshall (Natural History Museum, London) and Associate Professor Ian Whittington (South Australian Museum) aided parasite identification. Dr Leslie Chisholm and Thierry Laperousaz (South Australian Museum) curated accessioned parasite material. Use of the catch and effort data is courtesy of the State of Queensland, Australia through the Department of Agriculture, Fisheries and Forestry. We acknowledge use of climate data, methodology, and R scripts developed by Cassandra James (James Cook University) for James et al. (2013). Future climate scenarios were sourced from the Tyndall Centre (http://climascope.tyndall.ac.uk).

BACKGROUND

Tropical Australia is a region impacted upon by complex climate patterns, including monsoonal intra-year variability in rainfall and evaporation rate, mid-latitude seasonal oscillations, intra-annual fluctuations in the Madden Julian Oscillation, as well as longer global climate impacts such as the Interdecadal Pacific and El Niño Southern Oscillation Indices (Balston 2007; 2009a,b). Predicted climate change up to 2100 is expected to further introduce an additional layer of stochastic variability onto this already complex climate pattern through average annual temperature increases of 0.3 - 5.2 ºC and more frequent and intense extreme rainfall events (ie -5% - 15% in the wet tropics; Balston 2009b). As a consequence, tropical aquatic environments will be impacted through changes in water temperature, freshwater flow regimes, salinity levels driven by evaporation, precipitation and saltwater incursions, and nutrient pulses, all of which may cause changes in recruitment and migration patterns of fish, alter growth rates, and increase exposure to stress-induced disease epidemics (Frye 1983; Kapetsky 2000; Van Putten and Rassam 2001).

Barramundi (*Lates calcarifer*) is an iconic, catadromous, fish species that supports important commercial, aquaculture and recreational fisheries throughout the breadth of tropical Australia. Due to this diversity in stakeholder interest, the barramundi fishery is important socio-economically for regional communities and, as such, understanding how climate change will impact on fishery productivity and connectivity and the clear identification of adaptation options is of upmost importance to the future prosperity and resilience of tropical communities.

The link between climate variability and inshore/estuarine/freshwater fisheries throughout tropical Australia has to date been poorly evaluated and is primarily restricted to the examination of recruitment impacts by freshwater flows on localized populations of marine prawns and barramundi (Balston 2007). These studies have shown that recruitment and abundance demographics are dependent on climate induced hydrological factors, such as riverine freshwater flow rates and water temperatures. Correlated with demographic changes in these species were commercial catches. For example, warm sea temperatures, high rainfall leading to increased freshwater flows, and low evaporation, correlated strongly with increases in commercial barramundi catches from Princess Charlotte Bay, QLD, two years after the climate event (Balston 2007). Conversely, high evaporation and altered flow regimes under

simulated climate change scenarios relative to those predicted to occur by 2030 and 2070 indicate an overall decrease in anticipated barramundi catches within Princess Charlotte Bay (Balston 2007). However, the importance of these environmental factors to future barramundi catch rates under climate change scenarios has not to date been examined for populations across the full species distribution in northern Australia.. If tropical fish faunal assemblages, including barramundi, respond to altered climate scenarios as seen for temperate Australian fishes (Last et al., 2010), we can expect corresponding dramatic reorganisation of species distribution's and abundance's across the lower-latitudes as we move towards climate conditions predicted to occur by 2030.

Barramundi populations across the almost 17 degrees of latitude over which they occur in northern Australia have been shown to possess genetic structuring (Shaklee and Salini 1985, Salini and Shaklee, 1988; Chenoweth et al., 1998; Doupé et al., 1999; Keenan 1994; Marshall 2005). Barramundi is considered a eurythermal species, yet until recently very little was known about how it tolerates the broad range of temperatures (16 to 36°C) encountered across its extensive geographic range, or whether genetic strains exhibited different tolerances to environmental variables such as water temperature, hypoxia, or disease. Within the last 4 years, however, research at James Cook University has begun to show that barramundi originating from different populations may possess differing degrees of adaptive genetic variability to at least temperature. Edmunds et al., (2009), for example, highlighted possible differences in the capacity of barramundi populations from Darwin (NT) and Bowen (QLD) to deal with anoxia and lactate processing through altered expression of LDH-B, an important gene involved in lactic acid metabolism. Similarly, Newton et al., (2010) using a novel fin cell assay, demonstrated that a hatchery population of barramundi originating from Darwin (NT) was capable of surviving a prolonged upper thermal stress better than a population from the extreme south-eastern part of the species' range (Gladstone, Qld). Transcriptome studies also highlighted significant differences in gene regulation patterns among these same two populations when co-reared at both high and low temperatures (Newton et al., 2013). These studies indicate that there may be a large amount of natural adaptive genetic variability among barramundi populations that if properly defined and managed may allow for some limitation of climate change impacts to end-users of the fishery, either through better understanding of how different genetic strains will respond, or through the direct identification of adaptation options taking advantage of this genetic variability. Through the gathering of key data, some

of the adaptation options that would be useful to stakeholders in the barramundi fishery include a) the identification of wild and cultured fisheries under threat from altered biophysical parameters, b) possible new opportunities in these fisheries where population demographics may benefit under altered climate scenarios, c) identification and delineation of thermally tolerant barramundi strains that maintain metabolic homeostasis and exhibit efficient bioenergetic processing of artificial foods for aquaculture purposes, d) improved understanding of the threat posed by parasites and/or disease that will likewise respond to altered climate and the development of integrated management protocols to limit parasite impact on cultured populations.

The potential impacts of climate change on temperate aquatic faunal assemblages are well studied and the bulk of our present-day understanding of climate change impacts is therefore biased towards the temperate regions (see Last et al., 2010). There is currently a real void in knowledge on how tropical assemblages will respond to climate change and what options may be available for businesses and regional communities to minimize impacts; particularly for important tropical-specific fisheries such as barramundi, pearl oysters, marine prawns and reef fishes. The strategic challenge therefore is to gather relevant data that can be fed into "holistic" models so that threats and opportunities for tropical species under perceived future climate scenarios can be clearly delineated. Discussions with key end-users such as the Northern Territory Department of Primary Industries and Fisheries, Queensland Department of Agriculture, Fisheries and Forestry, recreational fishing groups and the Australian Barramundi Farmers Association have all highlighted a real lack of knowledge on how aligned industries will cope and adapt to changes in the barramundi fishery. The project proposed for barramundi here sought to gather essential new biological data relevant to wild and cultured fisheries and to integrate this new data with current biophysical climate change models to allow adaptation plans to be developed based on rigorous science outputs.

NEED

Barramundi-associated industries are integral to the socio-economic health of tropical communities. This species supports a strong commercial and aquaculture fishery (~$80 million) and has high societal value being the major recreationally targeted fish in tropical waters (valued at ~$50 million) and is intrinsically important to indigenous culture. In QLD, barramundi is the fastest growing aquaculture sector (~ 21% p.a).

For barramundi there is a need to understand future climate patterns, their impact on distribution, carrying capacities and local abundances within the commercial/recreational fisheries, as well as the threats and opportunities for aquaculture. Current climate-orientated models are restricted to the QLD wild fishery and these predictions need to be extended to other northern Australian regions, and the aquaculture landscape. In QLD, catch rates are linked to climate variability (Balston 2009a,b) and the abundance/connectivity of climate sensitive wetland/mangrove habitats (Meynecke et al 2008). Pond-based aquaculture often already experiences summer water temperatures above those for optimum growth. However, no estimates on climate induced vulnerability of the whole fishery, or on current land and sea-based aquaculture (geophysical, physiological and nutritional impacts), are available, and the capacity for the aquaculture industry to selectively breed for tolerance to altered temperature regimes is unknown. These needs strongly align with those identified in the Marine Biodiversity Adaptation Plan as highest priority for the various sectors.

OBJECTIVES

1) Define current genetic stock structure of barramundi using microsatellite markers and overlay genetic structure with environmental data to identify climatic scenarios stocks may be exposed to in the future.

2) Define current thermal tolerances and associated physiological/energetic consequences of thermal adaptation in genetically divergent barramundi stocks across tropical Australia.

3) Develop predictive models incorporating new physiological and genetic data with available population genetic, environmental and fisheries data to identify potentially vulnerable wild stocks and associated stakeholders under realistic climate change predictions. Opportunities for expansion of fisheries and aquaculture will be determined.

4) Quantify parasite impacts on marine aquaculture of barramundi under different temperature and salinity conditions and develop adaptive management strategies to minimize impacts under climate change scenarios.

MATERIALS AND METHODS

Scientific inquiry undertaken in this project was divided up into four distinct components. Firstly, an extensive population genetic analysis using microsatellite DNA markers was performed so that the present day genetic stock structure of barramundi in Australia could be resolved (Component A). This genetic analysis identified six major genetic stocks of barramundi in Australia. Fingerlings from up to five of these genetic stocks were acquired and then subject to cellular and metabolic physiological tests (Component B) to examine evidence for local adaptation to temperature and hypoxia amongst barramundi from the different stocks. Component C comprised an extensive survey and associated risk analysis of marine parasites affecting barramundi and the response of barramundi from different stocks to infection to the important marine pathogen (*Neobenedenia*). Component C also examined the impacts that altered thermal and salinity regimes will likely have on the life cycle of this parasite. Finally, available population genetic, biological and environmental data were used to model the response of both the wild fishery and aquaculture production under various future scenarios of climate (Component D).

Component A: The population genetic structure of Australian barramundi and its relationship to landscape features.

Background

Genetic connectivity among spatially separated populations, and levels of genetic diversity, are important factors that may contribute to the spread of genes advantageous under climate change conditions. While previous studies showing significant genetic structuring of barramundi populations in Australia exist (Shaklee and Salini 1985; Salini and Shaklee 1988; Keenan,1994; Chenoweth et al., 1998; Doupé et al., 1999; Marshall 2005) these have used diverse genetic methods and widely varying spatial coverage and scale. The most comprehensive past study was conducted with samples collected some 25 years ago and suffered a substantial north-eastern bias. The few later studies of the western parts of the species' distribution used different genetic markers and had little representation of the north-eastern populations. At the outset of this project, therefore, there was a real need to apply a consistent set of powerful genetic markers to explore the present day genetic stock structure and diversity of barramundi across the full species' distribution in Australia.

Materials and Methods

Genetic Sampling

Small tissue samples (fin or muscle tissue preserved in either 70 % ethanol or a saturated salt solution containing 20% dimethyl sulfoxide and 0.25 M EDTA preservative) were obtained from 3017 barramundi from a diverse range of sources. These samples were distributed across 48 different collections (Figure 1, Table 1). Recreational fishers were by far the greatest source of tissues with donations of over 1200 samples obtained from both individual fishers and several sport fishing clubs. Commercial fishers, including fishing guides in a number of locations, provided up to 150 samples. State and Territory fisheries personnel (WA, QLD and NT) supplied an additional 427 samples. Present day collections were obtained between 2006 and 2013 and in some cases sampling was spread across multiple years to obtain sufficient samples sizes for analyses. The remaining samples (N = 1079) came from an archived muscle tissue collection used in earlier allozyme studies (Shaklee et al., 1993; Keenan 1994) and consisted of liquid nitrogen frozen samples that had been stored at -80 °C and that had been collected between 1988 and 1993 (Table 1).

Table 1: Collection details for wild caught barramundi genotyped for 16 microsatellite loci. Generic latitude and longitudes are given for each collection and those obtained from earlier allozyme studies (Keenan 1994) are indicated in bold. Temporally replicated collections are indicated by *, n = sample size.

Collection #	n	Source Year	Collection CODE	State	Location Description	Latitude	Longitude
1	31	2012	PIL	WA	Pilbara Region	-20.67839	117.1886
2	14	2008-11	BME	WA	Broome	-17.9375	122.723333
3	30	2006-12	STG	WA	St George Basin	-15	125.41
4	37	2006-12	ADM	WA	Admirality Gulf	-14.703333	125.64
5	17	2006-08	SWI	WA	Swift Bay	-14.455	124.823333
6	26	2006-12	DRY	WA	Drysdale River	-14.023333	126.94
7	25	2006-08	SMB	WA	Salmon Bay	-13.951667	127.161667
8	24	2007-09	KGR	WA	King George	-13.985	127.361667
9	24	2007	BER	WA	Berkley River	-14.35275	127.772017
10	24	2007	HEL	WA	Helby River	-14.74	128.178333
11	21	2011	NNC	WA	Nulla Nulla Creek	-15.51527	127.849357
12	63	2011	ORD	WA	Ord River	-15.534703	128.369778
13	26	2007-12	BPG	WA	Bonaparte Gulf	-15.173103	129.360498
14	**29**	**1990-91**	**MOYK**	**NT**	**Moyle River**	**-13.976**	**129.74409**
15*	**22**	**1990**	**DLYK90**	**NT**	**Daly River**	**-13.45815**	**130.100467**
16*	24	2008	DLY08	NT	Daly River	-13.45815	130.100467
17	**24**	**1990-91**	**BTIK**	**NT**	**Bathurst Island**	**-11.6621**	**130.22645**
18	**24**	**1990-91**	**DHBK**	**NT**	**Darwin Harbour**	**-12.5492**	**130.783083**
19	**24**	**1990-91**	**SHOK**	**NT**	**Shoal Bay**	**-12.305117**	**130.985417**
20	24	2012	MRR	NT	Mary River	-12.7265	131.680548
21	13	2012	ALG	NT	Alligator River	-12.202467	132.409333
22	32	2011	LVP	NT	Liverpool River	-12.142917	134.186083
23	**22**	**1990**	**ANBK**	**NT**	**Arnhem Bay**	**-12.34895**	**136.18745**
24	24	2011	ROP	NT	Roper River	-14.73425	135.403183
25	24	2011	MAC	NT	McArthur river	-15.821065	136.659495
26*	**24**	**1990**	**LICK90**	**QLD**	**Leichhardt River**	**-17.579885**	**139.795083**
27*	24	2011	ALB11	QLD	Albert River	-17.5667	139.75
28	24	2011	GIL	QLD	Gilbert River	-16.655067	141.2942
29	**24**	**1993**	**MITK**	**QLD**	**Mitchell River**	**-15.19861**	**141.59138**
30	**21**	**1993**	**HOLK**	**QLD**	**Holroyd River**	**-14.2**	**141.6333**
31*	**24**	**1993**	**ARCK93**	**QLD**	**Archer River**	**-13.3767**	**141.7067**
32*	33	2011	ARC11	QLD	Archer River	-13.3767	141.7067
33	16	2011	JAR	QLD	Jardine River	-10.928053	142.214285
34	30	2011	JCK	QLD	Jacky Jacky Creek	-10.94	142.51
35	24	2006-11	ESC	QLD	Escape River	-10.995338	142.652585
36	24	2011	PCB	QLD	Princess Charlotte Bay	-14.16451	144.11564
37	15	2011	BIZ	QLD	Bizant River	-14.51306	144.065529
38	48	2009-11	JOR	QLD	Johnstone River	-17.512596	146.058021
39	50	2011-12	HCC	QLD	Hinchinbrook	-18.414883	146.2016
40	23	2011	CLE	QLD	Cleveland Bay	-19.284331	146.866419
41*	**24**	**1988**	**BOWK88**	**QLD**	**Bowling Green Bay**	**-19.25**	**147.25**
42*	24	2008	BOW08	QLD	Bowling Green Bay	-19.25	147.25
43*	**24**	**1989**	**BURK89**	**QLD**	**Burdekin River**	**-19.66759**	**147.54496**
44*	24	2008	BUR08	QLD	Burdekin River	-19.66759	147.54496
45	15	1988	BRSK	QLD	Broad Sound	-22.188334	149.572076
46*	**44**	**1988**	**FTZK88**	**QLD**	**Fitzroy River**	**-23.51275**	**150.825753**
47*	48	2013	FTZ13	QLD	Fitzroy River	-23.51275	150.825753
48	**18**	**1989-90**	**MARK**	**QLD**	**Mary River**	**-25.443283**	**152.911383**

Extraction

Due to the diverse nature of the samples obtained and, in some cases, relatively poor tissue preservation, it was necessary to extract DNA from a large number of samples to obtain workable sample sizes for genotyping. A total of 2312 samples were therefore extracted using a modified CTAB chloroform/isoamyl extraction buffer (Adamkewicz and Harasewych 1996). Briefly tissue was digested at 55 °C overnight in modified extraction buffer containing 200 µg of proteinase K, 100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA and 2% CTAB (hexadecyltimethylammonium bromide) followed by standard chloroform/isoamyl alcohol extraction and isopropanol precipitation (Sambrook and Russell 2001). Extracted DNA was resuspended in TE buffer (10 mM Tris-Cl ph8.0, 1 mM EDTA) and checked for quality and relative quantity of high molecular weight DNA by agarose gel electrophoresis on 0.8 % 1x TBE gels containing 0.3x GelGreen DNA stain (Biotium) following standard laboratory procedures (Sambrook and Russell 2001). Aliquots of the highest quality DNA extracts (N=1273) were diluted to ~ 5 ng/µl with nuclease free water and both diluted and neat DNA extracts were stored at -20 °C.

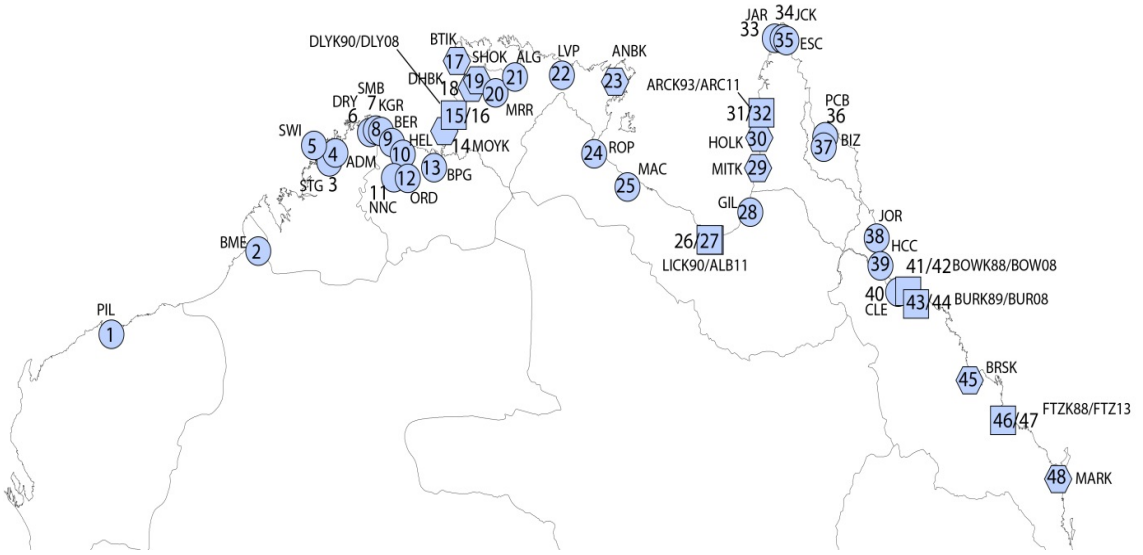

Figure 1: Spatial distribution of 48 barramundi sample collections genotyped for 16 microsatellite loci in the present study. Keenan (1994) sample collections obtained from 1988-1993 are indicated as hexagons, sample collections obtained in the present study between 2006 – 2013 are indicated by circles while temporally replicated collections sampled during both of these sampling periods are indicated as squares. Collection numbers are given within

symbols and collection codes (as per Table 1) are given next to symbols. Sample sizes for each location are given in Table 1.

Microsatellite Genotyping

A total of 1273 samples across the 48 sample collections were processed for microsatellite genotyping following initial DNA quality checks (Table 1). A total of 17 microsatellite loci were amplified for each of these samples in two multiplex polymerase chain reactions (PCRs), the P1 suite (Appendix A1) and the G suite (Appendix A2)(note 16 microsatellites were finally used for actual genetic analyses). The P1 multiplex suite consists of nine markers initially described by Zhu et al., (2006), while the G multiplex suite consists of eight markers and was developed within the Aquaculture Genetics Research Group at JCU from a variety of published primer sequences (Yue et al., 2001; Yue et al., 2002; Wang et al., 2006; Zhu et al., 2006). Modifications to published primer conditions are outlined in Appendices A1 and A2 and these modifications include a change of fluorescent tag on forward primers, attachment of a PIG tail sequence (GTTTCTT) to the 5' end of all reverse primers to minimise stutter patterns and facilitate scoring (Brownstein et al., 1996), and modifications to final primer concentrations to ensure more even loading of products during fragment analysis.

All PCR reactions were performed in low profile microtitre plates (Biorad or Fisher Biotech) sealed with microseal B film (Biorad) and contained multiple fluorescently labelled PCR primers. Both P1 and G suite multiplex PCR reactions contained 1 x TypeIT microsatellite PCR master mix (Qiagen Taq DNA polymerase, 1 x PCR buffer, 3 mM MgCl2 and 0.2 mM dNTPs), 1 x P1 or G primer mix (Appendix A1 and A2) and ~5 ng/µl gDNA in a 10 µl total reaction volume. Thermal cycling was conducted in a Biorad S1000 thermal cycler, under the following conditions for both marker suites: one cycle of 95 ºC for 5 min followed by 10 cycles of 95 ºC for 30 s, 57 ºC for 90 s, 72 ºC for 30 s followed by 20 cycles of 95 ºC for 30 s, 55 ºC for 90 s, 72 ºC for 30 s and concluding with a final step of 60 ºC for 30 min. Successful PCR amplification was verified via agarose gel electrophoresis on 1.5% 1xTBE agarose gels containing 0.3x GelGreen DNAstain (Biotium) prior to purification of PCR products using MicroCLEAN reagent (MicroZone).

Fragment analysis to determine the precise microsatellite alleles for each sample and locus was undertaken at the Georgia Genomics Facility (USA) on an Applied Biosystems (ABI) 3730 DNA Analysis System. Each sample was run together with a GeneScan 500 Liz ladder

(Applied Biosystems) and final allele scoring was conducted using GeneMarker 2.2.0 software (Soft Genetics). Alleles were labelled by the effective number of repeat units using the approach of Amos et al., (2007). This approach was chosen over the alternative of labelling alleles based on estimated fragment size as estimated fragment sizes can vary greatly due to a variety of sources of technical variability including rounding of non-integer estimated fragment sizes, allele binning, day-to-day differences in migration rate of fragments between genotyping runs and migration rate differences across different genotyping platforms (see for example; Alberto 2009; Amos et al., 2007; Davison and Chiba 2003; Dewoody et al., 2006). The labelling of alleles as effective number of repeat units, combined with the inclusion of several positive control and allele calibration samples on each genotyping run, allowed the standardisation of microsatellite allele calling across runs in the present study. This method of allele labelling and allele size calibration was also found to improve standardisation across different projects utilizing a variety of laboratory technicians and fragment analysis platforms (Smith-Keune, personal observation).

Two loci exhibited allele sizes that were consistent with both changes in microsatellite repeat number, as well as additional insertion/deletions (indels) in the regions flanking the microsatellite repeats (i.e. presence of 1 bpr out-of-phase alleles). Loci affected by indels do not conform to the Step Wise Mutational model (Ohta and Kimura 1973) typically accepted as the most appropriate for microsatellites (Goldstein et al., 1995). The two dinucleotide loci Lca008 and Lca016 showed allele size distributions indicative of out-of-phase (1 bpr) alleles, these alleles were not binned with neighbouring alleles, but were treated as separate alleles, as previous studies have shown that out-of-phase alleles do not contain the same number of repeat units as the nearest sized fragment (Smith et al., 2003; Benzie and Smith-Keune 2006). For the purposes of error checking (below) these loci were treated as mononucleotide loci. To determine whether major patterns of genetic structure were affected by the inclusion of these loci some analyses were run with and without these loci included (see below).

To facilitate merging of data in future studies, an excel file of allele labels used in the present study can be found in Electronic Appendix E1. All electronic appendices are lodged with the JCU Tropical data hub (https://research.jcu.edu.au/researchdata) and are made publically searchable and available upon acceptance of journal manuscripts arising from this research. Appendix E1 includes the fragment sizes associated with all reported alleles under the electrophoresis conditions used in the present study and it should be noted that changes to the

electrophoresis conditions may alter allele fragment sizes. The use of calibration samples in future studies is therefore strongly recommended for cross laboratory calibration (see for example Ellis et al., 2011). Calibration samples can be provided on request. To further facilitate future studies the complete 16 locus genotypes for all 1273 individuals used in the present study will also be made publically available as Electronic Appendix E2.

Microsatellite Data Quality Checks

Due to high levels of polymorphism and the characteristic stutter patterns associated with microsatellite markers, a number of quality control checks were undertaken to ensure that stringent genotype calling guidelines had produced reproducible and accurate genotype calls. Although 17 microsatellite loci were initially genotyped, only 16 were retained following these data quality checks. The Lca287 locus was excluded from final analysis as a high frequency of null alleles and other scoring difficulties was revealed in data checks performed with Microchecker software v 2.2.3 (van Oosterhout et al., 2004). All samples with unusual fragment peaks, or with more than four loci of missing data, were noted during initial genotyping and were re-genotyped from an independent PCR reaction. Within the final dataset there was less than 1% missing data for each of the 16 loci retained. In total, only 44 of the 1273 individuals analysed required re-genotyping to correct potential genotyping errors and within this set of notable individuals the actual genotyping error rate (excluding the Lca287 locus) was low at just 1.8%, with only 10 true errors detected in 544 valid comparisons. As the detected errors were spread across 6 loci, occurred across 9 different populations, and all re-genotyped individuals had initially been flagged as potentially problematic, it is expected that the true genotyping error rate is much lower than 2% and it is unlikely that any systematic bias in scoring has occurred that would affect population genetic comparisons.

It was necessary to check for repeated sampling of the same fish within each location due to the catch-and-release fishing methods employed by many of the recreational fishers contributing samples to this project. It is highly unlikely that two unrelated fish sampled from the same randomly mating population would by chance have the same 16 locus genotype as the Probability of Identity (*PI*) estimates ($PI = 2 \times \left[\sum (pi \times 2)^2\right] - \sum pi^4$, where pi is the frequency of the i-th allele at a locus), calculated in GenAlEx 6.41 (Peakall and Smouse 2006), were extremely low and ranged from 3.0×10^{-11} to 1.2×10^{-8}. Checks for matching 16

locus genotypes, that would indicate the repeated sampling of the same fish, were also conducted using GenAlEx 6.41 (Peakall and Smouse 2006). Only one instance of a repeated sample was found in the modern collections, while 11 repeated genotypes (repeated twice each) were identified from older collections and these most likely represent labelling errors and/or multiple sampling of muscle tissue from the same fish for earlier allozyme studies. In these instances only a single genotype was retained for population genetic analysis.

Many of the population genetics tests performed assume that the included loci are unlinked and are in Hardy Weinberg Equilibrium within the sampled populations. Statistical tests for linkage disequilibrium (LD) and Hardy Weinberg Equilibrium (HWE) were therefore performed in GenePop v4.2 (Rousset 2008). False discovery rate (FDR) correction (Benjamini and Hochberg 1995) was used to control the false discovery rate to 5% and determine statistical significance due to the large number of multiple comparisons made. There was no evidence for linkage between any marker pairs and only a single incidence of a significant deviation from HWE was observed following FDR correction. This single incidence of deviance from HWE involved locus Lca21 in the Alligator River (NT) and is likely a reflection of the small sample size from this location (n = 13). All 16 markers included in the final population genetic analysis are therefore considered to meet the assumptions of HWE and LD.

Marker Neutrality

As some population genetic analyses assume neutrality of genetic markers, and the identification of genetic markers potentially under the influence of selection is important for identifying locally adapted populations or stock, the neutrality of the microsatellite loci used here were examined using coalescent simulations of locus specific *F*-statistics under the hierarchical island model (Slatkin and Voelm 1991) and the Step Wise Mutational Model (SMM) first described by Ohta and Kimura (1973) as implemented in Arlequin v3.5.1.2 (Excoffier et al., 2005). A total of 20,000 simulations were performed assuming 21 groups (equivalent to subpopulations – see results/discussion) and 100 demes per group. The hierarchical island model was chosen as opposed to the non-hierarchical finite island model, as the finite island model has been show to lead to a substantially higher number of false positives (Excoffier et al., 2009). A single outlier locus (Lca040), suspected of being in a region of the genome under directional selection, was detected (Figure 2). To determine

whether major patterns of genetic structure were affected by the inclusion of this locus some population genetic analyses were run with and without Lca040 included (see below).

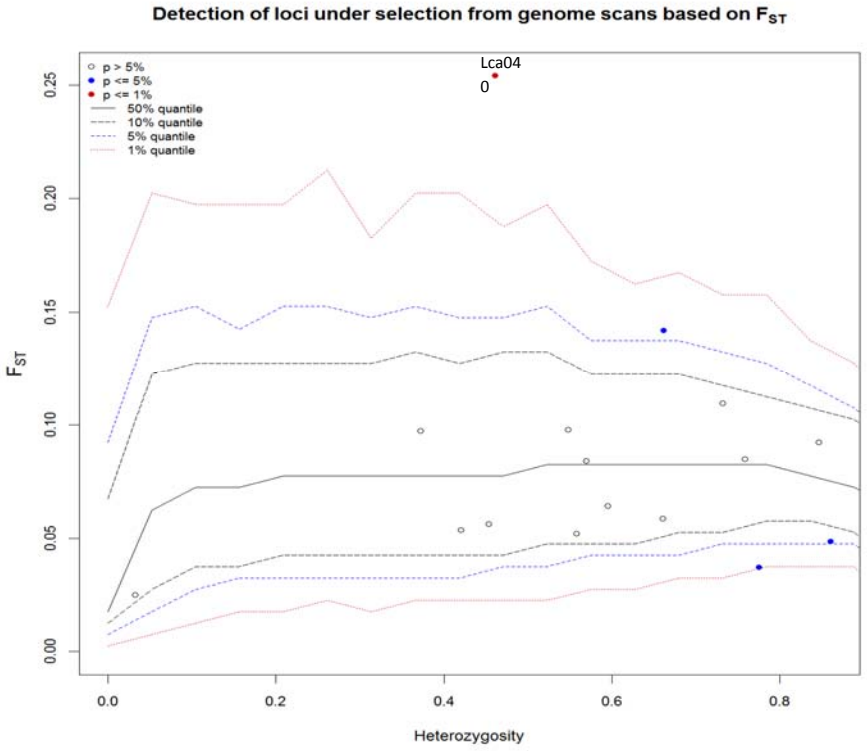

Figure 2:

Summary of F_{ST} outlier analysis (Excoffier et al., 2009) used to detect potentially selected loci. The analysis performed utilized a hierarchical island model based on 21 stocks (groups) and 100 demes simulated per group with 20,000 coalescent simulations performed in Arlequin v 3.5.1.3. A single locus (Lca040) suspected of being in a region of the genome under selection (F_{ST} = 0.254, H_o = 0.431, p = 0.001) is indicated at top of figure.

Temporal stability

Previous studies have shown temporal stability of allozyme allele frequencies at times scales from several months (Shaklee and Salini 1985) up to seven years (Keenan 1994), however, there are no published reports of temporal stability of more rapidly evolving microsatellite loci for barramundi over the times scales sampled in the present study. Due to the inclusion of modern collections, sampled between the years 2006 to 2013, as well as historical collections sampled between 1988 and 1993, it was necessary to confirm temporal stability of microsatellite allele frequencies in replicated samples prior to defining the genetic structure of

wild barramundi. Six locations were repeatedly sampled at intervals between 18 and 25 years including the Daly River in the Northern Territory and five locations in Queensland (Table 1). Within the Gulf of Carpentaria the Archer River was sampled twice while historical samples taken from the Leichardt River can be compared to the modern collection from the Albert River due to the close proximity of the river mouths (<10 km apart). On the east coast of Queensland both Bowling Green Bay and the Burdekin River were each sampled twice, while the Fitzroy River in south-east Queensland was sampled three times, once in 1988, again in 2008 and a third sample was obtained in 2013. Pairwise comparisons of temporally replicated samples were used to confirm temporal stability of microsatellite loci over time scales up to 25 years (see discrimination of subpopulations below).

Population genetic differentiation – discrimination of subpopulations

The extent of genetic divergence among the different collections, and amongst groups of collections identified as belonging to the same locally inter-breeding subpopulations, was examined using global *F*-statistics (Weir and Cockerham 1984), calculated as the weighted average among loci implemented as part of the AMOVA analysis of Arlequin v3.5.1.2 (Excoffier et al., 2005). In addition, the extent of genetic divergence among pairs of sample collections was assessed using pairwise F_{ST} values and associated *p*-values to identify locally interbreeding subpopulations (Electronic Appendix E3). Statistical significance of both global and pairwise F_{ST} values was determined through 10,000 permutations of the data. Due to the large number of pairwise comparisons the false discovery rate correction of Benjamini and Hochberg (1995) was again used to limit the false discovery rate to 5% while still providing sufficient statistical power to discriminate genetically distinct collections. To facilitate interpretation a graphical matrix of pairwise F_{ST} values was generated using the in-built R-scripts in Arlequin v3.5.1.2 (Excoffier et al., 2005).

Pairwise comparisons of temporally replicated samples were used to confirm temporal stability of microsatellite loci, while pairwise comparisons of geographically nearest neighbour collections were examined to identify genetically similar collections that could be pooled into discrete subpopulations. A statistically significant value of F_{ST} >0.01 was used as the threshold to differentiate collections such that collections with both significant and non-significant pairwise $F_{ST} < 0.01$ were pooled together into a single combined subpopulation. This threshold was chosen after careful inspection of the overall pattern of significant pairwise F_{ST} values (Appendix E3). Only 3 of 114 pairwise F_{ST} values $\leq$ 0.01 were

statistically significant (these most likely reflect false positives), while only 2 of 1014 pairwise F_{ST} values >0.01 were non-significant after FDR correction. An alternative approach is to use Fisher's exact-tests of allele frequencies differences between collections to define subpopulations, an approach analogous to that used to define subpopulations in earlier allozyme studies (Keenan 1994). Indeed this approach can be more powerful when sample sizes are unbalanced (Goudet et al., 1996). To test for congruence in defining subpopulations between the pairwise F_{ST} method employed above, and Fisher's exact-tests for genic (allelic) differentiation (Raymond and Rousset 1995), the latter tests were performed for all 48 collections (and later also for defined subpopulations) in GenePop v4.2 (Rousset 2008) and results compared from the two methods. Tests for deviations from HWE for identified subpopulations were also performed in GenePop v4.2 (Rousset 2008) to ensure that subpopulations conformed to Hardy-Weinberg expectations for large randomly mating populations. Global *F*-statistics (Weir and Cockerham 1984) and pairwise F_{ST} values between all pairs of subpopulations were then calculated as described for comparisons between individual collections.

Subpopulation genetic diversity

As genetic diversity is widely accepted as an important factor for adaptability of natural populations to changing environmental conditions the genetic diversity of identified subpopulations of barramundi was explored using a variety of diversity indices. The mean number of alleles, Nei's unbiased heterozygosity (UH_e) (Nei, 1978), observed heterozygosity (H_o) and average inbreeding co-efficient F_{IS} were all calculated in Arlequin v3.5.1.3 (Excoffier et al., 2005). Allelic richness that corrects the mean number of alleles to account for sample size differences among samples was calculated using FSTAT v2.9.3.2 (Goudet 2001).

Major genetic patterns – Isolation by distance

A number of previous studies have suggested that the genetic structure of barramundi in Australia conforms to a pattern of isolation-by-distance such that spatially limited gene flow results in an increase in genetic divergence as geographic distance increases between sampling sites (Marshall 2005; Keenan 1994; Chenoweth et al., 1998a). The present study is the most comprehensive genetic audit of Australian barramundi stocks, with sample collections covering almost the complete species distribution including an extended coverage

of Western Australia. To determine whether the previously described patterns of isolation-by-distance were upheld over the currently analysed sample collections Mantel's tests for correlation between genetic distance (as pairwise F_{ST} between collections and log of pairwise F_{ST} between collections) and both geographic distance and log geographic distance were performed in the web-based software package IBDWS v3.2.3 (Jensen et al., 2005). Geographic distance between sampling locations along the coast (coastal distance) was determined by setting a 5 km buffer around the Australian coastline in ArcGIS 10 and calculating the distance of the least-cost pathway between each population using a custom-built R script from the 'gdistance' library in R 2.14 (www.r-project.org).

Major genetic patterns – freshwater drainage divides

Major patterns of genetic stock structure were examined using hierarchical analysis of molecular variance (AMOVA), with collections grouped in a variety of ways to test explicit hypotheses about the conformance of genetic structure in barramundi to a freshwater fish model as proposed by Marshall (2005). AMOVA was initially conducted on the 48 individual barramundi collections (Table 1) to establish the baseline covariance components and percentage of overall variability that is due to differences among/within collections. Next the variability due to differences among defined subpopulations and among collections within subpopulations was examined to determine how well the defined subpopulations explained the genetic variance present in the dataset. Following this, in an approach analogous to that used by Marshall (2005), the 48 barramundi collections were assigned to the nearest freshwater fish bioregion (major provinces) of Unmack (2001) and to subprovinces nested within the freshwater fish bioregions as indicated in Appendix A3. The hierarchical AMOVA framework (Excoffier et al., 2005) was used to explore the variability due to differences among bioregions, and among collections within bioregions, and similarly to look at differences among subprovinces (Unmack 2001) and collections within subprovinces. As Marshall (2005) proposed that the genetic structure of barramundi in Australia has been driven largely by historical divisions among freshwater drainages, two additional analyses exploring covariance components and genetic variability among different levels of drainage basins and among sample collections within those basin levels was undertaken. Two levels of drainage basins were examined including Level 1 drainage divisions and Level 2 aggregated river basin groups as identified by the National Catchment Boundaries v1.1.4 (available from http://www.ga.gov.au/topographic-mapping/national-surface-water-information.html).

Major genetic patterns – geographical patterns of stock structure

Two different approaches were utilized to explore major geographical patterns of genetic stock structure in Australian barramundi collections. Firstly, a multivariate approach involving principal components analyses (PCA) of Nei's unbiased genetic distance matrix was conducted in GenAlEx 6.41 (Peakall and Smouse 2006). This approach allows a preliminary visual assessment of subpopulation clusters to determine if any major geographic patterns of stock structure can be identified. It should be noted, however, that Nei's genetic distance assumes the infinite allele model (Nei 1978), which may not be appropriate to most microsatellite loci (Goldstein et al., 1995). However, as the GenAlEx PCA is restricted to this distance matrix and is quick to run this approach was chosen to briefly explore the effect of including and excluding loci with out-of-phase alleles (i.e. Lca008 and Lca016) and excluding/including the Lca040 locus identified as being in a region of the genome potentially under directional selection. Changes in the percentage of variation explained by each of the first three PCA axes occurred when the potentially selected Lca040 locus was dropped from the analysis, although the cumulative percentage of variation explained by these axes was only slightly reduced from 81.9% to 79.2% (Table 2). Little change in the percentage of variation explained by the first three axes (individually or cumulatively) were observed when both Lca008 and Lca016 were removed (Table 2). Examination of PCA plots indicated no major changes to patterns of stock structure when these loci were removed from the analysis (data not shown) and as a result all population genetic analysis presented retain the full set of 16 microsatellite loci.

Table 2: Summary of percentage of variation explained by the first three Principal Co-ordinates from PCA analysis of Nei's Unbiased genetic distance performed in GenAlEx 6.4.1 (Peakall and Smouse 2006) using all loci or when excluding the potentially selected locus Lca040 or two loci with out-of-phase 1bpr alleles (Lca008 and Lca016).

	PCA Axis			
Markers Included	**1**	**2**	**3**	**Cum%**
All 16 loci	48.0%	21.8%	12.1%	**81.9**
Minus Lca040	39.5%	25.9%	13.9%	**79.2**
Minus Lca008/016	49.3%	21.3%	12.0%	**82.7**

An individual, model-based Bayesian clustering method was used to explore patterns of co-ancestry among all 1273 genotyped individuals as an alternative to genetic distance based PCA analysis to explore major patterns of genetic stock structure. The STRUCTURE algorithm of Pritchard et al., (2000) enables the detection of major geographical patterns of genetic stock structure and furthermore enables the detection of recent migrants between stocks, the detection of admixed individuals with ancestry from two or more genetically distinct genetic stocks (populations) and the probabilistic assignment of individuals of known or unknown sampling location to identified genetic stocks. Importantly for the current data set, the method is independent of the mutational process (i.e. does not rely on markers conforming to either a SMM or IAM) and can be set to make use of informative priors, such as fish catch location, to improve individual clustering solutions when population structure is weak. The STRUCTURE algorithm was applied in a hierarchical fashion using catch location as an informative prior and utilizing the correlated allele frequency and admixture models, an approach that has previously been found to be robust to the presence of non-admixed individuals and compares favourably with alternative Bayesian clustering algorithms (Francois and Durand 2010; Chen et al., 2007). As the number of genetic stocks (populations) is unknown at the outset of the analysis the algorithm is run repetitively assuming varying K number of populations and multiple runs of each K are performed to ensure consistent results are obtained. In this case STRUCTURE was run for K = 1 to 20 (the approximate number of subpopulations identified in earlier analyses) and 10 independent runs of each K were performed. A burnin period of 50,000 iterations and 100,000 Markov chain Monte Carlo (MCMC) repetitions was set for each run (see Pritchard et al. 2000 for details). The most probable number of clusters (K) was determined for each hierarchical level using the Evanno et al., (2005) method implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012). Where K = 2 (or greater) the dataset was split into corresponding geographical groups and re-run to determine progressively finer levels of stock structure. This was performed until no further structure was detected. Individual membership coefficients (q values) indicating the proportion of each individuals genome with ancestry in the identified clusters were averaged over the 10 replicate runs for the most probable K using the downstream program CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) and plots of average q values were generated with DISTRUCT 1.1 (Rosenberg 2004).

Previous authors have suggested reduced genetic diversity in some Australian barramundi stocks (Keenan 1994; Chenoweth et al., 1998b; Marshall 2005) that may have implications for future adaptability. Following the identification of the major genetic stocks we therefore explored the possibility of differences in genetic diversity for the major stocks identified in the present analysis using the 'Test Groups' function in FSTAT v2.9.3 (Goudet 2001). Tests for significant differences in allelic richness (A_R), observed heterozygosity (H_O) and average inbreeding co-efficient (F_{IS}) were undertaken using 5000 permuations and were performed for all stocks, and for a subset of stocks based on initial results.

Component B: Physiological tests

Physiological tests were divided up into three separate experiments to test for differences among barramundi genetic stocks to temperature induced stresses that may be indicative of localised adaptation. The first experiment used the dissociated live/dead cell method of Newton et al., (2010) to predict relative tolerance of representative hatchery populations from five different barramundi genetic stocks to acute temperature stress. The other two experiments examined oxygen consumption to determine the metabolic rate and aerobic scope of these same hatchery populations, as well as examining hypoxia tolerance under varying thermal regimes.

i) Live/Dead cell assays

Background

Newton et al., (2010) established the predictive power of dissociated caudal fin cells to differentiate barramundi from two locations (Darwin and Gladstone) within different genetic stocks when exposed to an upper thermal stress. In this trial the methodologies of Newton et al., (2010) were extended to define the relative thermal tolerance of barramundi from five of the six major Australian genetic stocks identified by the population genetics analyses. Newton's et al., (2010) methodology was also adapted to provide greater precision and larger numbers of cells that could be counted via flow cytometry.

The objective of this component of the study was to use the live/dead cell caudal fin assay developed by Newton et al., (2010) to establish if barramundi from each of five major genetic stocks a) exhibit tolerance differences to acute upper thermal stress and b) to determine the relative ranking of hatchery populations from each genetic stock in the face of this stress.

Materials and Methods

Experimental fish

Barramundi fingerlings (11.2 to 16.0 cm in standard body length) were obtained from hatcheries representing five major genetic stocks of barramundi in Australia (i.e. from Broome in WA, Darwin in the NT and Karumba, Townsville/Bowen and Gladstone suppliers in Qld). Supply of the experimental fish from the hatcheries was coordinated so all fish obtained were the same age and approximate size for the experiment. Each population was reared in a separate 5000 L interconnected recirculating freshwater tank maintained at 27 ±

0.7 °C for 2 months at the Marine and Aquaculture Research Facility Unit of James Cook University, Townsville, Australia before experimentation commenced. This allowed all fish to recover from the stress of shipping and to acclimate to the same thermal conditions. Within each tank, barramundi were graded into two enclosures to reduce cannibalism and standardize feed dispersion. Fish were fed once daily until satiation with appropriate-sized formulated pellets (Grobest Australia, Tingalpa, Australia), but were not fed for 24 h prior to experimental trials.

Preparation of dissociated caudal fin clips

Thirty five to 59 fish were sampled from each hatchery population (Broome n = 56, Darwin n = 35, Karumba n = 58, Townsville/Bowen n = 58, Gladstone n = 59). For each experimental replicate, fish were anesthetized with 20 mg L-1 of Aqui-S® (Aqui-S New Zealand Ltd, Lower Hutt, New Zealand) in separate, aerated buckets for 30 min prior to acquiring fin clips. Standard length (cm), body depth (cm), and wet weight (g) were measured before a fin clip of approximately 1 cm^2 was taken from the caudal fin of each juvenile barramundi. Single cell suspension was achieved using a method modified from Newton et al., (2010). Fin clips were added to 1 ml of sterile calcium and magnesium free phosphate buffered saline (CMF-PBS: 0.01 M PBS, 0.138 M NaCl, 0.0027 M KCl, pH 7.4), rinsed with Leibovitz's L-15 medium (Invitrogen, Mount Waverley, Australia), dissected into 1 mm^2 squares, and added to a 250 µm steel mesh filter placed within a petri dish. Dissected fin clips were gently pressed through the steel mesh filter with a 5 ml syringe plunger (5 times) and washed with 600 µl of Leibovitz's L-15 medium twice. 700 µl of dissociated caudal fin cells were pipetted into a 1.7 ml eppendorf tube and stored at room temperature until all daily samples were completed. Dissociated caudal fin clips were heat shocked at 43 ± 0.1 °C in a water bath for 60 min and subsequently filtered (63 µm) to remove cell aggregates that may potentially block up the flow cytometer. 600 µl of filtered samples was pipetted into a BD LSRFortessaTM Cell Analyser tube and stained with 2 µM Calcein-AM (CAM) (live cell counts) and 30 µM Propidium Iodide (PI) (dead cell counts) consecutively (Sigma-Aldrich, Castle Hill, Australia). Samples were maintained at room temperature in the dark for 30 min to allow for adequate time for dye staining and all samples were analyzed with the flow cytometer within 60 min of staining. One control fish was also used for fluorescence compensation on each experimental day. Three times the amount of caudal fin clip was taken from this control fish and this was evenly divided into three control samples. The three controls were treated as

follows: one positive control with no heat shock and no stain, two negative controls; one with no heat shock and stained with 2 μM CAM and a second heat shock and stained with 30 μM PI.

Flow cytometry

Flow cytometric data were collected with a BD LSRFortessa™ Cell Analyser and FACSDiva™ Software v6.0 (Becton, Dickinson and Company, San Jose, USA). Prior to experimentation, cytometer setup and baseline performance checks were performed using BD Cytometer Setup and Tracking Beads™. Parameter voltages were set as follows: 180V for forward angle light scatter (FSC), 150V for side-angle light scatter (SSC), 280V for FITC (i.e. CAM) and 340V for PI. Both CAM and PI stained cells were excited with a 488-nm blue laser and thresholds set to channel 1,000 in order to exclude undesirable cell debris from recorded data. To avoid spectral overlap between CAM and PI stained cells (i.e. live and dead cells, respectively), compensation was calculated using the three control samples before each experimental replicate. A FACS Diva™ worksheet containing a FSC-A vs. SSC-A logarithmic dot-plot (gated to include live/dead cells) and a PI-A vs. CAM-A logarithmic dot-plot (individual gates for live/dead cell populations) was used to collect data from 2,500 to 8,000 dissociated caudal fin cells from each sample.

Statistical analyses

Statistical analyses were performed using SPSS® Statistics Version 19 (IBM®, Australia). A one-way ANOVA with post hoc (Tukey) was used to determine significant differences among cell viability in the five hatchery populations representing five different genetic stocks.

ii) Routine metabolic rate, maximum metabolic rate and aerobic scope in hatchery populations of barramundi from different genetic stocks

Background

Future climate change scenarios highlight the need to better understand the impact of rising temperature on the barramundi fishery and aquaculture industries. The physiology of barramundi is thought to have a high level of plasticity to changes in temperature; however, the degree of plasticity may differ amongst different subpopulations or stocks. Research using loss of swimming equilibrium (LOSE) and dissociated caudal fin cell assays has indicated discrete subpopulation adaptation to local thermal conditions (Newton et al., 2010).

Physiological traits such as routine metabolic rate (RMR), maximum metabolic rate (MMR) and resultant aerobic scope (AS) are indicators of fitness and the ability of a species to successfully occupy an ecological niche (Fry 1971; Priede 1985). Previous work has shown that the RMR of genetically divergent subpopulations of barramundi will increase similarly and predictably with increasing temperature; however, RMR is the minimal maintenance or routine metabolic rate below that normal physiological function is impaired and it is reasonable to consider that this parameter will remain relatively constant (or conserved) within this species. If differences in physiological traits exist amongst different genetic sub-populations of barramundi these will likely be exhibited when animals are physiologically challenged. There are currently no data published describing the MMR of barramundi.

The objective of this component of study was to identify the maximum metabolic rate and aerobic scope of discrete subpopulations of barramundi over a broad ecologically relevant temperature range to ascertain if they exhibit differences indicative of adaptation to local thermal regimes. Experimental procedures were divided into two sections, a) validation of an open-top respirometry approach as the method to determine metabolic rate and aerobic scope and b) determination of RMR, MMR and AS in genetically divergent subpopulations of barramundi.

Materials and Methods

Validation of open-top respirometry as a method to determine MO_2

When measuring aerobic metabolism of aquatic animals open-top respirometry is rarely used in favour of closed cell respirometry. Presumably this is due to concerns about potential gas exchange across the air-water interface maintaining equilibrium of partial gas pressures that conceivably has the potential to confound oxygen consumption (MO_2) measurements. Open-top respirometers offer many advantages over traditional closed systems and do not require specialized construction and are therefore a relatively inexpensive and practical alternative to traditional closed respirometers. The open-top method also offers the flexibility for multiple animals to be measured at one time, reducing variance and stress to gregarious species housed individually. Leclercq et al., (1999) demonstrated that the aerobic metabolism of coral reef communities (corals and microfauna) in open-top saltwater 'mesocosms' (195 L aquaria) was similar to that determined using a traditional closed-top respirometry technique. Similarly, Pirozzi and Booth (2009b) found that gas exchange across the air-water interface in static open 200 L tanks was so slow as to have no significant effect on routine metabolic rate (RMR) measurements of the marine finfish mulloway, *Argyrosomus japonicas*, and yellowtail kingfish, *Seriola lalandi*. There have, however, been no direct demonstration of open and closed-top respirometry in measuring the MO_2 of finfish in freshwater systems and given that to understand the effect of temperature on metabolic activity of barramundi required large numbers of fish from different subpopulations to be evaluated it was considered that open-top respirometry may be an effective approach to gather the required MO_2 data.

The first objective of this subcomponent therefore was to validate the open-top respirometry method by comparing the RMR of barramundi in both open and closed-top freshwater respirometers in order to determine if the open-top approach is suitable for large scale determination of MO_2 in freshwater barramundi.

General respirometer design

Open and closed respirometers were 114 L high density polyethylene (HDPE) tanks covered with a HDPE lid that had a 25% section removed to allow exposure to natural light while limiting potential disturbance. The external tank walls were wrapped in black plastic to limit light and stimulation from the surrounding environment. Each tank was fitted with a 40 mm PVC pipe, perforated with 16 mm holes, positioned centrally and perpendicular to the base of

the tank. This was used to conceal the oxygen probe so as not to stimulate fish activity during oxygen measurements. The design of the closed-top respirometer was as above with the addition of a circular, 12 mm thick, acrylic lid insert machined 4 mm larger than the inside diameter of the top of the HDPE tank. Before sealing into place, an entry and exit point for fish was cut out and fixed with mechanical fixings and surgical rubber to create an air-tight seal. A threaded poly riser and purge valve to remove remaining air from the respirometer was fitted to the lid. A tapered nipple was pressed into the centre of the lid to create an air-tight seal around the oxygen probe cable. The lid insert was a friction fit and once pressed into place was sealed with a marine grade adhesive/sealant.

Study Animals and husbandry

Barramundi fingerlings were sourced from a local hatchery and grown to a mean weight of 189.2 ± 15.9 g (mean ± SE). Eight barramundi were stocked at ambient water temperature (24 ^{o}C) in triplicate for each of the three temperature treatments (18, 28 and 38 ^{o}C) in both open-top and closed-top respirometers. Each tank was supplied with constant freshwater flow (~ 8 L min^{-1}) from a 15,000 L recirculating aquaculture system (RAS). All fish were fed a maintenance ration once a day of a commercial diet (Ridley AquaFeed Pty Ltd, Narangba, QLD, Australia).

Water quality parameters, ammonia (NH_3/NH_4^+; ≤ 0.25 ppm), nitrite (NO_2^-; ≤ 0.25 ppm), dissolved oxygen (DO; > 95% saturation), pH (6.8 –7.0) and temperature were recorded daily. The temperature was controlled with two heater chiller units that achieved a temperature control of ± 0.5 $^{\circ}$C of the set temperatures. All fish were fasted for 48 h prior to MO_2 readings to ensure MO_2 was not confounded due to post-prandial effects. The study was carried out in freshwater.

Experiment Protocol

After initial stocking all fish were held for one week to allow sufficient time to acclimate to the tanks. Following this period the temperature was either increased or decreased in increments of 1 $^{\circ}$C day^{-1} until the experiment temperature was reached. MO_2 was measured using a Luminescent Dissolved Oxygen (LDO) meter (HQ30d-LDO101-03; Hach, Loveland, USA) which was re-calibrated for each temperature treatment as per manufacturers instructions.

The mass-specific routine metabolic rate (RMR; mg O_2 $kg^{-0.8}$ h^{-1}) of barramundi was established at the three temperatures (18, 28 and 38 °C). Dissolved oxygen (DO) (mg l^{-1}) was recorded at the start and end of the experimental period after water supply to each respirometer tank was removed leaving the fish in static water. Start DO levels in each static tank were close to saturation (> 97%) and > 50% at the conclusion of the experimental period. DO was recorded over approximately 1 h periods before water supply was returned for a minimum of 1 h to re-saturate DO levels and to flush out any waste metabolites. MO_2 readings were repeated three times following the same protocol. After measuring mean MO_2 fish were reciprocally transplanted into the alternate respirometry system, with fish from the open-top respirometers transplanted into the closed-top respirometers and *vice versa*. Fish were allowed to settle for a further 24 h and RMR re-measured as above. Reciprocal MO_2 readings of fish and systems in this manner allowed for the mutual validation of open and closed-top respirometry systems by accounting for any behavioral discrepancies within groups of fish. Each temperature treatment was independent, with different fish used for each temperature treatment. Fish were removed from the system and re-weighed after completing MO_2 readings at each temperature treatment. After fish were removed background biological oxygen demand (BOD; mg O_2 l^{-1} h^{-1}) was determined for each replicate tank.

Confirmation of air-tight closed-top respirometer

To confirm that the closed-top respirometers were air-tight, re-aeration rates (mg O_2 l^{-1} h^{-1}) were measured in respirometers excluding barramundi over ~ 24 h for each temperature treatment using oxygen depleted water. Triplicate closed-top respirometers were first disinfected with liquid sodium hypochlorite (NaOCl) (5 ml/l with 30 min contact time) (Lekang 2007) to eliminate any biological oxygen demand in tanks before being rinsed and refilled. The temperature of the experiment tanks was maintained by placing them in a water bath. Dissolved oxygen was then displaced out of the respirometer water to ~ 50% saturation by bubbling nitrogen gas into each respirometer. Dissolved oxygen levels were measured for each temperature treatment (18, 28 and 38 °C) at 1, 3, 6, 12 and 24 hour intervals to determine the re-aeration rate. There was no measureable change in DO concentration over 24 h in any replicate tank at all temperatures implying the closed-top respirometers were sufficiently air tight to prevent O_2 resaturation.

Routine metabolic rate, Maximum metabolic rate and aerobic scope in four hatchery populations of barramundi from different genetic stocks

The routine metabolic rate (RMR), maximum metabolic rate (MMR) and aerobic scope (AS) was determined using open-top respirometry at three different temperatures (18, 28, and 38 °C) for four genetically divergent barramundi stocks sourced from hatcheries in Broome, Karumba, Townsville region and Gladstone. Barramundi from the Darwin genetic stock were not examined due to insufficient numbers of fish of the same age and size remaining from the original population sourced.

Maximum metabolic rate was determined for each individual fish after its RMR was established and whereafter each individual fish was swum until exhaustion. Here fish were placed in a temperature controlled aerated 350 L conical tank and flow rate increased in a stepwise fashion using an Onga L413 pump. The following protocol was applied to each fish:

- 5 min at 10 L/min
- 15 min at 40 L/min
- 15 min at 80 L/min
- 15 min at 120 L/min
- 160 L/min until exhaustion

Exhaustion was determined as the point when fish were no longer able to swim against the water flow. Exhausted fish were then immediately transferred to an open-top respirometer where MO_2 readings were taken at 0 h to determine MMR and every 15 min for 3 h and every hour thereafter until fish returned to MO_2 readings within the standard error of the mean RMR for that particular population. Dissolved oxygen (DO) saturation was maintained at > 70% by flushing respirometers intermittently as needed. This was carried out at low flow rates to avoid activity associated with water movement.

Mass-specific RMR was calculated by using a scaling body weight exponent of 0.8 (Brett and Groves 1979) and corrected for background biological oxygen demand (BOD) of the tank with the mass-specific RMR calculated using the equation:

$$\text{RMR (mg } O_2 \text{ kg}^{-0.8} \text{ h}^{-1}) = (V/BW/n) \times (\Delta O_2 - O_{2BOD}) \quad \text{(Eq.1)}$$

Where V = tank volume (l); BW = mean body weight (kg); n = number of fish tank $^{-1}$; ΔO_2 = net change in O_2 concentration (mg l^{-1} h^{-1}) including fish respiration and background BOD; O_{2BOD} = background BOD rate (mg l^{-1} h^{-1}). Background BOD was observed to increase with increasing temperature, ranging from 0.015 ± 0.003 mg O_2 l^{-1} h^{-1} at 18 °C to 0.231 ± 0.019 mg O_2 l^{-1} h^{-1} at 38 °C, equating to < 0.05% of MO_2 at 18 °C and < 0.3% at 38 °C.

Temperature quotient (Q_{10}) values were calculated as a function of mean temperature across a 10 °C range as:

$$Q_{10} = (MR_2/MR_1)^{10/(T_2-T_1)} \quad \text{(Eq. 2)}$$

Where $MR_{1;}$ = RMR value at T^1 and MR_2 = RMR value at T^2. T^2 is the highest temperature used and T^1 the lowest temperature.

The energetic demands for the RMR for barramundi were calculated using the oxyenergetic coefficient of 13.59 J mg^{-1} O_2 (Brett and Groves 1979; Elliott and Davison 1975).

Mean aerobic scope (AS) was calculated as MMR – RMR for each stock at each temperature. The factorial difference (F*x*) was also calculated as MMR / RMR.

Data Analysis

All oxygen consumption (MO_2) data was expressed as mg O_2 $kg^{-0.8}$ body weight h^{-1}. A two-factor ANOVA was used to test for the interactive effect of barramundi stock and temperature on RMR, MMR and AS. All results were regarded as significant at P<0.05. Tukey-Kramer post hoc tests were used for multiple comparisons of significant effects.

iii) Hypoxia trials

Background

Altered weather patterns resulting from climate change have the potential to exacerbate periods of environmental hypoxia. Higher water temperatures reduce the solubility of oxygen in water while concurrently increasing the metabolic demand for oxygen in aquatic ectotherms (Weiss 1970; Diaz and Breitburg 2009). As such, the unprecedented rate of temperature rise predicted under current climate change scenarios may push many aquatic species close to, or above, their upper thermal thresholds (Tewksbury et al., 2008;

Morrongiello et al., 2011; Huey et al., 2012). This may be particularly pronounced at low latitudes near the equator, where species may be adapted to mean temperatures that may span only 1-2°C annually (Tewksbury et al., 2008). Thus, it is likely that the elevated temperatures predicted to occur in the future will progressively increase the prevalence and severity of hypoxia in aquatic systems, with flow-on effects to larger scale processes such as population growth, fitness and ecosystem dynamics (Pollock et al., 2007).

Physiological responses to hypoxia are regarded to be dependent on the frequency and severity of the hypoxic event (Farrell and Richards 2009). Below a certain level of dissolved oxygen (DO), most fish are unable to regulate their rate of oxygen consumption independently of the ambient oxygen and consequently enter a state of oxygen conformity (Prosser and Brown 1961; Fernandes et al. 1995; Schurmann and Steffensen 1997). The level of DO at which this conformity occurs is referred to as the critical oxygen saturation (O_2 crit) level and is commonly used as a measure of the hypoxia tolerance of a species (Prosser and Brown 1961; Schurmann and Steffensen 1997).

At present there is no understanding of the response or tolerance of barramundi (*Lates calcarifer*) to hypoxic episodes, despite the fact that hypoxic events have been implicated in large-scale fish mortalities, including barramundi in tropical Australia (Townsend and Edwards 2003). Without this knowledge it is impossible to predict how barramundi as a species will cope with these sudden hypoxic events. Additionally, given the genetic stock structure of barramundi in Australia, whereby stocks are exposed to differing thermal regimes, it is possible that adaptive differences to temperature and associated hypoxia exist which may provide resilience within the species to coping with sudden climate change induced hypoxia events.

The objectives of this particular component of study was to a) quantify the hypoxia tolerance of barramundi from different genetic stocks at an optimal (26°C) temperature, b) determine the hypoxia tolerance of barramundi from different stocks at elevated (36°C) temperature, and c) examine the extent of intraspecific variability in hypoxia tolerance in genetically distinct populations of Australian barramundi and determine whether hypoxia tolerance is related to latitudinal position.

Materials and Methods

Experimental Animals and Holding Conditions

Barramundi juveniles were obtained from five commercial hatcheries located at Broome, Darwin, Karumba, Bowen/Townsville and Gladstone. All five hatcheries use broodstock sourced from local rivers that are separated by a minimum of 700 km. Prior to experiments, fish were on-grown to a size of ~ 200 g over approximately 10 months in fresh water at the Marine and Aquaculture Research Facility Unit of James Cook University, Townsville, Australia. All fish were fed ~ 1% body weight per day using a commercial pelleted feed (Ridley AquaFeeds, Narangba, Australia), and maintained at 26 ± 1°C under a 12:12 hour photoperiod. Dissolved oxygen was maintained > 75% saturation in the holding tanks and water quality was monitored daily. Individual fish were weighed and measured prior to and following experiments.

Experimental Design

Prior to experiments, fish were removed from their holding tanks and acclimated to one of two temperatures in a separate system where water temperature could be closely controlled. Temperature treatments were selected based on a review of atmospheric and sea-surface temperatures obtained from Australian government databases (AIMS 2012; BOM 2012) and from a comprehensive review of river temperatures from previously published data (Pusey et al., 1998; Stuart and Berghuis 2002; Webster et al., 2005). Based on this information, two temperature treatments were selected; 1) a 'typical' temperature (26 °C), which is representative of the annual mean across the species distribution in Australia (and the temperature at which the fish had been held long-term), and 2) a 'warm' temperature (36 °C), which is regarded to be representative of the upper limit that wild populations currently experience, but still within the known tolerance limits for the species (Bermudes et al., 2010). Fish used in the 36 °C treatment were acclimated by increasing the temperature by 1 °C per day and then held at 36 °C for a minimum of 7 days prior to experimentation. All fish were fed as stated above, but food was withheld for 24 h prior to conducting experiments to minimise the effect of specific dynamic action on oxygen consumption measurements.

Respirometry

All measurements were performed using intermittent flow-through respirometry. Respirometers (volume = 10.3 L) were fitted with a small Perspex window to allow fish to be observed during respirometry trials. Each respirometer was connected to two pumps; a single recirculating pump to keep water within the chamber mixed, and a flush pump to supply the chamber with aerated water between measurements. Respirometers and flush pumps were submerged in a shallow 1000 L tank with vigorous aeration to provide both stable temperature conditions during experiments (26.2 ± 0.5 °C and 36.4 ± 0.2 °C) and to supply the chambers with ~100% saturated water during the flush cycle. Temperature-compensated oxygen concentration ($mg.L^{-1}$) of the water within each chamber was continuously recorded (0.5 Hz) using oxygen sensitive REDFLASH® dye on contactless spots (2 mm) adhered to the inside of each chamber and linked to a Firesting Optical Oxygen Meter (Pyro Science, Aachen, Germany) via fibre-optic cables. Oxygen sensing equipment was recalibrated daily using a one-point calibration and electrical zero. Following initial measurements of background respiration in the respirometers, individual fish that had been fasted for 24 h were placed in respirometers in the evening and allowed to acclimate to the respirometers for 16 h. During the acclimation period, the flush pumps attached to each respirometer were set to a 30:15 min on:off cycle and oxygen consumption ($mg\ O_2.kg^{-1}\ .min^{-1}$) was measured from the decline in oxygen in each respirometer during each 15 min off cycle. Resting oxygen consumption was calculated for each fish as the mean of the lowest three measurements recorded during the period of acclimation.

Following the chamber acclimation period and resting oxygen consumption measurements, each flush pump was turned off and fish allowed to deplete the oxygen within their respective respirometers to 5% air saturation. Oxygen consumption was calculated for each consecutive 5 min period during the decline in oxygen (~1.5-4 hours depending on temperature). Upon reaching 5% air saturation, each flush pump was turned on again to restore oxygen levels to 100% saturation. Preliminary trials conducted using similar sized fish indicated that depletion to 5% saturation was sufficient to calculate O_2 crit, but still above the level that induced loss of equilibrium. Two values were identified from each O_2 crit experiment; 1) pre-hypoxia, that was calculated from the mean of the lowest three 5 min measurements recorded between 100% and 75% saturation after flush pumps had been turned off, and 2) O_2 crit, that was determined using previously established methods (Corkum and Gamperl 2009; Nilsson et al.,

2010). Briefly, O_2 crit was determined by fitting two linear regression lines to the measurements (one line based on the calculated pre-hypoxia, and another line based on the decrease in oxygen consumption observed during the later stages of the O_2 crit test), and calculating the intersection point of the two lines (Ott et al. 1980; Nilsson et al., 2004). After the completion of each trial, additional background respiration measurements were obtained for each chamber in the absence of fish.

Data Analyses and Statistics

All oxygen consumption rate measurements (mg O_2. kg^{-1}. min^{-1}) were calculated using commercial software (LabChart v.7, ADInstruments, Sydney, Australia) from the slope of the decline in oxygen concentration according to the formula:

$$\dot{M}o_2 = \frac{\left(\left(\text{slope}_a \times 60\right) \times \left(V_c - V_f\right)\right) - \left(\left(\text{slope}_b \times 60\right) \times V_c\right)}{V_f}$$

where slope_a and slope_b are the declines in oxygen ($mg.L^{-1}.s^{-1}$) measured in the presence and absence of fish within the chamber, respectively. V_c and V_f are the volumes (L) of the chamber and fish, respectively.

Resting, pre-hypoxia, and O_2 crit measurements were obtained for 114 barramundi from the five distinct genetic stocks, with individual fish considered as an individual replicate for population and temperature treatments. Individual replicates where O_2 crit was not able to be calculated (n = 7) were excluded from further analyses. The temperature coefficient (Q_{10}) for resting for each population over the 10 °C temperature increment was calculated using the formula:

$$Q_{10} = \frac{R_2}{R_1}^{10/(T_2 - T_1)}$$

where R is resting oxygen consumption, T is temperature, 1 represents values at 26 °C, and 2 represents values at 36 °C.

Statistical analyses were performed using SPSS v. 20 (IBM, Chicago, IL, USA). A general linear model was used to assess the effect of the two main factors (sub-population and temperature) on resting, pre-hypoxia and O_2 crit. These analyses were followed by one-way ANOVA with Student-Newman Keuls post hoc tests for O_2 crit and the non-parametric Kruskal-Wallis test with Dunn's post hoc comparison for resting oxygen consumption and pre-hypoxia to assess sub-population differences at each temperature. Homogeneity of variance and normality were assessed using Levene's test and a normal quantile-quantile (Q-Q) plot respectively. Data are presented as means ± standard deviation and results were considered statistically significant at $P < 0.05$.

Component C: Parasite surveys and risk analyses

Most attention in aquatic finfish species to date in the face of climate change has focused on the resilience and adaptability of fish species to increased temperatures and other environmental parameters. However, climate change is also expected to impact significantly on other organisms within aquatic ecosystems including fish parasites. As parasites and their control are one of the biggest economic impactors in barramundi finfish aquaculture it is prudent to identify potential risks of increased pathogen prevalence and pathogenicity. Accordingly, this component sought to identify and quantify the risk posed by specific parasites to barramundi aquaculture populations. It addressed this aim in the following ways; 1) a risk assessment was conducted to identify the likelihood and consequence of metazoan parasite epizootics in mariculture of barramundi and thereby determined parasite species of the greatest risk to industry viability; 2) the effects of temperature and salinity as a proxy of future climate variations on the life cycle of high risk parasites was investigated; 3) a strategic management timetable for the treatment of one of the most important marine parasites *Neobenedenia* sp. infecting farmed *Lates calcarifer* was developed; 4) examined whether genetic strains of barramundi exhibit different susceptibility to ectoparasite infection; and 5) examined the pathology associated with *Neobenedenia* infection. The broader objective of this component was to generate information enabling efficient parasite management practices that can be tailored to temperature and salinity conditions at particular locations that will serve to minimize reinfection on farms.

i) Risk assessment to identify the likelihood and consequence of metazoan parasite epizootics in mariculture of barramundi

Background

Parasite risk assessments are commonly used to identify economic (production, trade) and environmental risk (Diggles 2003; Nowak 2004; Hutson et al., 2007b). Although the impact of emerging parasite species can be difficult to predict, scrutiny of available information and responsive research on high risk species can improve vigilance in disease surveilance, prevention and managment. Accurate parasite identification is critical, because knowledge of specific species' biology will determine the most appropriate management methods (e.g. farm location; fallowing; water quality and filtration; net changes; antifoulants; therapeutic chemicals). Parasite identification is also significant in biosecurity as some pathogenic species

in aquaculture are known to have inadvertently translocated from one region to another, leading to the spread of disease (e.g. Johnsen and Jensen 1991). The objective of this sub-component was to undertake a survey of metazoan parasites that have been implicated with infection of marine barramundi so that a risk assessment can be performed to identify possible future disease threats to the barramundi mariculture industry.

Metazoan parasites present considerable concern for sustained global production of finfish. In the past two decades, reliable hatchery production of Latids has enabled considerable commercial expansion, with global production of Latids (primary species *Lates calcarifer*) tripling over the past decade (> 69, 000 t in 2011) with major producers operating in the Indo-Pacific (FAO, 2013). The mass production of any organism is always accompanied by parasites and pathogens that threaten economic viability and food production capability (Seng 1997; Seng 2006; Guo and Woo 2009; Rückert et al., 2008). In addition to mass mortality, parasites can cause deformities, reduce growth rates, decrease consumer confidence and demand greater investment in stock management and disease management infrastructure (Lackenby et al., 2007; Fajer-Ávila et al., 2008).

Materials and Methods

Metazoan parasites included in the risk analyses were recovered from necropsies performed on wild and farmed fish specimens and/or have been documented in scientific literature. Wild and farmed barramundi were collected from Australian waters and examined fresh for live parasite recovery (Table 3). Parasites were collected and preserved using standard parasitological techniques (Hutson et al., 2007a) and identified to lowest possible taxon following published parasite descriptions, taxonomic keys, voucher material and additional parasitological expertise (see acknowledgements). Representative parasite specimens, mounted on slides and in alcohol for future DNA analysis, were deposited in the South Australian Museum, North Terrace, Adelaide, 5000, South Australia, Australia (see Table 4 and 6). Published host-parasite records for *L. calcarifer* (including records for misapplied host names and synonyms as listed by Fishbase) were sought from the scientific literature, the Natural History Museum host-parasite database (Gibson et al., 2005), and Australian Museum collections (the South Australian Museum, Adelaide; the Australian Museum, Sydney).

Risk analyses

Several risk analyses provide frameworks to identify hazards and quantify risks posed by parasites in aquaculture (Diggles 2003; Fletcher et al., 2004; Nowak 2004; Murray and Peeler 2005). This assessment evaluates the likelihood of parasite establishment in farmed fish by considering the biological pathway (i.e. life cycle) necessary for parasite species to infect farmed fish species (Hutson et al., 2007b). A framework was developed to assess the potential negative consequence of establishment and proliferation of parasite species (see below). This risk assessment estimated two parameters, likelihood and consequence, that were considered independently. Likelihood and consequence estimates were then combined in a risk matrix to determine overall risk. Risk was estimated for each parasite identified to genus in our examination of wild and farmed fishes and from previously published records. Parasite-host records deemed to require further validation (see Table 4 and 6) were not included in the risk analyses. Analyses did not include parasite records from freshwater environments (*L. calcarifer* are diadromous), protozoan parasites or other disease-causing agents such as viruses, bacteria and fungi.

Likelihood

The likelihood of parasite establishment and proliferation on or in *L. calcarifer* was estimated by reference to the biological pathways necessary for each parasite species to infect and reproduce on the farmed species. Five likelihood criteria were used to estimate the likelihood of parasite establishment and proliferation based on parasite biology. Likelihood definitions ranged from negligible to extreme (adapted from Fletcher et al., 2004) (Table 4).

Consequence

Information was gathered from scientific literature on parasites (family, genus and/or species) to indicate any potential negative consequence of establishment and proliferation. Data were sought that directly related to four risk criteria including: 1) potential to cause mortality, 2) potential to cause pathology or disease, 3) potential impact on marketability and 4) potential impact on consumer health. Parasites were scored for each of these four criteria. Parasites that met all four criteria were assigned an *extreme* consequence, parasites that met three criteria were assigned a *high* consequence, parasites that met two criteria were assigned a *moderate* consequence, parasites that met one criterion were assigned a *low* consequence and parasites that met no criteria were assigned a *negligible* consequence. Using this information, we

determined the consequence of parasite establishment and proliferation as adapted from risk criteria by Fletcher et al., 2004.

Overall risk

In order to identify the most serious metazoan parasite risks for mariculture of *L. calcarifer*, total risk was estimated by considering the likelihood and consequence of parasite establishment and proliferation in sea-cage farming following the Australian Quarantine Inspection Service (AQIS) (AQIS 1999) risk estimation matrix (Table 5).

Table 3: Sample locations for wild and farmed Lates calcarifer examined in Australia. (QLD = Pacific Ocean, Queensland; WA = Indian Ocean, Western Australia). Land based aquaculture in Bowen, QLD, comprises concrete raceways with seawater intake.

Species	**Origin**	**Location**	**Latitude and longitude**	***n***	**L_T range (mm)**
L. calcarifer	wild	Cleveland Bay, QLD	19°17′03″S 146°51′59″E	19	420 – 560
	sea-cage	Port Hinchinbrook, QLD	18°23′31″S 146°8′12″E	26	515 – 650
	land based	Bowen, QLD	19°56′28″S 147°55′49″E	15	435 – 540
	sea-cage	Cone Bay, WA	16°28′20″ S 123°32′35″E	8	310 – 540

Table 4: Definitions for likelihood and consequence (adapted from Fletcher et al., 2004).

Likelihood level	**Descriptor**
Extreme	Parasites with direct infective stages that have been recorded in farmed fish
High	Parasites with direct life cycles not previously recorded in farmed fish
Moderate	Parasites with complex life cycles that exhibit direct infective stages
Low	Parasites with two or more host species in the life cycle, that require the primary host to consume an infected intermediate
Negligible	Parasites that have not been previously recorded from the host fish species
Consequence and risk level	
Extreme	Catastrophic consequences to the entire industry, total mortality or eradication of fish is considered, trade
High	Establishment and proliferation of parasites could have serious biological consequences, prolonged high mortality
Moderate	Substantial seasonal morbidity and mortality rates with significant cost to the farmer to warrant intermittent concern
Low	Establishment and proliferation of parasite species is manageable with low economic significance to the industry
Negligible	Establishment and proliferation of parasite species could have no significant consequences, with low or no

Table 5: Risk estimation matrix following the Australian Quarantine and Inspection Service (AQIS 1999).

	Consequence (2)				
Likelihood (1)	Negligible	Low	Moderate	High	Extreme
Extreme	Negligible	Low	Moderate	High	Extreme
High	Negligible	Low	Moderate	High	High
Moderate	Negligible	Negligible	Low	Moderate	High
Low	Negligible	Negligible	Negligible	Low	Moderate
Negligible	Negligible	Negligible	Negligible	Negligible	Low

ii) The effects of temperature and salinity on the life cycle of high risk parasites

Background

Integrated parasite management strategies of farmed stock require comprehensive and accurate knowledge of parasite life cycles and the influence of environmental parameters to be effective (Tubbs et al., 2005). As disease-causing organisms will be similarly impacted by climate change, understanding the influence environmental parameters such as temperature and salinity will have on parasite life-cycles provides information on how parasites will respond to environmental changes, as well as identify particular integrated pest management intervention areas to limit the parasites impact.

One method of managing parasites in aquaculture is to administer treatments at times that disrupt or break the life cycle of the parasite. The life cycle is interrupted by initially killing all adult parasites on the host. Future treatments are temporally coordinated to occur after all

eggs have hatched, but before new parasite recruits reach sexual maturity and contribute new eggs into the system (Tubbs et al., 2005). Essential to this method is the accurate determination of embryonation periods and time to sexual maturity at a range of environmental parameters allowing for treatments to be tailored to particular localities and farms (Hirayama et al., 2009). Results of the effective administration of this method include the exposure of hosts to fewer infective stages and reduction in re-infection rates, ultimately necessitating fewer treatments (Tubbs et al., 2005; Lackenby et al., 2007; Chambers and Ernst 2005).

Monogeneans in the Family Capsalidae are a particular threat to the aquaculture industry as some species have been responsible for epizootic events (Bauer and Hoffman 1976; Paperna and Overstreet 1981; Deveney et al., 2001; Whittington 2004). Capsalid monogeneans of the genus *Neobenedenia* are particularly harmful ectoparasites of tropical and subtropical fin fishes in marine aquaria and aquaculture (Whittington 2012). *Neobenedenia* spp. infect over 100 teleost species, many of which are important aquaculture species (Ogawa and Yokoyama 1998; Hirazawa et al., 2004; Ogawa et al., 2006), including barramundi, in Indonesia (Rückert et al., 2008) and Australia (Deveney et al., 2001; Hutson et al., 2012). Their direct life cycle, short generation time and filamentous eggs, which entangle on structure, cause difficulties in managing infections and large numbers of parasites can become present in a system over a short period of time (Jahn and Kuhn 1932; Ogawa et al., 1995; Ogawa 2006). Grazing habit on host fish causes inflammation, dermal ulceration and allows the ingress of secondary infections (Kaneko et al., 1988).

The *L. calcarifer* aquaculture industry also faces severe challenges from parasitic copepod epizootics. Most parasitic copepods exhibit monoxenous (direct, single host) life cycles which facilitate the rapid completion of their life cycles and prolific reproduction in aquaculture environments. Species in *Lernanthropus* (de Blainville 1882) present considerable concern for sustained global production of finfish. Damage to fish occurs through the parasite's attachment appendages that constrict the flow of blood through the gills, and high burdens can cause anaemia and a loss in respiratory surface area. Extensive research has been dedicated to *L. kroyeri* that infects farmed fishes in the Mediterranean, but research on tropical *Lernanthropus* spp., such as *Lernanthropus latis*, is comparatively sparse. This subcomponent examined the hatching success of *Neobenedenia* sp and *Lernanthropus latis* infecting *Lates calcarifer* in current and predicted water temperature and salinity scenarios.

Materials and Methods

Parasite collection

A laboratory infection of *Neobenedenia* sp. Originating from near Ayr, north Qld, was established on *L. calcarifer* to ensure a continuous source of parasites for experimentation (see Hutson et al., 2012). Representative *Neobenedenia* sp. specimens (hereafter as *Neobenedenia*) were accessioned to the Australian Helminth Collection (AHC) at the South Australian Museum, Australia (SAMA), North Terrace, Adelaide, South Australia 5000 (SAMA AHC 35461). The species of *Neobenedenia* investigated in this study is presently unidentified (see Hutson et al., 2012).

Lates calcarifer examined for *Lernanthropus latis* were collected from four localities in tropical north Australia between October 2010 and June 2011 (Table 6). Fish from the wild (Cleveland Bay, Townsville; 19.284331: 146.866419) were captured by a commercial fisherman using a gill net. Fish from two Queensland farms were captured from their sea-cage (Bluewater Barramundi, Hinchinbrook: 18.392292: 146.136547) or raceway (Good Fortune Bay, Bowen: 19.940108: 147.932147) using hand nets and cast nets, respectively. Freshly killed sea-caged fish from Western Australia (Marine Produce, Cone Bay: 16.472297: 123.543144), were refrigerated and freighted to the laboratory for examination. Gills were removed from fish and placed into individual petri dishes filled with filtered seawater. Gill arches were separated with dissection scissors and individual gill filaments were observed under a stereomicroscope (~20× magnification). Parasites were removed from filaments with fine forceps. Representative male and female specimens were fixed in 70% ethanol for morphology.

Temperature and salinity treatments

Temperature (22, 24, 26, 28, 30, 32 and 34 °C) and salinity (0, 11, 22, 35 and 40‰) treatments were selected to represent seasonal temperature and salinity variation experienced in tropical monsoonal climates in Queensland, Australia. Two degree increments were chosen from the minimum average winter sea surface temperature (22 °C) to the average summer ocean temperature (34 °C) predicted by the year 2050 at Lucinda, Queensland (18°31'41.271"S: 146°19'53.04"E) (Australian Institute of Marine Science, 2008) under the Intergovernmental Panel on Climate Change (2007a; 2007b) scenario of 'business as usual' emission levels. Selected increments included the average summer ocean temperature (30 °C)

and maximum summer ocean temperature (32 °C) (AIMS, 2008). A range of salinities including freshwater (0‰), hyposaline (11 and 22‰), seawater (35‰) and hypersaline solutions (40‰) were chosen to represent extreme salinity fluctuation as observed in open, semi-closed and closed aquaculture systems in tropical climates.

Time to first and last hatch and hatching success of Neobenedenia eggs

The effect of temperature and salinity on the time to first and last hatch and hatching success of *Neobenedenia* eggs was examined. *Neobenedenia* eggs were collected from infected experimental fish as per Militz et al., (2013). Eggs were divided into groups of approximately ten using fine-tipped forceps and placed into individual glass cavity blocks (40 mm^2) with six replicates made for each temperature/salinity combination. The blocks were filled with solution to the brim and a glass cover placed on top in order to reduce lysed or trapped oncomiracidia in the surface tension (Ernst and Whittington, 1996). Eggs were incubated (Sanyo: ML-351 Versatile Environmental Incubation Chamber) with fluorescent lighting for a 12:12 LD cycle. One third of the solution was changed each day with minimal disturbance to the eggs. Blocks were monitored every 24 h at 1100. When hatching was observed, oncomiracidia were removed with a pipette and the day of hatching was recorded. Hatching experiments were continued until 48 h passed without hatching in any treatment when any unhatched eggs were not considered viable. Hatching success of *Neobenedenia* eggs was measured as the number of oncomiracidia removed from each cavity block divided by the total number of eggs

Table 6: Host localities indicating number of fish examined, parasite prevalence (P), mean intensity (I) and mean abundance (A) of adult Lernanthropus latis. WA = Western Australia. QLD = Queensland. Cone Bay, WA fish collected in March, 2011, Cleveland Bay, QLD fish collected between March–June 2011, Hinchinbrook, QLD fish collected in October, 2010 and Good Fortune Bay, QLD fish collected in May, 2011. Measurements in millimetres (mm); mean followed by range in parentheses.

Location	**Origin**	**# Fish**	**Fish size**	**Female**			**Male**			**Total**		
				P	**I**	**A**	**P**	**I**	**A**	**P**	**I**	**A**
Cone Bay, WA	Sea-cage	10	438 (310–540)	100%	3	2.6	0%	0	0	100%	3	2.6
Cleveland Bay, QLD	Wild	20	515 (420–560)	80%	6	4.8	25%	1.8	0.7	80%	6	4.8
Hinchinbrook, QLD	Sea-cage	26	574 (515–650)	89%	6	5	8.6%	1.3	0.11	89%	6	5
Good Fortune Bay, QLD	Land based	10	491 (435–540)	0%	0	0	0%	0	0	0%	0	0

Incubation and hatching of Lernanthropus latis eggs

Live ovigerous females from wild hosts collected at Cleveland Bay, Townsville between March and June 2011, were used for hatching and infection experiments. Over the sampling period, sea surface temperature ranged between 22 and 27 °C. *Lernanthropus latis* bear uniserate egg sacs in which disc-shaped eggs are closely packed. Paired egg sacs were gently detached from the genital complex using fine-tipped forceps and placed in filtered sea water (35 ‰). Eggs within the sac were counted under a stereomicroscope using a hand-held counter. Egg sacs were randomized and placed in 100 mL plastic containers with 80 mL of 0, 11, 22, 35 or 40 ‰ saline solution and were incubated at 22, 30, 32 or 34 °C (Sanyo: ML- 351 Versatile Environmental Incubation Chamber). Each treatment had four replicates.

Incubators were lit with fluorescent lighting for a 12:12 day:night cycle. The water was aerated throughout the incubation process using a battery powered aerator. Eggs were monitored every 6 hours. Monitoring ceased following a 24 h period without hatching in any treatment. Following hatching, a representative sample of five individual nauplii was aspirated with a pipette every six hours and fixed in 70% ethanol to document larval development. Hatching success was defined as the number of eggs that hatched into swimming nauplii.

Neobenedenia oncomiracidia (larva) longevity

The effects of temperature and salinity on the life span of *Neobenedenia* oncomiracidia were examined. *Neobenedenia* eggs did not hatch in 0 and 11‰ treatments (see above) and consequently these salinities could not be investigated in oncomiracidia longevity experiments. Approximately 100 eggs were incubated in a petri dish at each salinity (22, 35 and 40‰) at each temperature (22, 24, 26, 28, 30, 32 and 34 °C) (Model UP150 refrigerated incubator) with fluorescent lighting for a 12:12 LD cycle. Eggs were monitored every 24 h at 1000 for hatching. Once hatched, individual oncomiracidia were gently removed from petri dishes using a plastic pipette and placed into separate glass cavity blocks in the salinity in which they were incubated. Eggs incubated in 22‰ at 24, 26 and 28 °C did not yield enough oncomiracidia for sufficient replication, consequently, oncomiracidia used in 22‰ treatments were sourced from 35‰ at the same temperature. The solution in each cavity block was filled to the brim. Water was not changed throughout the experiment in order to minimize disturbance to oncomiracidia. Six replicates were made for each temperature/salinity combination. Each oncomiracidium was monitored every 2 h to assess survival.

Oncomiracidia were considered dead once they showed no signs of motion and failed to respond to a gentle stream of water from a plastic pipette. Once determined to be dead, oncomiracidia were still examined in the subsequent monitoring period to confirm death. Oncomiracidia longevity was expressed as the time period from treatment immersion to death.

Infection success and time and size at sexual maturity

The effect of temperature and salinity on oncomiracidia infection success and time to sexual maturity was examined in vivo. *Neobenedenia* eggs did not hatch in 0 and 11‰ treatments and consequently these salinities could not be investigated in infection or time to sexual maturity experiments. Approximately 200 eggs were incubated in each temperature (22, 24, 26, 28, 30, 32 and 34 °C) and salinity (22, 35 and 40‰) combination to provide a source of oncomiracidia for the infection experiments. Eggs incubated at 22‰ and 32 °C and 34 °C did not yield enough oncomiracidia for sufficient replication. Oncomiracidia used in 22‰ treatments were sourced from 35‰ and oncomiracidia used in 32 and 34 °C treatments were sourced from 30 °C.

Immediately following hatching, ten vigorously swimming oncomiracidia were gently aspirated with a glass pipette and slowly ejected from the pipette into a 10 L aquarium with an individual *L. calcarifer* (mean 180 LT (150–200 mm)) sourced from Good Fortune Bay Hatchery, Kelso, Queensland) in 6 L of UV filtered seawater. Fish were reared in freshwater and were naïve to *Neobenedenia* infection. Five replicates were made for each temperature/salinity treatment. Aquaria were aerated using battery powered aerators. Pieces of large gauge (1 mm) tulle (20×10 cm) were placed into the container and inspected under a stereo-microscope daily for tangled eggs, denoting the presence of at least one sexually mature parasite.

When egg production was detected, the fish was bathed the following day twice in dechlorinated freshwater for 5 min and the epithelial surface gently rubbed in order to dislodge attached parasites (see Militz et al., 2013). The solution was filtered through 60 µm mesh to collect dislodged individuals which were counted under a stereomicroscope. Infection success was determined as the proportion of parasites collected to the number of oncomiracidia introduced to the fish. *Neobenedenia* were stained with Haematoxylin, dehydrated through an alcohol series and mounted on microscope slides in Canada balsam. Total length, total width, anterior hamuli and accessory sclerites were measured using a

micrometer and ImageJ 1.44p (Java 1.6.0_20). Measurements are given as: mean (minimum – maximum range) and follow Lackenby et al., (2007). Time to life cycle completion was calculated as the minimum time taken for eggs to hatch and resulting oncomiracidia infect fish, reach sexual maturity and begin laying eggs of their own.

Strategic management timetable

A strategic management timetable was formulated to determine the most effective time for treating fish in order to limit recontamination by fecund *Neobenedenia*. Assumptions were: 1) an initial treatment (on day 0) kills 100% of attached parasite stages on treated stock and 2) that oncomiracidia reinfect treated fish within 24 h. Reinfection is assumed within 24 h as eggs may be retained on structure within the aquaculture system and oncomiracidia are ciliated and capable of swimming back into a previously treated area. Furthermore, acute treatments do not inhibit egg hatching (Sharp et al., 2004; Militz et al., 2013). The most appropriate time for a second treatment was calculated using data collected on time (in days) to first and last hatch of *Neobenedenia* sp. eggs and time to sexual maturity. The appropriate time for subsequent treatments was determined to occur within a period that allowed enough time for eggs deposited by adult parasites pre-treatment to hatch, but less time than that needed for post-treatment parasites to mature on fish.

Statistical analyses

Data did not fulfil the conditions of normality and homogeneity of variance and were analysed by permutational analysis of variance in the PERMANOVA function of PRIMER 6.0. PERMANOVA compares the observed value of a test statistic (F-ratio) against a recalculated test statistic generated from random permutation of the data. A PERMANOVA with 9999 permutations based on Euclidean distance was used to statistically evaluate experimental treatments, followed by pairwise comparisons. Significance was accepted at $p<0.05$.

A one-way analysis of variance (ANOVA) was used to assess the effects of temperature on the measurements of total length, total width, anterior hamulus length and accessory sclerite length. Analyses were performed using the S-Plus 8.0 software package from Spotfire®. Significance was accepted at $p<0.05$. Post hoc comparisons of group means were performed using Tukey's HSD test. Linear models were used to describe the relationship between treatment (temperature and salinity) and response variables (hatching and infection success, time to last hatch and time to sexual maturity). Quantile regression models (tau = 0.95) were

created for 'Time to last hatch' and 'Time to sexual maturity' data. Resulting models and equations used to generate each relationship are detailed in Table 7. Linear and quantile regression models were created using the statistical program R 3.0.0. using the 'quantreg' package (Koenker 2013).

Table 7: Equation values for linear models: 'hatching success', 'infection success' and quantile regression models: 'time to last hatch' and 'time to sexual maturity data'. '-' denotes no value, 'temperature' data is represented by the term 'a' and 'salinity' data is represented by the term 'b'.

Terms	Hatching success (%)	Infection success (%)	Time to last hatch (days)	Time to sexual maturity (days)
Intercept	5.016	$1.660e^{4}$	$3.218e^{2}$	$-7.623e^{1}$
a	$2.891e^{1}$	$-2.292e^{3}$	$-4.574e^{1}$	4.136
b	$2.120e^{1}$	$-1.184e^{1}$	$1.839e^{1}$	4.056
ab	-1.633	$5.388e^{-1}$	-1.632	$-2.006e^{-1}$
a^2	$-4.862e^{-3}$	$1.172e^{2}$	2.403	-
b^2	-1.207	$1.796e^{-1}$	$-2.120e^{-1}$	-
ab^2	$9.572e^{-2}$	-	$1.073e^{-2}$	-
a^2b	$2.983e^{-2}$	-	$3.975e^{-2}$	-
a^2b^2	$-1.761e^{-3}$	$-2.258e^{-4}$	-	$6.881e^{-5}$
a^3	-	-2.637	$-5.527e^{-2}$	$-9.563e^{-4}$
b^3	-	-	$2.823e^{-3}$	$-5.319e^{-4}$
a^3b^2	-	-	$-2.436e^{-6}$	-
a^2b^3	-	-	$-4.647e^{-6}$	-
a^4	-	$2.198e^{-2}$	$4.702e^{-4}$	-
b^4	-	-	$-4.473e^{-5}$	-
a^4b	-	-	$-6.875e^{-6}$	-
ab^4	-	-	$1.173e^{-6}$	-
a^4b^4			$2.965e^{-11}$	-
a^b	-	-	-	$-5.658e^{-62}$

iii) Genetic susceptibility of barramundi stocks to *Neobenedenia* infection and mode of this parasites pathology

Materials and Methods

Naive *Lates calcarifer* from three populations (Darwin, Bowen/Townsville and Cairns) were used in infection experiments. Fish were held in the Marine Aquaculture Research Facility Unit (MARFU) at James Cook University, Townsville (19°19'42"S 146°45'40"E) in freshwater and fed ~ 5% bodyweight per day until required. Fish were caught using a small net and acclimated to seawater over two hours. Individual fish were gently netted into a 10L container with 6 L of filtered seawater (35‰). Fish were acclimatised for 24 h in an air-conditioned room (25 °C) exposed to natural light and constant aeration. Thirty replicate *L. calcarifer* were used from each of the three genetic stocks. Approximately 50 eggs were allocated to each 10L container when eye spots had developed. Fish were held for six days to allow oncomiracidia to hatch from eggs and attach to their host and commence development. Fish were not fed during this period and mortalities were excluded from data analysis. Individual fish were removed from the container by hand and placed in freshwater for 5 min. The solution was gently swirled to detach dead worms from the fish's body surface. The freshwater solutions were examined for juvenile *Neobenedenia* sp. under a stereomicroscope. Fish were weighed to the nearest gram and measure (L_T = total length).

iv) Histopathology

Materials and Methods

Histopathology

Histopathology was compared between microhabitats of uninfected and infected fish by semi-qualitative morphological differences. Forty *L. calcarifer* were held in separate aquaria with 10 L of seawater (35 gL-1) after the seawater acclimation period. Twenty fish were experimentally infected with *Neobenedenia* by gently pipetting 25 oncomiracidia into each aquarium. Aeration was turned off for one hour after the introduction of the parasite to facilitate infection (Hirazawa et al., 2010). The remaining 20 fish were considered controls and treated similarly, although without exposure to *Neobenedenia* sp.. Water changes were done thoroughly in each tank every day to maintain optimal water parameters and avoid reinfection. Twenty-one days post-infection all 40 fish were euthanized with an overdose of Aqui-S aquatic anaesthetic without causing parasite detachment (Sharp et al., 2004). Each

euthanized fish was then immersed in seawater (35 gL-1) in a shallow tray and tissue samples were collected from the fish using a surgical scalpel blade. Samples were taken from the mandible, operculum and middle body surface. Each tissue sample collected (~1 cm2) from infected fish had a single individual parasite attached to the epithelium. Comparable tissue samples were taken from the same microhabitats in uninfected fish. Samples were fixed in 10% buffered neutral formalin solution for 48 h prior to being routinely processed, paraffin embedded, and 4 µm sections stained with haematoxylin and eosin solution.

Photomicrographs were taken from one plane of section per sampling site, which were divided into 10 fields (x400) along the length of the haptor attachment site to the epidermis. Each plane of section per sampling site was examined in the following manner: epidermal thickness was measured from the basal epithelial layer to the external or apical layer, and the number of squamous epithelial layers and mucous cells were counted. These criteria were based on previous reports of pathological changes in the skin morphology of scaled fish parasitized by *Neobenedenia* (Leong and Colorni 2002; Ogawa et al., 2006; Hirayama et al., 2009; Hirazawa et al., 2011). Epidermal thickness, epithelial layers and the number of mucous cells were measured/counted from each photomicrograph and an average was calculated from five fields for each tissue section in infected and uninfected fish. Photomicrographs of control samples were analysed in the same manner.

Statistical analyses

Histopathology statistical analysis was performed using S-plus 8 from Spotfire®. A Shapiro-Wilk test was used to test for normality of the data. Two-sample t tests were used to analyse differences in epithelial morphology between uninfected and infected fish within each microhabitat. A two-way ANOVA test was used to test for interaction between the condition of the fish (infected and uninfected) and the defined microhabitats. A negative binomial regression was used for the same purpose in the case of epithelial layers and mucous cells. Significance in all tests was accepted at $P<0.05$.

Component D: Modelled effects of future climate change on wild fisheries and aquaculture production

i) Modelling climate change impacts on the wild barramundi fishery

Background

Recent studies have shown that commercial barramundi catches are correlated with connectivity (proximity) of estuaries (Meynecke et al., 2008) and the temperature and precipitation of the 2 to 4 year period prior to the commercial catch (Balston 2009b). This suggests populations are likely recruitment-limited, where recruitment success is defined by short-term weather or longer-term climate cycles. Several studies have looked at correlations between commercial catches and short-term environmental variables, however, we know of no studies that have successfully assessed the impact of long term climate and the potential change in climate on the Australian barramundi fishery. Barramundi, as with all ectotherms, may be sensitive to climatic changes (Deutsch et al., 2008) and, as such, the barramundi fishery could potentially face quite rapid, dramatic change over the coming decades.

Physiological mechanisms

Thermal performance curves for many species often best describe basic physiological functions, including growth, reproduction, movement, and performance (Huey and Stevenson 1979). As a major driver of growth rate in fish, temperature can contribute to spatial variation in these physiological functions. Temperature change can facilitate migration of marine fish (Cheung et al., 2010), which can expose fish to changes in photoperiod. Temperature is proposed to greatly impact recruitment in barramundi, as barramundi survival and growth is limited to temperatures above 15 °C and ~ 40 °C at all life stages (Katersky and Carter 2005; Glencross 2008). Temperature, however, is not the only limiting factor. Barramundi larvae grow faster over the first 8-10 days with increasing photoperiod, as they are induced to feed more over longer light periods (Barlow et al., 1995). Additionally, prey density has also been shown to be higher under more intense light regimes, thus enabling greater feed intake and therefore increased growth (Fermin and Seronay 1997). Seasonal flow changes the salinity of estuaries, which may affect recruitment by changing survival of larvae and eggs, which are most successful in high-salinity environments (Moore 1982). A link has also been identified between timing of spawning and start of wet season (Moore 1982).

Physical mechanisms

It is long accepted that the physical, abiotic environment, to greater or lesser degrees, structures the distribution and abundance of species. Barramundi's use of different habitat at different life history stages means that the physical environment plays an important role in structuring local abundance patterns and influences recruitment success. Barramundi require freshwater and brackish water to reproduce and survive, restricting ideal habitats to perennial streams and rivers draining into the ocean (Dunstan 1959). As the habitats required vary with life stage, the juxtaposition of wetlands, streams and marine environments must be such that the species can effectively move between habitats as required (Moore and Reynold 1982). Post-larvae recruit to wetlands where freshwater flow is thought to influence survival and growth through increasing spatial and temporal extent of nursery habitat (Staunton-Smith et al., 2004). Juvenile barramundi utilise freshwater and brackish swamps or supralittoral tidal pools (Russell and Garrett 1983) before migrating into tidal creeks in estuaries (Russell and Garrett 1985), then to freshwater to mature, so the connectivity of these environments is vital to recruitment. It is suggested that enhanced recruitment occurs in years with high spring and summer flow that enhances larval and juvenile recruitment and survival (Staunton-Smith et al., 2004). Additionally, growth of barramundi correlated positively to freshwater flow in a monsoonal wet-dry tropical system (Robins et al., 2006). Flow has even been considered as a mechanism of colonisation. Larvae and juveniles in nursery habitats can be washed out and dispersed to other catchments via flood plumes during periods of extreme flow in the tropical monsoon regions (Keenan 1994). Therefore, changes in rainfall can have major implications for barramundi survival, recruitment and migration. While these correlations have been shown to influence recruitment, the studies to date have largely focussed on short-term events at focal study sites; little work has been done to assess the spatiotemporal patterning of barramundi populations.

Studies looking at physiological and physical mechanisms of recruitment, survival and growth on barramundi population structures have largely focussed either on spatial or temporal patterns of recruitment or catch; few have looked at linking spatiotemporal patterns of population changes with key climate and landscape features.

It is realistic to expect short-term weather events (climate variables) will influence localized catch from year to year; this has been the focus of several key studies examining temporal changes in populations of barramundi. Most studies have considered effects of short-term climate variability on inter-annual variation in indicators of population changes, such as Catch

Per Unit Effort (CPUE), though two have modelled effects of long-term climate indices. These studies found CPUE in a couple of populations along the coast of Queensland to be related to the Southern Oscillation Index (SOI) (Meynecke et al., 2006), an index of the Quasi-Biennial Oscillation at lags of three to four years and the latitude of the sub-tropical ridge one to four years prior to catch (Balston 2009a). One study showed that climate-derived variables such as rainfall, stream flow and temperature influenced nutrient availability and nursery habitat extent and suitability which are critical to successful recruitment of early life stages (Balston 2009a). Short-term weather events show similar results to studies associated with long-term climate, but they are not all consistent in identifying climate-related drivers of population size. Meynecke et al., (2006) found that up to 80% of the barramundi catch variation in a population in the Gulf of Carpentaria could be explained by rainfall, while abundance of juveniles in a catchment in the Northern Territory decreased with flow and adult abundance increased (Stewart-Koster et al., 2011). However, even though Balston (2009b) found wet season freshwater flow to correlate significantly with the variability in catch adjusted for effort (CAE), both at year of catch and a 2 year lag, flow was not included in Balston's temporal model explaining short-term temporal changes in CAE. Instead, the variables that explained the majority (62%) of the temporal variability in catch at the location studied were precipitation in the dry season and annual evaporation. The only previous study to examine potential future impacts of climate change determined influence of freshwater flow on the structure of an Australian barramundi population and measures of catchability within a single basin near the southern extent of barramundi distribution along the eastern seaboard. Tanimoto et al., (2012) positively linked river discharge in a single basin to mean values of exploitable biomass, annual catch, maximum sustainable yield and spawning stock size through a monthly age- and length- structured population for barramundi. This model was then projected onto the A1FI SRES scenario, which modelled a decrease in these variables with reduced total freshwater flow in a modified system with high water abstraction (Tanimoto et al., 2012). Periodic episodes of high flow appear to be necessary for high recruitment.

Most models linking barramundi catch with climate variables have not been conducted spatially, despite known range limitations driven by physiological temperature limits. In fact, temperature has been considered an unlikely driver of abundance (Robins et al., 2005) and has frequently not been included in studies. Where flow has been the focus of most temporal studies, its impact on abundance has only been studied at the spatial scale of a single catchment (Tanimoto et al., 2012; Balston 2009b; Stewart-Koster et al., 2011). Physical

availability of habitat and geomorphic attributes, where examined, have been studied most rigorously in a spatial context. First, Meynecke et al., (2007) determined the importance of availability of saltmarsh, flats and mangrove habitats by showing they correlated with catch of 13 regions in Queensland (Meynecke et al., 2007). Barramundi CPUE was best explained by connectivity indices for mangroves, salt marsh and channels, including measures of the number of wetland patches, mangrove connectivity and wetland connectivity (Meynecke et al., 2008). The most ambitious spatially-explicit model developed for barramundi to date essentially consisted of temporal models of sea surface temperature, rainfall, and SOI for eight populations defined by Bureau of Meterorology rainfall districts (BOM, 2004) (Meynecke and Lee 2011).

In this sub-component, we assess the potential impacts of climate change on the distribution and abundance (Catch per Unit Effort) for barramundi across Australia. We initially examine the potential changes in distribution using a presence-only species distribution model and examine how distribution is likely to change. Then we assess the ability of long-term climate-derived indices to predict the spatial distribution of Catch per Unit Effort for commercial fisheries across Queensland, before projecting this across Northern Australia and into the future. These spatially-explicit models on population viability (e.g., changes in catches with space) were assessed for barramundi as a whole and for each of the genetic lineages identified in this report.

Materials and Methods

Barramundi observations were sourced from Atlas of Living Australia (HYPERLINK "http://www.ala.org.au/"www.ala.org.au) and several other personal / institutional databases; this data was sourced, verified and vetted as per James et al., (2013). Commercial catch and long term monitoring data was provided by the Queensland Government Department of Agriculture, Forestry and Fisheries (QDAFF). Annual catch and effort data was collected between 1988 and 2012 (Figure 3) at 0.1 degree resolution; these were aggregated to 0.5 degree grid cells along the Queensland coast. Catch Per Unit Effort for each grid cell and year was calculated; the 25 year average was used in this analysis (Figure 4).

Monthly, annual and quarterly measures of current and future climate measures (e.g., air temperature, precipitation, runoff and potential evaporation) have been sourced from James et al., (2013). All environmental layers were sourced at ~5 km or ~1 km grids and were aggregated to reaches as defined by the Australian Hydrological Geospatial Fabric (AHGF)

(Geofabric) products of the Bureau of Meteorology (BOM). Current and future climate measures represent 30 year averages centred on 1990 (current; 1976-2005) and decadal from 2015 to 2085. Seasonal variables were calculated as defined by Vance et al. (1998): pre-wet (October–December); wet (January–March); early dry (April–June); and dry (July–September). Climate variables frequently exhibit high levels of colinearity between independent variables (e.g. evaporation is correlated with temperature). A correlation matrix of independent climate variables was generated to determine cases of collinearity (Table 8).

The climate change scenarios used here are based on the IPCC 5th assessment report greenhouse gas emission scenario that represents a 'business-as-usual' pathway. Identified as RCP85 (Representative Concentration Pathway), we used 18 global climate models bounded by this scenario to examine a probabilistic outcome for the potential impacts on climate change on wild barramundi and the associated fishery.

Environmental data was extracted using 0.1 degree sites of capture due to the wide range of temperature variation within a 0.5 degree grid cell, which overlap with land and therefore include land and temperatures the fish are highly unlikely to experience (such as mountain tops). The mean within each 0.5 degree grid cell for each year was determined and the mean across years was calculated to determine the climate within which fish are caught.

Species distribution models (SDMs) incorporating baseline climate and hydrological data and species occurrences were created using MaxEnt (Phillips et al., 2006), as per methods here described by James et al., (2013). MaxEnt uses a maximum entropy algorithm to statistically relate presence-only distribution records to environmental variables. Here our observation records for a species were the unique stream segments in which the species had been observed. These observations were correlated with attributes of the stream segments (and associated catchments). These environmental attributes included mean annual temperature and its seasonality, temperatures of the warmest and coldest months, annual precipitation and its seasonality, precipitation of the wettest and driest quarters, length of dry periods and its associated severity index, and accumulated mean annual runoff.

MaxEnt was parameterised with default settings (Phillips and Dudik 2008), with the exception of the removal of threshold and hinge features (see Elith et al., 2011 for explanation of Maxent features); removal of these features produce more ecologically realistic response curves and provides more general predictions (Austin 2007).

Observation data is spatially biased often toward roads, trails and areas of greater human density. To account for any spatial biases, we used a 'target-group' method for selecting our background (Phillips and Dudik 2008). A target group background selects background points (or pseudo-absences) only from where species of the same taxonomic group have been observed. It is assumed that any sampling bias (spatially or temporally) in our observation records for a single species can also be observed in our background points; in effect cancelling out the effect of any spatial or temporal sampling bias in the modelling exercise (Elith and Leathwick 2009; Phillips and Dudik 2008). Model performance was evaluated by the area under the receiver operating characteristic curve (AUC). AUC measures each model's consistency and predictive accuracy (Ling et al., 2003). An AUC score of 1 is a perfect model fit of the data; 0.5 is no better than random (Elith et al., 2006; Phillips et al., 2006). AUC values $\geq$ 0.7 indicate 'useful' models, whereas values $\geq$ 0.9 indicate models with 'high' performance (Swets 1988). Models were screened for low AUC ($<$ 0.7) so that underperforming models were not included in further analyses. The Species distribution model was projected onto 18 GCMs representing RCP85 in the year 2085 and the tenth, fiftieth and ninetieth percentiles were calculated.

The default MaxEnt distribution output is a continuous prediction of environmental suitability for the species. A binary distribution output was created by applying an appropriate threshold obtained from the MaxEnt results output file. The threshold showing the most realistic distributions for the species, as defined by expert opinion and comparisons with known distributions, was the 'equate entropy of threshold and original distributions logistic threshold' — a value between 0 and 1 that minimises the omission rate while maximising area predicted; values above this threshold are assumed suitable for the species.

The resulting distribution from MaxEnt is all stream segments that are environmentally suitable — a potential distribution that is akin to a fundamental niche. This potential distribution is often unrealistic in that there may be many areas which the species cannot use due to biotic limitations (e.g., dispersal limitations), or due to environmental factors not included in the models. To account for this we have created a more realistic distribution from the potential distributions. The 'realised' species distributions for fish were clipped to the fish biogeographic provinces defined by Unmack (2001). Biogeographic provinces in which the fish species was not observed were removed from the realised distribution models. Future projections were also limited to being 'realistic'. This was done by clipping potential future distributions to connected sub-catchments that intersected the current realised distribution.

Potential population changes were assessed using linear regressions. The linear regression analysis was used to test the relationship between each selected climate variable and commercial barramundi CPUE (Table 9). Variables were either log transformed, or squared, to ensure normality of variables. Due to the large number of possible combinations of environmental variables, these were simplified within each overarching climate variable to use only the variable with the strongest correlation to CPUE (i.e. minimum temperature, maximum temperature, rainfall, potential evaporation) and to reduce colinearity. Further, variables with high collinearity were not used together (ie. Max temp (mean) and Max temp (Oct-Dec) were not used together in a model). The simplest model with the least variables and highest explanation of variance (R^2) was selected. All models were constructed and visualised using the SDMTools (VanDerWal et al., 2012) and maptools (Lewin-Koh and Bivand 2013) libraries in the statistical program R (r-project.org).

The model developed using current climate data was projected onto future global climate models using the High Performance Computers (HPC) at JCU. A projection was constructed for each of 18 Global Climate Models for three future time-steps at 2030, 2050 and 2085. The 10th, 50th, and 90th, percentiles were calculated to show variability across the models.

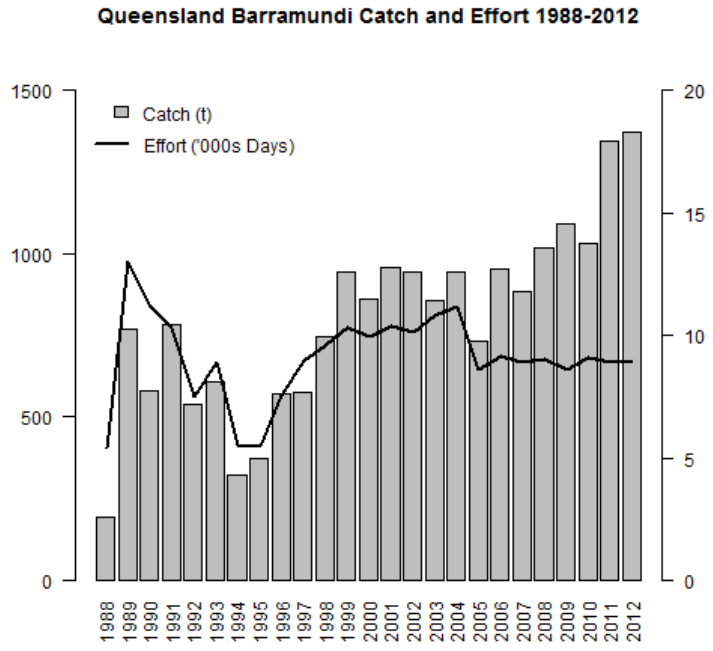

Figure 3: Queensland commercial barramundi catch and effort (1988–2012), supplied by Queensland Government Department of Agriculture, Forestry and Fisheries (QDAFF).

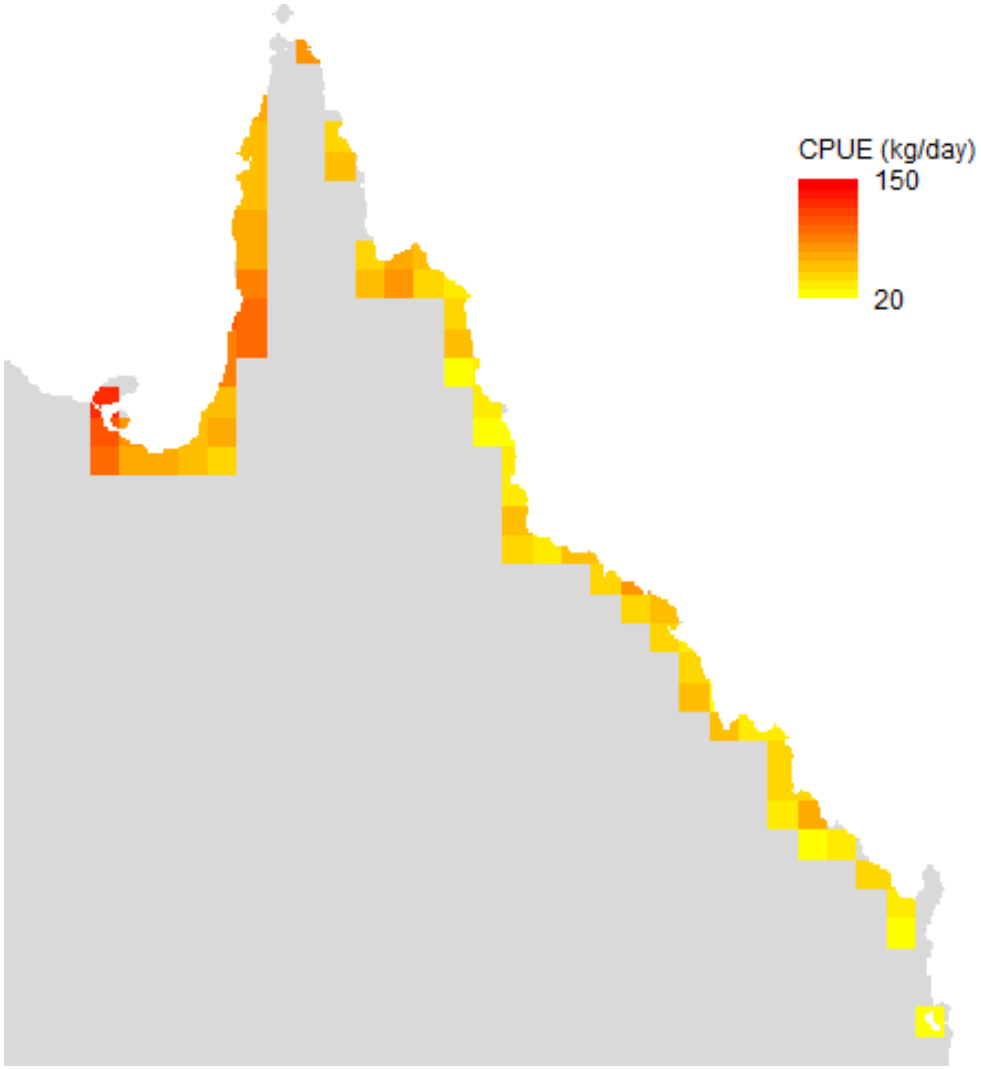

Figure 4: Queensland commercial barramundi mean catch per unit effort for each commercial fishing grid (1988–2012) supplied by Queensland Government Department of Agriculture, Forestry and Fisheries (QDAFF).

Table 8: Pearson coefficients of correlation (*r*) of climate parameters used in the Queensland analyses used to identify possible cases of collinearity between variables. Correlations are significant at the $P < 0.05$ level (bold).

	Min temp (mean)	Min temp Jan-Mar	Min temp Apr-Jun	Min temp Jul-Sep	Min temp Oct-Dec	Max temp (mean)	Max temp Jan-Mar	Max temp Apr-Jun	Max temp Jul-Sep	Max temp Oct-Dec	Rainfall (mean)	Rainfall Jan-Mar	Rainfall Apr-Jun	Rainfall Jul-Sep	Rainfall Oct-Dec
Min temp Jan-Mar	**0.93**														
Min temp Apr-Jun	**0.97**	**0.82**													
Min temp Jul-Sep	**0.97**	**0.82**	**0.99**												
Min temp Oct-Dec	**0.95**	**0.99**	**0.85**	**0.86**											
Max temp (mean)	**0.83**	**0.87**	**0.72**	**0.73**	**0.9**										
Max temp Jan-Mar	**0.71**	**0.84**	**0.55**	**0.56**	**0.85**	**0.94**									
Max temp Apr-Jun	**0.85**	**0.88**	**0.76**	**0.77**	**0.91**	**0.99**	**0.91**								
Max temp Jul-Sep	**0.84**	**0.85**	**0.76**	**0.77**	**0.89**	**0.99**	**0.89**	**0.99**							
Max temp Oct-Dec	**0.79**	**0.85**	**0.67**	**0.69**	**0.89**	**0.99**	**0.94**	**0.98**	**0.98**						
Rainfall (mean)	0.14	-0.08	**0.29**	**0.27**	-0.06	-0.17	**-0.34**	-0.13	-0.11	-0.19					
Rainfall Jan-Mar	**0.47**	0.24	**0.59**	**0.58**	**0.28**	0.2	-0.02	0.24	**0.28**	0.18	**0.91**				
Rainfall Apr-Jun	-0.24	**-0.39**	-0.09	-0.11	**-0.41**	**-0.55**	**-0.6**	**-0.52**	**-0.51**	**-0.57**	**0.86**	**0.57**			
Rainfall Jul-Sep	**-0.52**	**-0.61**	**-0.4**	**-0.42**	**-0.64**	**-0.73**	**-0.72**	**-0.72**	**-0.71**	**-0.74**	**0.69**	**0.32**	**0.94**		
Rainfall Oct-Dec	-0.02	-0.22	0.13	0.11	-0.21	**-0.29**	**-0.44**	**-0.26**	-0.23	**-0.31**	**0.97**	**0.82**	**0.88**	**0.76**	
Pot. Evap. (mean)	**0.78**	**0.83**	**0.68**	**0.68**	**0.85**	**0.93**	**0.87**	**0.93**	**0.93**	**0.93**	-0.15	0.21	**-0.51**	**-0.69**	**-0.28**

Table 9: Pearson coefficient of correlation (*r*) between Queensland barramundi Catch per Unit Effort and climate variables. Highlighted correlations significant at the $P < 0.01$**.

	CPUE (mean)
Min temp (mean)	**0.5****
Min temp (min)	0.31
Min temp Jan-Mar	**0.62****
Min temp Apr-Jun	**0.37****
Min temp Jul-Sep	**0.38****
Min temp Oct-Dec	**0.63****
Max temp (mean)	**0.62****
Max temp (max)	**0.65****
Max temp Jan-Mar	**0.61****
Max temp Apr-Jun	**0.62****
Max temp Jul-Sep	**0.6****
Max temp Oct-Dec	**0.63****
Rainfall (mean)	-0.32
Rainfall (max)	**-0.33****
Rainfall (min)	-0.29
Rainfall Jan-Mar	-0.04
Rainfall Apr-Jun	**-0.55****
Rainfall Jul-Sep	**-0.62****
Rainfall Oct-Dec	**-0.39****

ii) Modelling climate change impacts on barramundi aquaculture

Background

Despite sustained growth of the Australian aquaculture industry, the implications of climate change for aquaculture species have been given minimal consideration. Average global temperature has risen by 0.6 °C over the past century, and there is high confidence that global temperature increases of between 1.4 and 6 °C from the year 2000 levels can be expected by the year 2100 (Navicenovic et al., 2000). Increases in temperature can be expected to exercise strong control over species and individuals habitat use, behaviour, phenology and migration among other attributes (Parmesan and Yohe 2003; Root et al., 2003), and changes in rainfall and evaporation may drastically affect the viability of certain locations for species to persist (De Silva 2009), thus changing viability of locations for aquaculture However, temperature increases in temperature may be beneficial for aquaculture by enabling diversification to novel species, or increased production of cultured species that are currently temperature limited (Troadec 2000; McCarthy et al., 2001). It has long been suggested that an

increase in mean air temperatures could lead to an increase in aquaculture and fisheries yield (Meisner et al., 1987). Therefore, it is important to investigate the potential influence of climate variables such as temperature on future viability of Australia's growing aquaculture industry.

Barramundi is an increasingly important aquaculture species across the monsoonal tropical north of Australia where increasing temperature and changes to rainfall and evaporation will potentially threaten, or enhance, some current aquaculture operations. Increases in temperature may further provide opportunities for aquaculture in new areas. With pond-based aquaculture of barramundi, the current geographical extent is limited to northern coastal Australia due to more southerly regions experiencing the lower thermal limit of the species in the coldest months.

Temperature is perhaps the most important factor influencing growth in fish (Huey and Stevenson 1979). Increases in temperature cause increased metabolism and feeding (Brett and Groves 1979 and Jobling 1994), and energy that is not spent on metabolic processes promotes increased growth in warmer temperatures (Katersky and Carter 2007). However, every species exists within a thermal tolerance range, and growth is maximised at an optimal temperature (Huey and Stevenson 1979). Barramundi have a wide thermal tolerance of 15 – 40 °C and several studies have found that maximum growth efficiency occurs between 27 – 36 °C (Katersky and Carter 2005, Glencross 2008). It has as yet not been explored what proportion of the Australian landmass will remain below the upper thermal limit of the species, or whether the extent of Australia that experiences optimal temperatures for barramundi aquaculture will increase or decrease under climate change scenarios.

Here in this subcomponent we examine the implications of increasing global temperatures on pond-based Australian barramundi aquaculture. We apply the growth function determined by Bermudes et al., (2010), that describes weight gain by barramundi as a function of fish weight and water temperature, to current geographic temperature data to determine thermal suitability across Australia. We then compare the results of known fish growth from specific areas in the recent past with modelled fish growth across the same location and time period to validate performance of the model. Using the RCP 8.5 emissions scenario of 18 global climate models (GCMS), we apply the factorial growth model to each to determine the median growth of barramundi for 2030, 2050 and 2080.

Materials and Methods

The predictive growth equation

Here we use the robust estimate of barramundi weight gain first developed by Bermudes et al., (2010). The equation defining gain, in terms of grams per fish per day, was developed as a predictive growth equation using data that included a range of assessment periods and fish size ranges from numerous fish cohorts, farms and experiments covering temperatures ranging from 18 ºC to 39 ºC. In excess of 800 data points were collected. These data were used to define the equation used in this assessment:

$$Gain\ (g/fish/d) = (K + xT + yT2 + zT3) * (live\text{-}weight)\ aT + b$$

In this equation, *K*, and *b* are constants, and *x, y, z* and *a* are coefficients determined using the regression function. *T* is temperature (operational range of 16 ºC to 39 ºC) and weight is the geometric mean weight of the fish in grams.

K = 2.249522916
x = -0.327485829
y = 0.014951694
z = -0.000203425
b = 0.72000
a = -0.00950

This predictive growth equation was only tested to 3000 g and thus we use it here to look at fish from fingerling to 3 kg.

Current suitability and future climates

We determined current suitability for pond-based barramundi aquaculture across continental Australia based on the modelled weight of one fish after one year at a given temperature (January-December). Mean air temperature was chosen as a surrogate for spatial pattern of monthly pond temperature. The contemporary monthly temperatures used here were derived

from Australia Water Availability Project (AWAP; http://www.bom.gov.au/jsp/awap/) representing monthly temperatures of 1975-2005. These are mapped at ~5 km resolution across Australia. Given the variety of ponds used for barramundi aquaculture, the assumption that average air temperature will equate pond temperature is based on the idea that the aquaculture ponds are relatively shallow and while offering a buffered environment from extreme temperatures, the average water temperature should represent the average air temperature; this assumption is currently being validated, but a recent study showed that 84-94% of the water temperatures examined could be explained simply by air temperature (Johnson et al., 2013).

We applied the Bermudes et al., (2010) growth model to continental monthly temperature between the thermal tolerance limits of barramundi (15 ^{o}C and 40 ^{o}C). Regions that reached below a monthly mean temperature of 15 ^{o}C were defined as unviable locations for barramundi aquaculture. For the annual weight gain model presented here, we defined the start date at January 1st and the end date at December 31st, on the basis that stocking is known to occur between September and May on farms within all currently viable Australian latitudes. We defined each month as having 30 days. Juvenile fish were characterised as having an initial stocking weight of 20 g, which represents a typical stocking weight.

The Bermudes et al., (2010) growth equation was validated using average ranges of fish weight reported from four farms in northern Queensland. The four farms represented a wide range of latitudes and altitudes at which barramundi are currently farmed. The respective locations were: Innisfail, Mareeba, Julaten, and Port Douglas. We used a stocking weight of 20 g, as all farms reported a stocking weight of 15 – 20 g, or we inferred a weight within this range from published fish length at stocking. Mean daily air temperature from the AWAP data was used to determine the weight of a fish at the end of 12 months for each month in the potential stocking season of September to May.

Future climates represented projections of monthly mean temperature data of 18 Global Climate Models (GCMs) for the RCP 8.5 emission scenario ("business-as-usual"). We calculated weight of a fish after one year for all 18 GCMS, then calculated the median weight at all locations across all GCMs. We also calculated the maximum and minimum weight at all locations. Where temperature in any run fell below 15 ^{o}C, we set growth to zero, and where temperature fell below 15 ^{o}C across all runs of all GCMs, that 5 km^2 location was considered 'unsuitable'. Therefore, for future scenarios, areas marked with <1000 g weight gain in one

year are generally high risk areas where at least one model projected that the area would reach below the lower thermal limit of 15 ^{o}C.

Projected weights of fish after one year were categorized by 500 g intervals, up to 3500 g, and total geographic area for each category was calculated using patch characteristics in VanDerWal et al., (2012). This was completed for current, 2030, 2050 and 2080 time-steps, and suitability maps were produced for the minimum, mean and maximum across all GCMs for each year.

We assessed potential evaporation and rainfall with respect to the "suitable areas" predicted currently and in the future. Where evaporation is greater than precipitation, it is unlikely to be suitable for aquaculture without significant external water resources. We therefore clip all projections to areas where precipitation is greater than potential evaporation for at least 3 months of the year assuming this would allow recharge of water resources.

All climate surfaces, current and future, were sourced from James et al., (2013) and used the methods therein to create derived products.

All analyses were performed using R, version 2.14.0 (http://www.R-project.org), and the SDMTools library, version 1.1-5 (VanDerWal et al., 2012). Total area for each growth category was calculated using patch characteristics in SDMTools.

RESULTS/DISCUSSION

Component A: The population genetic structure of Australian barramundi and its relationship to landscape features.

Temporal stability

Prior to defining the present day genetic structure of barramundi from the combined modern and historical samples it was necessary to demonstrate temporal stability of microsatellite datasets. Temporal stability of microsatellite allele frequencies for the 16 loci examined were confirmed by both pairwise F_{ST} analysis and Fisher's exact tests of genic differentiation (Table 10). Temporal allele frequencies were not significantly different in the Daly River, Leichardt/Albert River, Archer River, Burdekin River or Bowling Green Bay temporal collections, over time scales of up to 25 years. This temporal stability justifies the merging of microsatellite datasets from samples collected over these timescales. The only exception to temporal stability involved a difference in samples collected in the estuarine reaches of the Fitzroy River in south-east Queensland. Our analysis showed that a 2008 collection was anomalous in having a significant pairwise F_{ST} in comparisons with the earlier 1988 Keenan collection and a later 2013 collection. There was, however, no evidence for differentiation between the 1988 and 2013 Fitzroy River collections from both pairwise F_{ST} and Fisher's exact tests for allele frequency differences (Table 10). This suggests that the temporal shift in allele frequency was short lived and may have been the result of sampling error in the 2008 Fitzroy River (QLD) collection.

Heavy rainfall led to significant flooding of the Fitzroy River basin in late 2007/early 2008 and InfoFish tagging data indicated that a large proportion of tagged fish recaptures in the Fitzroy River estuary in 2008 were stocked fish that were flushed into the Fitzroy River estuary (Sawynok and Platten, 2009). Sample datasheets from fisheries personnel involved in collecting the 2008 genetic samples indicate that the samples came from a single commercial fish catch, with at least one of the samples provided possessing a stocking tag. As not all stocked fish in this area receive tags at the time of release (Jonathon Staunton-Smith, QDAFF, pers comm.), and physical tags are frequently lost, it is likely that the 2008 collection from this area contained more than one stocked fish. The temporary shift in allele frequency is therefore thought to be due to sampling error associated with a single commercial fish catch and the presence of stocked fish in the catch. For the purposes of defining stock structure the

2008 Fitzroy River collection was therefore omitted. All other historical and modern collections were retained as described in Table 1 (Materials and Methods section).

Table 10: Summary of temporal stability tests for microsatellite datasets obtained from temporally replicated sample collections. Significant results are in bold, ns = not significant. Significance was determined after FDR correction to control false discovery rate to 5% (Benjamini and Hochberg 1995).

		Pairwise *F*st		Fisher's Exact Test		
Sampling location	Sampling years	Fst	*p* value	χ2	df	*p value*
Daly River	1990 vs 2008	-0.002	0.632 ns	26.343	30	0.657 ns
Leichardt/Albert Rivers	1990 vs 2011	0.003	0.277 ns	33.391	32	0.399 ns
Archer River	1993 vs 2011	-0.005	0.887 ns	19.727	32	0.956 ns
Bowling Green Bay	1988 vs 2008	-0.001	0.523 ns	37.736	32	0.223 ns
Burdekin River	1989 vs 2008	0.000	0.359 ns	39.160	32	0.179 ns
Fitzroy River	1988 vs 2008	**0.033**	**0.001***	not performed		
Fitzroy River	2008 vs 2013	**0.036**	**0.001***	not performed		
Fitzroy River	1998 vs 2013	0.000	0.437 ns	23.505	32	0.862 ns

Overall population genetic differentiation

The presence of significant population genetic structure across the spatial scale examined, from the Pilbara region in Western Australia to the Mary River in south-east Queensland, was confirmed by a significant ($P < 0.001$) global F_{ST} of 0.079 (i.e ~ 8% of genetic variation in this species in Australia is defined by genetic differences among collections)(Table 11). The overall level of divergence reported here is similar to that reported in earlier allozyme studies. Keenan (1994) reported an overall F_{ST} value of 0.064 from an analysis of 50 barramundi collections sampled from the Ord River in the north of Western Australia to the Mary River in south east Queensland. The present study has expanded the sampling area down to the Pilbara region in the more south-western parts of the species distribution. A previous mitochondrial DNA study suggested that the Pilbara region of Western Australia is home to a divergent and

evolutionarily significant stock of barramundi (Marshall 2005). The increased global F_{ST} reported here for barramundi in Australia may result from the use of a different type of genetic marker (microsatellites vs allozymes), but may also reflect the inclusion of genetically divergent subpopulations from areas to the west and south-west of the previous allozyme studies.

Table 11: Summary tables for AMOVA analyses examining partitioning of genetic variance within and among barramundi collections/subpopulations. A) among/within 48 individual collections of barramundi as described in Table 1 or B) among/within subpopulations defined by combining neighbouring collections with pairwise $F_{ST} < 0.01$ as described in methods section.

Source of variation	d.f.	Sum of Squares	Variance Component	*p*-value	Percent of variation
A: among/within collections					
Among collections	47	1094	0.36	**0.000**	7.9
Within collections	2498	10552	4.22	**0.000**	92.2
Total	2545	11646	4.58		
B: among/within subpopulations					
Among subpopulations	20	967	0.39	**0.000**	8.5
Within subpopulations	2525	10679	4.23	**0.000**	91.5
Total	2545	11646	4.62		

Discrimination of barramundi subpopulations

One of the key aims of population genetics is to define locally interbreeding subpopulations for the purposes of fisheries management, monitoring and conservation. Discrimination of genetically distinctive subpopulations is critical for informed stocking programs and sea-cage aquaculture, as knowledge of the location of genetically discrete stocks can assist in avoiding translocation of fish between different stocks, either through deliberate stock enhancement efforts, or accidental aquaculture release. It is also useful to identify genetically discrete barramundi stocks for aquaculture as different stocks may possess varying quantitative traits of value to commercial aquaculture ventures.

Two different approaches were used here to identify distinct barramundi subpopulations (locally interbreeding units and potential management units). These subpopulations will later be classified into broader genetic stock groups (potential evolutionarily significant units – see sections below). The two statistical approaches used here were pairwise F_{ST} comparisons (Figure 5, Appendix E3) and Fisher's Exact tests of allele frequency differences (Appendix E4). In all but one case (the Johnstone River) identical subpopulation groups were identified using the two statistical approaches (Appendix E4). For the Johnstone River, the Exact tests suggested differentiation from the more southern neighbouring collection in Hinchinbrook Channel (χ^2 49.822, df 32, P = 0.046) (however, the statistical significance was marginal). Pairwise F_{ST} between these two sample collections was also low (F_{ST} = 0.006, P = 0.015) and fell below the 0.01 threshold set for discriminating collections (Appendix E4). The marginal significance of the Fisher's exact test and the low pairwise F_{ST} suggests a false positive result and consequently the Johnstone River collection has been designated as belonging to the same subpopulation (subpopulation 18) as collections from Hinchinbrook Channel to Bowling Green Bay to the south (Figure 5 and 6a).

In total, 21 genetically distinguishable subpopulations were identified from the analysis of the full 16 locus microsatellite dataset (Table 12, Figure 5 and Figure 6a). The most comprehensive earlier genetic study conducted by Keenan (1994) utilized 13 allozyme markers and described 16 subpopulations (designated I-XIV) over a smaller geographic area from the Ord River in the north of Western Australia to the Mary River in south-east Queensland (see Figure 6b). Despite the difference in genetic markers and statistical methods employed there is remarkable similarity in the patterns of subpopulation structure described by these two studies, with only three areas of minor difference identified (Figure 6a and 6b). One area of minor difference between the two studies is at the tip of Cape York. Similar minor differences in subpopulation boundaries between collections to the east and west of the tip of Cape York were noted by Keenan (1994) when comparing to the subpopulation boundaries reported by Shaklee et al., (1993). These differences were attributed to differences in statistical interpretation of the data (Keenan 1994). Our subpopulation designations at the top of Cape York support the earlier classifications of Shaklee et al., (1993) rather than those of Keenan (1994). Our treatment of the present microsatellite dataset also resulted in a minor difference in subpopulation discrimination around the Darwin/Bathurst Island region of the Northern Territory (Figure 6). Keenan (1994) designate Bathurst Island as subpopulation V, different from Darwin Harbour and Shoal Bay both designated as subpopulation III (Figure 6b). Here we do not discriminate Bathurst Island from these two locations, as the pairwise

comparisons of Bathurst Island and Darwin Harbour, and Darwin Harbour to Shoal Bay were non-significant (Appendix E3 and E4). Furthermore the comparisons between Bathurst Island and Shoal Bay were only marginally significant (pairwise $F_{ST} = 0.011$, $P = 0.031$ and Fisher's Exact test $\chi^2_{df\ 30} = 44.76$, $p = 0.041$) and are considered likely to be false positives.

Table 12: Pairwise F_{ST} comparisons between 21 subpopulations of barramundi identified following genotyping of 16 microsatellite loci. Subpopulation numbers are defined in Figure 5 and associated collection codes are as shown in Table 1. Pairwise F_{ST} values are below the diagonal while *p*-values are given above the diagonal, all significant pairwise F_{ST} values and associated *p*-values that remained significant after FDR correction for multiple tests (Benjamini and Hochberg 1995) are in bold, non-signficant values are in italics.

	Subpopulation Number																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
2	**0.08**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
3	**0.08**	**0.08**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
4	**0.11**	**0.12**	**0.03**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
5	**0.11**	**0.11**	**0.04**	**0.05**	*	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
6	**0.10**	**0.11**	**0.05**	**0.04**	**0.02**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
7	**0.12**	**0.13**	**0.05**	**0.06**	**0.01**	**0.02**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
8	**0.12**	**0.13**	**0.06**	**0.07**	**0.03**	**0.04**	**0.02**	*	**0.04**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
9	**0.12**	**0.12**	**0.05**	**0.06**	**0.01**	**0.02**	**0.01**	**0.01**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
10	**0.13**	**0.15**	**0.08**	**0.10**	**0.05**	**0.05**	**0.06**	**0.06**	**0.04**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
11	**0.11**	**0.15**	**0.09**	**0.09**	**0.05**	**0.04**	**0.06**	**0.07**	**0.05**	**0.04**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
12	**0.12**	**0.14**	**0.08**	**0.09**	**0.04**	**0.05**	**0.05**	**0.04**	**0.03**	**0.05**	**0.03**	*	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
13	**0.10**	**0.11**	**0.07**	**0.09**	**0.05**	**0.05**	**0.03**	**0.03**	**0.03**	**0.05**	**0.03**	**0.01**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
14	**0.08**	**0.11**	**0.06**	**0.07**	**0.04**	**0.03**	**0.03**	**0.03**	**0.02**	**0.05**	**0.02**	**0.01**	**0.01**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
15	**0.12**	**0.14**	**0.08**	**0.08**	**0.05**	**0.04**	**0.04**	**0.03**	**0.02**	**0.04**	**0.03**	**0.03**	**0.02**	**0.01**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
16	**0.12**	**0.12**	**0.08**	**0.09**	**0.06**	**0.06**	**0.07**	**0.05**	**0.05**	**0.06**	**0.04**	**0.04**	**0.05**	**0.03**	**0.02**	*	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
17	**0.18**	**0.18**	**0.11**	**0.14**	**0.11**	**0.11**	**0.11**	**0.10**	**0.09**	**0.11**	**0.13**	**0.11**	**0.11**	**0.09**	**0.07**	**0.07**	*	**0.00**	*0.27*	**0.00**	**0.00**
18	**0.15**	**0.16**	**0.10**	**0.12**	**0.10**	**0.11**	**0.10**	**0.08**	**0.08**	**0.11**	**0.11**	**0.10**	**0.10**	**0.08**	**0.09**	**0.07**	**0.01**	*	*0.07*	**0.00**	**0.00**
19	**0.16**	**0.17**	**0.10**	**0.12**	**0.09**	**0.09**	**0.11**	**0.09**	**0.07**	**0.10**	**0.11**	**0.11**	**0.10**	**0.08**	**0.07**	**0.05**	*0.00*	*0.00*	*	**0.00**	**0.00**
20	**0.19**	**0.19**	**0.14**	**0.19**	**0.15**	**0.15**	**0.16**	**0.17**	**0.14**	**0.16**	**0.15**	**0.16**	**0.15**	**0.12**	**0.14**	**0.12**	**0.06**	**0.04**	**0.06**	*	**0.00**
21	**0.17**	**0.17**	**0.12**	**0.17**	**0.13**	**0.13**	**0.13**	**0.13**	**0.12**	**0.13**	**0.14**	**0.14**	**0.12**	**0.10**	**0.11**	**0.09**	**0.04**	**0.03**	**0.06**	**0.01**	*

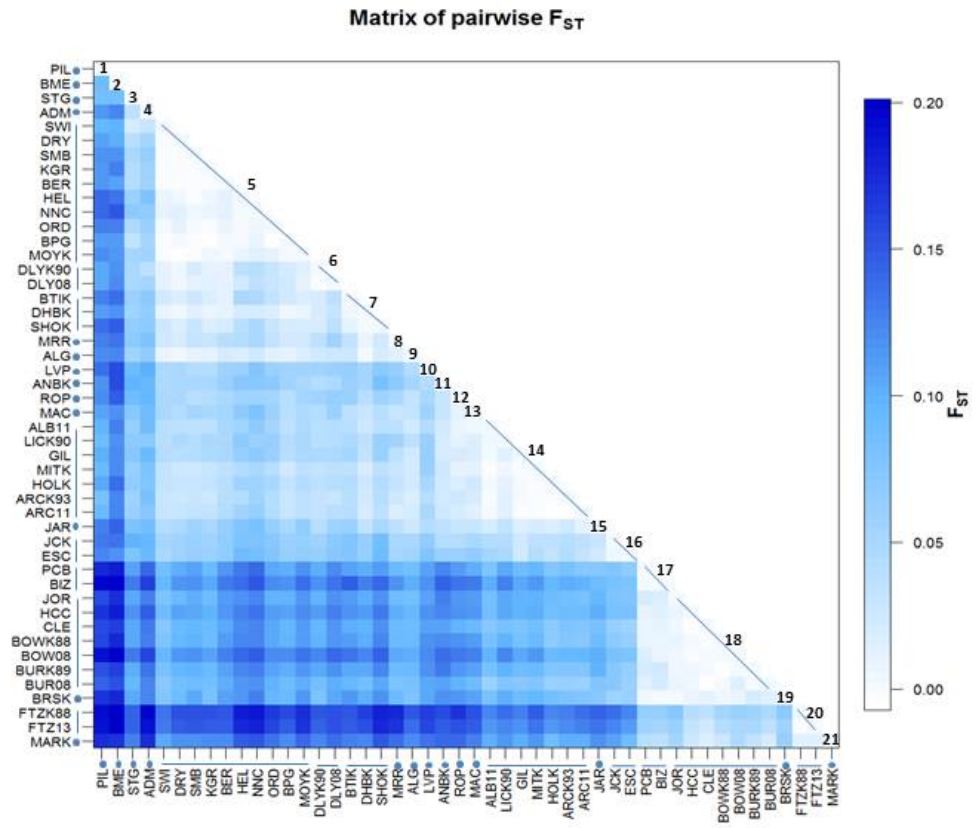

Figure 5: Pairwise matrix of F_{ST} values for 48 collections of barramundi genotyped with 16 microsatellite loci. Collection codes are as in Table 1 and collections belonging to the same subpopulation, as determined by pairwise F_{ST} <0.01 between neighbouring collections, are joined by a solid line along the diagonal, as well as along the x and y axis. Individual collections that form distinct subpopulations are indicated by a circle next to the collection code. Subpopulation numbers are given along the diagonal with a solid line connecting collections from the same subpopulation.

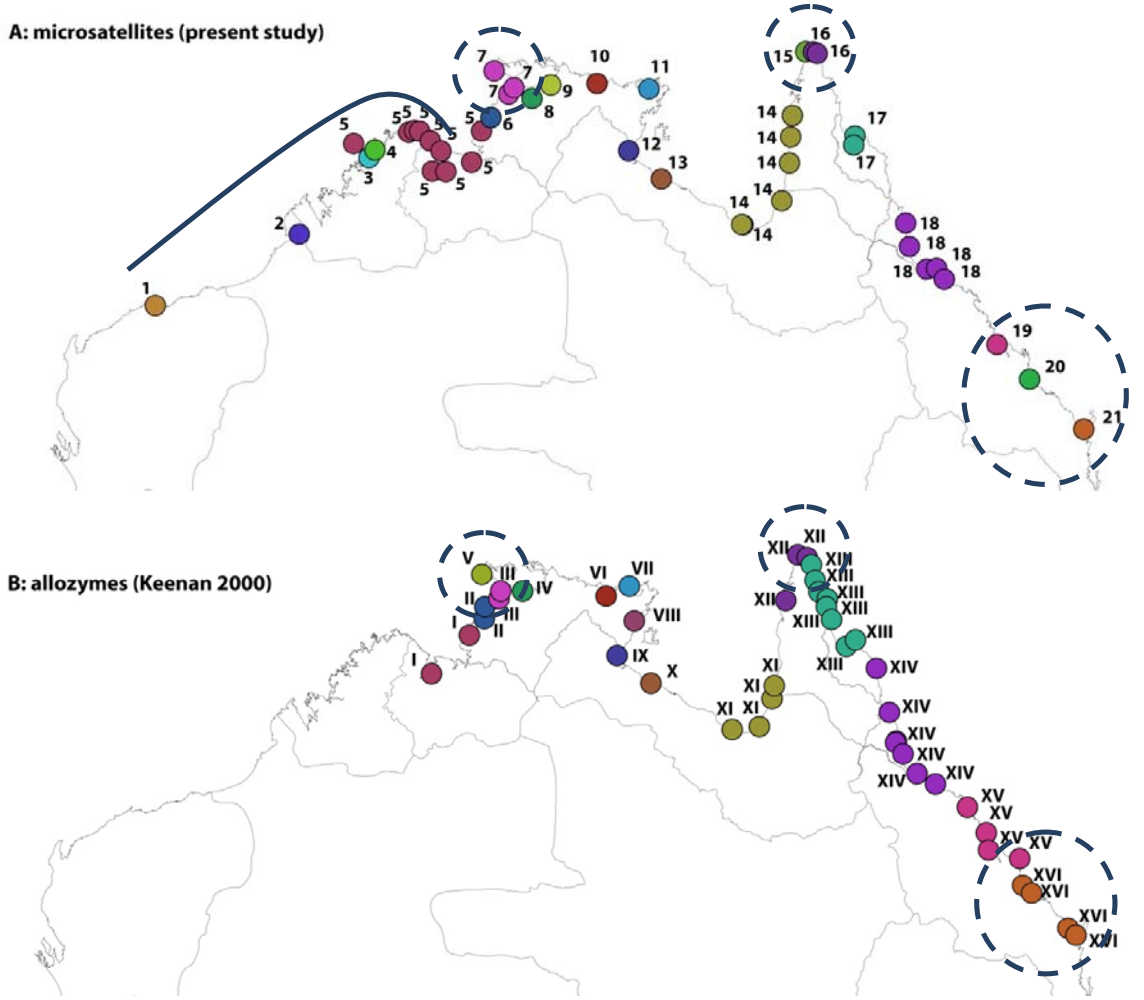

Figure 6: Maps of northern Australian coastline with major freshwater fish bioregions indicated as per Unmack (2001) showing A) 21 genetically distinguishable subpopulations of barramundi identified using 16 microsatellite loci and pairwise F_{ST} comparisons, where neighbouring collections are described as belonging to different subpopulations if the pairwise F_{ST} is >0.01 and remains significant following FDR correction for multiple tests, and B) location of 16 subpopulations (I – XVI) described from allozyme electrophoresis study of Keenan (1994). The extended range of the present day samples is indicated by the solid blue line while areas of difference between the current study and that of Keenan (1994) are highlighted by dashed circles.

The main area of difference between our subpopulation designations and that of the two earlier allozyme studies (Keenan, 1994, Shaklee et al., 1993) is in the south-east corner of Queensland. Our microsatellite comparison of a subset of Keenan's historical Fitzroy River and Mary River samples indicates a statistically significant, but minor divergence (pairwise F_{ST} = 0.014, P = 0.017 and Fisher's Exact test $\chi^2_{df\ 32}$ = 47.473, P = 0.004), that was not detected in Keenan (1994) or Shaklee et al., (1993). This minor difference between the historical Mary River sample collection (1989-90) and the Fitzroy River was up-held by the modern 2013 Fitzroy River collection, with similar pairwise comparison results (pairwise F_{ST} = 0.015, P = 0.009 and Fisher's Exact test $\chi^2_{df\ 32}$ = 66.836, P = 0.0003). It should, however, be noted that our sample size for the historical Mary River collection and also that to the north of the Fitzroy River in Broad Sound were small (n = 18 and 15 respectively) and likely to be prone to random sampling effects. Differences in subpopulation designations in south-east Queensland were also noted between Shaklee et al., (1993) and Keenan (1994). Shaklee et al., (1993) recognised one subpopulation (designated Stock G) from Repulse Bay – Mackay to the Rockhampton-Bundaberg area, while Keenan (1994) recognised two subpopulations (XV and XVI) within the same area, with the second subpopulation extending down to the Mary River (see Figure 6b). Our current microsatellite analysis identifies potentially three subpopulations (19, 20 and 21) from Broad Sound to the Mary River (Figure 6a). As our sampling in this area of Queensland was not as comprehensive as earlier studies, and in light of the recent flooding of stocked fish from the Fitzroy River basin, additional sampling in this area would be beneficial to clarify the current nature of subpopulation genetic structure in south-east Queensland.

When the 48 collections genotyped in the present study were combined into 21 subpopulations, as described above, the resultant subpopulations conformed to Hardy-Weinberg Equilibrium across all loci, with just two single loci in different subpopulations deviating from HWE proportions following FDR correction for multiple tests. Locus Lca057 deviated significantly (P = 0.0003) from HWE for subpopulation 5, consisting of collections from Swift Bay WA to the Moyle River in the NT, while Lca021 deviated significantly (P = 0.0001) from HWE for subpopulation 11 which is comprised of the small (n = 13) Alligator River collection from the NT. AMOVA analysis indicates that a greater proportion of variation is explained when collections are grouped as subpopulations compared to that observed among collections themselves (Table 11). A globally averaged F_{ST} of 0.085 ($P < 0.001$) was observed among subpopulations indicative of 8.5 % divergence across the sample range, compared to the 7.9 % global average observed among individual collections (Table 11). With

only two exceptions, pairwise F_{ST} comparisons among the 21 identified subpopulations indicated divergences ranging from 1.2% (pairwise F_{ST} = 0.012, P = 0.012) for subpopulation 5 in the Ord River region of WA compared to subpopulation 9 in the Alligator River region of the NT, to as high as 19.7% (pairwise F_{ST} = 0.197, P < 0.000) for subpopulation 2 in Broome WA compared to subpopulation 20 in the Fitzroy River in south-east Queensland. Only two pairwise F_{ST} comparisons among defined subpopulations were below the 0.01 threshold set for combining collections into subpopulations (Table 12). These two non-significant comparisons both involved subpopulation 19, which is comprised of only 15 individuals from the single historical Broad Sound collection, in comparison to the two more northern subpopulations, 17 and 18 (see Table 12 and Figure 6a for subpopulation locations). Despite the small sample size and single collection in this area in the present study it is considered most likely that wild-caught barramundi in the Broad Sound area belong to a subpopulation that extends northwards at least as far as Repulse Bay, but not as far north as Bowling Green Bay (our subpopulation 18) as was described by Keenan (1994).

Allelic patterns and subpopulation genetic diversity

In the past population genetic studies have favoured the use of apparently neutral genetic markers due to the widespread acceptance of the neutral theory of molecular evolution (Kimura, 1968) and the proliferation of 'neutral' population genetic models. In more recent times population genetics has realised the added power and benefit of including selected loci (Narum et al., 2010; Nosil et al., 2009), particularly in high gene-flow fish species with typically weak genetic structure (Andre et al., 2010; Hemmer-Hansen et al., 2007; Larsen et al., 2008). The inclusion of one F_{ST} outlier locus (Lca040) in the present analysis therefore warrants some further discussion and exploration. The Lca040 microsatellite is located on linkage group 22 of a first generation female barramundi linkage map (Wang et al., 2007). This locus stands out as an F_{ST} outlier (Excoffier et al., 2009) in the current population genetic dataset, with a significantly higher F_{ST} than expected under a neutral hierarchical island model (Figure 2, materials and methods section). The spatial distribution of Lca040 alleles shows a clinal pattern of increasing diversity across the north-western parts of the species distribution with a strong predominance of the '207' allele in the more westerly subpopulations including a complete lack of polymorphism in the two most south-westerly subpopulations (subpopulations 1 and 2, Figure 7a). This pattern differs to the more random differences in diversity and allele frequency seen with other microsatellite markers (see Electronic Appendix E5) including those possessing similar numbers of alleles for example Lca154 (Figure 7b) and

Lca371 (Figure 7c). These allelic patterns and the F_{ST} outlier results suggest that the Lca040 locus may be in a region of the barramundi genome that is subject to some degree of directional selection. Some caution in this interpretation is advised, however, due to the relatively low number of markers included in the analysis. In the burgeoning age of genomics, outlier detection is typically conducted with 100's to 1,000's of genetic markers (Excoffier et al., 2009) and a more extensive and genome wide analysis of population genetic structure in barramundi would be highly beneficial to identify genetic markers capable of ascertaining locally adapted subpopulations. Subsequent to this report, James Cook University are in the process of using new genotype by sequencing approaches to generate large numbers of SNP markers to address questions of local adaptation in barramundi.

The lack of polymorphism in the Lca040 locus in subpopulations 1 and 2, including collections from the Pilbara region and Broome respectively, likely contributed to a diminished allelic richness (mean A_R = 3.1 and 3.2 respectively) compared to other subpopulations (Table 13). In general, however, there was little support for major differences in genetic diversity indices such as observed or expected heterozygosity across the spatial range sampled and there was no evidence for localised inbreeding effects (i.e. no significant F_{IS}) in any of the subpopulations identified (Table 13). As indicated previously, all identified subpopulations conformed to Hardy Weinberg expectations (Table 13).

Table 13: Genetic diversity statistics for 21 subpopulations of barramundi identified with 16 microsatellite loci. SUBPOP subpopulation number, Values are means ± s.d.; N sample size, N_a number of alleles (Excoffier et al., 2005), A_R allelic richness (Goudet 2001), UH_e unbiased heterozygosity (Excoffier et al., 2005), H_o observed heterozygosity (Excoffier et al., 2005), HWE *p*-value from Hardy-Weinberg Equilibrium tests (Rousset 2008), F_{is} average inbreeding co-efficient (Excoffier et al., 2005). Where statistical testing has been performed 'ns' indicates not significant following FDR correction for multiple comparisons (Benjamini and Hochberg 1995). Collection codes are as listed in Table 1.

SUBPOP	N	N_a	A_R	UH_e	H_o	HWE *p*-value	F_{is}	Collection Codes
1	31	3.6 ± 2.2	3.1 ± 1.7	0.489 ± 0.257	0.559 ± 0.219	0.531 ^{ns}	-0.007 ^{ns}	PIL
2	14	3.3 ±1.6	3.2 ± 1.6	0.457 ± 0.288	0.515 ± 0.272	0.334 ^{ns}	0.013 ^{ns}	BME
3	30	4.5 ± 2.2	3.9 ± 1.7	0.537 ± 0.240	0.572 ± 0.220	0.723 ^{ns}	-0.043 ^{ns}	STG
4	37	4.4 ± 1.9	3.6 ± 1.5	0.494 ± 0.253	0.491 ± 0.222	0.476 ^{ns}	0.068 ^{ns}	ADM
5	279	6.7 ± 4.2	4.1 ± 2.0	0.532 ± 0.240	0.563 ± 0.205	0.038 ^{ns}	0.004 ^{ns}	SWI, DRY, SMB, KGR, BER, HEL, NNC, ORD, BPG, MOYK
6	46	5.4 ± 2.9	4.2 ± 1.8	0.538 ± 0.230	0.565 ± 0.169	0.657 ^{ns}	0.015 ^{ns}	DLYK90 ,DLY08
7	72	5.5 ± 3.0	4.0 ± 2.0	0.506 ± 0.245	0.525 ± 0.195	0.532 ^{ns}	0.027 ^{ns}	BTIK, DHBK, SHOK
8	24	4.6 ± 2.5	4.0 ± 2.1	0.499 ± 0.250	0.536 ± 0.225	0.999 ^{ns}	-0.007 ^{ns}	MRR
9	13	4.3 ± 2.0	4.3 ± 2.0	0.515 ± 0.259	0.574 ± 0.246	0.999 ^{ns}	-0.048 ^{ns}	ALG
10	32	4.1 ± 1.8	3.6 ± 1.5	0.524 ± 0.227	0.569 ± 0.104	0.799 ^{ns}	0.049 ^{ns}	LVP
11	22	4.6 ± 2.0	4.1 ± 1.6	0.518 ± 0.189	0.523 ± 0.236	0.830 ^{ns}	-0.010 ^{ns}	ANBK
12	24	4.6 ± 2.1	4.1 ± 1.9	0.497 ± 0.242	0.558 ± 0.224	0.945 ^{ns}	-0.055 ^{ns}	ROP
13	24	4.8 ± 2.3	4.2 ± 1.9	0.525 ± 0.237	0.547 ± 0.212	0.510 ^{ns}	0.024 ^{ns}	MAC
14	174	7.6 ± 4.0	4.5 ± 2.1	0.538 ± 0.221	0.528 ± 0.221	0.602 ^{ns}	0.018 ^{ns}	ALB11, LICK90, GIL, MITK, HOLK, ARCK93, ARC11
15	16	4.4 ± 2.3	4.2 ± 2.1	0.525 ± 0.198	0.539 ± 0.230	0.638 ^{ns}	-0.028 ^{ns}	JAR
16	54	5.6 ± 2.6	4.1 ± 18	0.556 ± 0.214	0.554 ± 0.211	0.454 ^{ns}	0.003 ^{ns}	JCK, ESC
17	39	4.4 ± 2.2	3.7 ± 1.6	0.555 ± 0.169	0.543 ± 0.198	0.144 ^{ns}	0.022 ^{ns}	PCB, BIZ
18	217	6.3 ± 3.6	4.1 ± 1.9	0.556 ± 0.185	0.567 ± 0.185	0.327 ^{ns}	-0.020 ^{ns}	JOR, HCC, CLE, BOWK88, BOW08, BURK89, BUR08
19	15	3.6 ± 1.8	3.6 ± 1.8	0.537 ± 0.203	0.568 ± 0.154	0.800 ^{ns}	-0.016 ^{ns}	BRSK
20	92	5.3 ± 2.9	3.6 ± 1.5	0.520 ± 0.193	0.528 ± 0.201	0.808 ^{ns}	-0.016 ^{ns}	FTZK88,FTZ13
21	18	4.4 ± 2.3	4.0 ± 2.0	0.554 ± 0.199	0.552 ± 0.199	0.877 ^{ns}	0.004 ^{ns}	MARK

Table 14: Genetic diversity statistics for major subpopulation groups (stocks) including, AR allelic richness, Ho observed heterozygosity and Fis average inbreeding co-efficient. Two sided p-values obtained after 5000 permutations in FSTAT v2.9.3.2 (Goudet, 2001) are given with significant values (*P < 0.05, **P < 0.01) indicated in bold.

Stock	**Stock Description**	**Subpopulations**	A_R	H_o	F_{is}
1	South-Western	1-2: PIL-BME	3.163	0.477	0.004
2	Western	3-4: STG-ADM	3.754	0.494	0.037
3	North-Western	5-9: SWI - ALG	4.129	0.521	0.009
4	Central	10-16 LVP - JAR	4.118	0.531	0.007
5	Eastern	17-19: PCB – BRSK	3.781	0.562	-0.012
6	South-Eastern	20: FTZ - MARK	3.802	0.532	-0.013
		P-value:	**0.006****	**0.016***	0.311
	P-value without stock 1:		0.508	0.152	

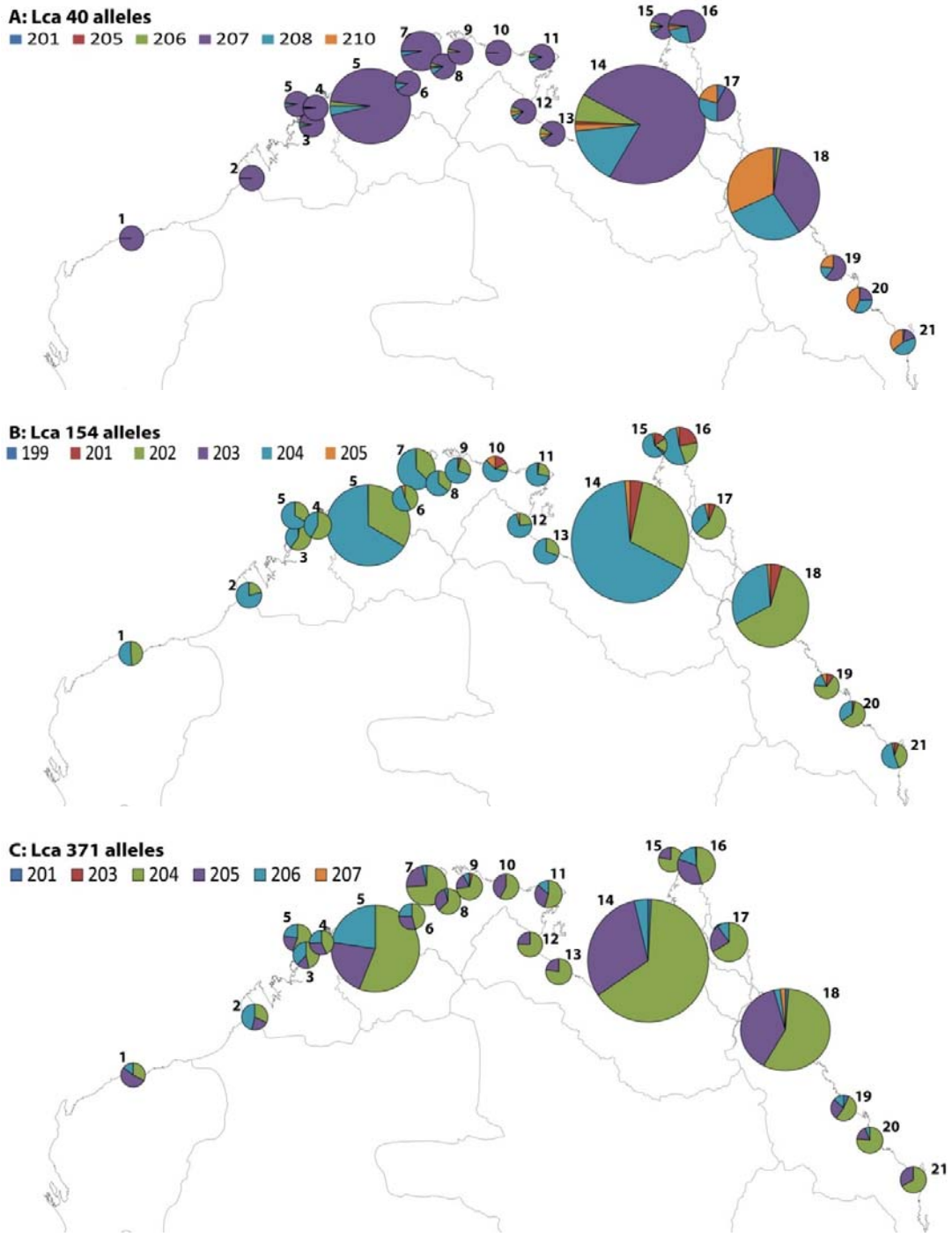

Figure 7: Spatial distribution of microsatellite alleles across 21 barramundi subpopulations for three markers A) Lca040, B) Lca154 and C) Lca371. Numbers beside pie charts correspond to subpopulation numbers as defined in Figure 5 and pie charts are scaled to reflect the size of the subpopulation. Full details of allele frequencies for all 16 markers in each subpopulation can be found in electronic Appendix E3.

Major geographical patterns of genetic structure

To explore the major geographical patterns of genetic structure present in the current microsatellite dataset a number of different analyses were conducted. Firstly an examination for isolation-by-distance patterns, indicative of increasing genetic distance with increasing geographic separation, was undertaken with Mantel's tests. The highest regression coefficient was obtained for the regression of geographic distance against genetic distance as pairwise F_{ST} (Z = 151224.7285, r = 0.7588, r^2 = 0.576, *P*-value <0.001) that supports a pattern of isolation-by-distance for barramundi across the full species distribution in Australia (Figure 8). This is similar to the conclusions of earlier authors utilizing allozymes (Keenan, 1994) and mitochondrial DNA markers (Chenoweth et al., 1998a; Marshall 2005) over more restricted spatial scales. Given the linear and near continuous distribution of barramundi along the northern Australian coast-line the detection of such a pattern is largely to be expected. The demonstration of Isolation-by-Distance does not, however, preclude the development or presence of other informative patterns of stock structure.

In a previous study by Marshall (2005) mitochondrial DNA and five microsatellite markers were used to explore genetic variability among and within three major freshwater fish bioregions originally described by Unmack (2001). It was proposed from a hierarchical AMOVA analysis that the genetic structure of barramundi in Australia fit a freshwater fish model with genetic divisions stated as occurring principally among river drainages (Marshall 2005). This earlier study, however, had a restricted number of widely spaced sample collections, each of which was treated as a separate population, with 11 locations sampled across a broad spatial scale from the Pilbara region in Western Australia to the Fitzroy River (Queensland). Given the extensive geographic coverage of the present microsatellite dataset and good spatial distribution of collections throughout the species distribution we attempted to replicate this analysis and explore the hypothesis of a freshwater fish model of genetic structure for barramundi.

Similar to Marshall (2005) we conducted AMOVA analysis (Appendix A4) to partition the observed genetic variance among various groups of collections including grouping collections by Unmack (2001) freshwater fish bioregions (Figure 9C), among the sub-provinces nested within those bioregions (Figure 9D) and among Level 1 (L1) drainage basins (Figure 9E) and the Level 2 (L2) basins located within L1 drainages (Figure 9F). The extent of among group

variability captured was compared to that attributable to differences among the collections overall (Figure 9A) and among genetic subpopulations as defined earlier (Figure 9B). The highest proportion of among group variability was observed when collections were grouped into the genetic subpopulations defined earlier, with 8.4 % variance among subpopulations and a negligible 0.2% variance between collections within subpopulations. Level 2 drainage basins also showed a similar level of among group genetic variance with 7.6 % variance among L2 basins and just 0.4% variance among collections within L2 basins (Figure 9F). It should however be noted that due to the relatively small size of L2 basins, and the spatial scale of sampling, there is little to no replication of sampling collections within most L2 basins (Appendix A3). Less among group variation (6.5 – 7.0 %), and more variation among collections within groups (1.2 – 3.1 %), was found when collections where grouped at the larger spatial scales corresponding to FW fish bioregions, FW fish subprovinces or Level 1 drainage divides respectively (Figure 9C-E). The presences of still significant levels of genetic variation among collections within groups based around freshwater fish bioregions and subprovinces, as well among collections within the major drainage basins, suggests that genetic structure in barramundi does not conform to a strict freshwater fish model. The high similarity in amount of genetic variance captured among collections overall (Figure 9A) and among the smaller L2 aggregated river basins (Figure 9F) is considered simply a reflection of the spatial distribution of sample collections within individual river basins.

Close examination of subpopulation locations overlaid on maps of Unmack's (2001) FW fish bioregions (see Figure 6A) show that the more widespread subpopulations transverse FW fish bioregion boundaries in a number of areas. This includes subpopulation 5 that extends from the Drysdale River in WA to the Moyle River in the NT, subpopulation 14 in the eastern part of the Gulf of Carpentaria, and subpopulation 18 along the central-eastern coast of Queensland (Figure 6A). This spatial pattern suggests a higher degree of coastal marine dispersal and/or connectivity of freshwater/estuarine habitats in these areas than elsewhere in the species distribution. While tagging studies typically indicate relatively restricted coastal movements of less than 25 km (Russell and Garrett 1988; Sawynok and Platten 2009), exceptions of between 50 - 100 km including movements to adjacent or neighbouring river systems have certainly been reported (Davis 1986; Sawynok and Platten 2009) and anecdotal reports of even larger flood-plume mediated coastal dispersal exist (Keenan, 1994). A recent fisheries modelling study has also suggested that the number of wetland patches and the extent of both wetland and mangrove connectivity are important factors influencing recruitment and subsequent catch-per unit effort (CPUE) in barramundi fisheries in the

southern Gulf of Carpentaria (Meynecke et al., 2008). Precipitation and temperature have also been shown to be key drivers of barramundi recruitment and CPUE in Queensland (Balston 2009a,b; Meynecke and Lee 2011). Because the persistence and connectivity of wetland habitat is linked to factors such as temperature, precipitation and evaporation these same factors operating at the spatial scale of river basins are also likely to influence the finer scale genetic structure of barramundi. As a spatial model of current habitat suitability and fisheries CPUE for northern Australia is presented later (Component D) further discussion of finer scale population genetic structure will also be addressed later.

Larger scale patterns of genetic stock structure were explored using both PCA of genetic distances between subpopulations (Figure 10) and using an individual-based Bayesian clustering approach (Figure 11). Both of these analyses produced a similar pattern of stock structure that suggests a strong influence of a number of historical biogeography barriers to gene flow across the species distribution. The dominant structure detected was that of an Eastern and Western stock (Figure 10 and 11) joined by a centrally admixed stock with individuals in the Gulf of Carpentaria from the Roper River (NT) to the Jardine River at the Tip of Cape York (QLD), and neighbouring subpopulations as far west as the Liverpool River (NT) and as far east as the Escape River in far north-east of Queensland, showing a gradient of mixed co-ancestry between these two stocks. The presence of an East/West split in barramundi stocks was suggested in earlier allozyme and some mitochondrial DNA studies (Keenan 2000; Keenan 1994; Chenoweth et al., 1998a,b; Salini and Shaklee 1988) although this was argued by later mitochondrial studies (Doupe et al., 1999; Marshall 2005). Long-term historical separation of Eastern and Western barramundi populations caused by the formation of an extensive land-bridge between Australia and Papua New Guinea at times of lower sea-level are the likely driver behind this East/West split (see Keenan 1994; Chenoweth et al., 1998b). Contemporary gene-flow into the Gulf of Carpentaria to produce a diverse Central genetic stock has also been indicated previously (Keenan 1994; Chenoweth et al., 1998b).

The extensive microsatellite dataset obtained in the present study strongly supports the presence of an Eastern, Western and a Central (admixed) stock of barramundi in Australia. Further geographic stock structure was, however, also revealed in the present analysis when looking at progressively finer spatial scales. The comprehensive 16 locus microsatellite dataset obtained in the present study in fact suggests the existence of 6 broader groups of subpopulations with at least two admixed stocks and 4 more discrete stocks identified. Based on the present dataset the most southerly subpopulations of barramundi on both the Eastern

and Western coasts of Australia were genetically distinctive from more northern subpopulations. For this reason subpopulations from the Pilbara (PIL) and Broome (BME) region of Western Australia are recognised as a separate stock described here as the South-West Stock (Figure 11C) while the subpopulations to the north of this area in the St George Basin (STG) and Admirality Gulf (ADM) appear as an admixed stock. The ancestry of individuals in the STG/ADM region is consistent with the mixing of the South-West Stock and an additional stock to north of this area, however as the level of admixture is ~50 % it is difficult to assign these subpopulations to either the South-West stock or the broader 'Western Stock'. This therefore leads to a further split of the larger 'Western Stock' into the South-West Stock (BME – PIL), the 'Western' stock (STG – ADM) and a larger North-Western stock (DRY – ALG, Figure 11C). The genetic distinctiveness of this more southern WA stocks is consistent with previous studies based on mitochondrial DNA and is indicative of past biogeographic barriers to gene flow in Western Australia (discussed in Marshall 2005; Doupe et al., 1999).

The relative distinctiveness of the SE Queensland collections, including both modern and historical collections from the Fitzroy River and historical collections from the Mary River, warrants further investigation given the inconsistencies in subpopulation structure between the present study and the earlier work of Shaklee and Salini (1993) and Keenan (1994). However the consistent differences seen between historical and modern collections of the Fitzroy River (QLD) from those to the north seems to suggest a further split of the broader Eastern Stock into a south-eastern stock and an 'eastern Stock' (Figure 11D) is possible. Historical differences in the shape of the Queensland coastline and the direction of riverine discharge (see figures and discussions in Keenan 1994, Chenoweth et al., 1998b; Unmack 2001) and a strong historical gradient in environmental (climatic) conditions along the east coast (see Unmack 2001) may have all contributed to past reductions in gene-flow. The distinction of the South-East Queensland subpopulations in the present analysis may be an indication that the effects of past barriers to gene-flow may not yet have been fully overcome by present day gene-flow but further and more comprehensive sampling in this region is required before stronger conclusions or interpretations can be made.

Past genetic studies have suggested a reduced genetic diversity of Eastern versus Western sub-populations, and a higher genetic diversity of central barramundi populations (Keenan 1994; Chenoweth et al., 1998b). Given the potential importance of genetic diversity to future adaptive potential we examined various indices of genetic diversity for the 16 microsatellite

loci genotyped in the present study. Unlike previous studies of allozymes (Keenan 1994) and mtDNA (Chenoweth et al., 1998b) we did not find any evidence for a bottle neck or reduction of genetic diversity in the nuclear genome of barramundi in Eastern Australia. While both the North-Eastern stock and Central (admixed) stock described here have slightly elevated allelic richness (Table 14), the results of statistical tests suggest that this difference is not significant. A statistically significant difference in genetic diversity in the form of both allelic richness (A_R) and observed heterozygosity (H_O) was, however, observed when the South-Western Stock (PIL-BME) was included in the analysis. This is consistent with a previous mtDNA study that first reported that barramundi in the Pilbara region should be considered an Evolutionary Significant Unit (Marshall, 2005) and that showed a latitudinal trend of decreasing genetic diversity of a mtDNA marker southward along the Western Australian coast. Our microsatellite analysis also suggests that the barramundi populations in the Pilbara – Broome region of WA should be considered as a genetically distinct stock with reduced genetic diversity relative to the other stocks described.

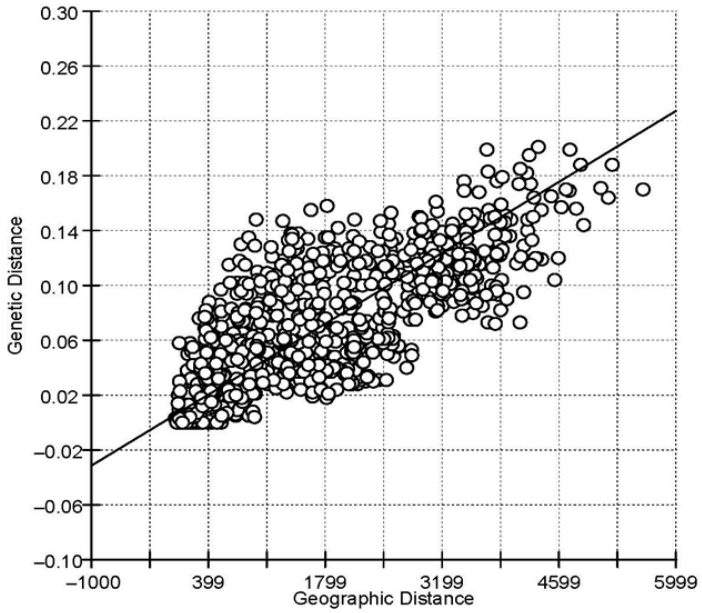

Figure 8: Isolation-by-distance plot of genetic distance (pairwise F_{ST}) against geographic distance for 48 barramundi collections sampled and genotyped for 16 microsatellite loci. Mantel test results: Z = 151224.7285, r = 0.7588, r^2 = 0.576, *p*-value <0.001. Regressions involving log-genetic and log-geographic distance had lower coefficients and are therefore not shown.

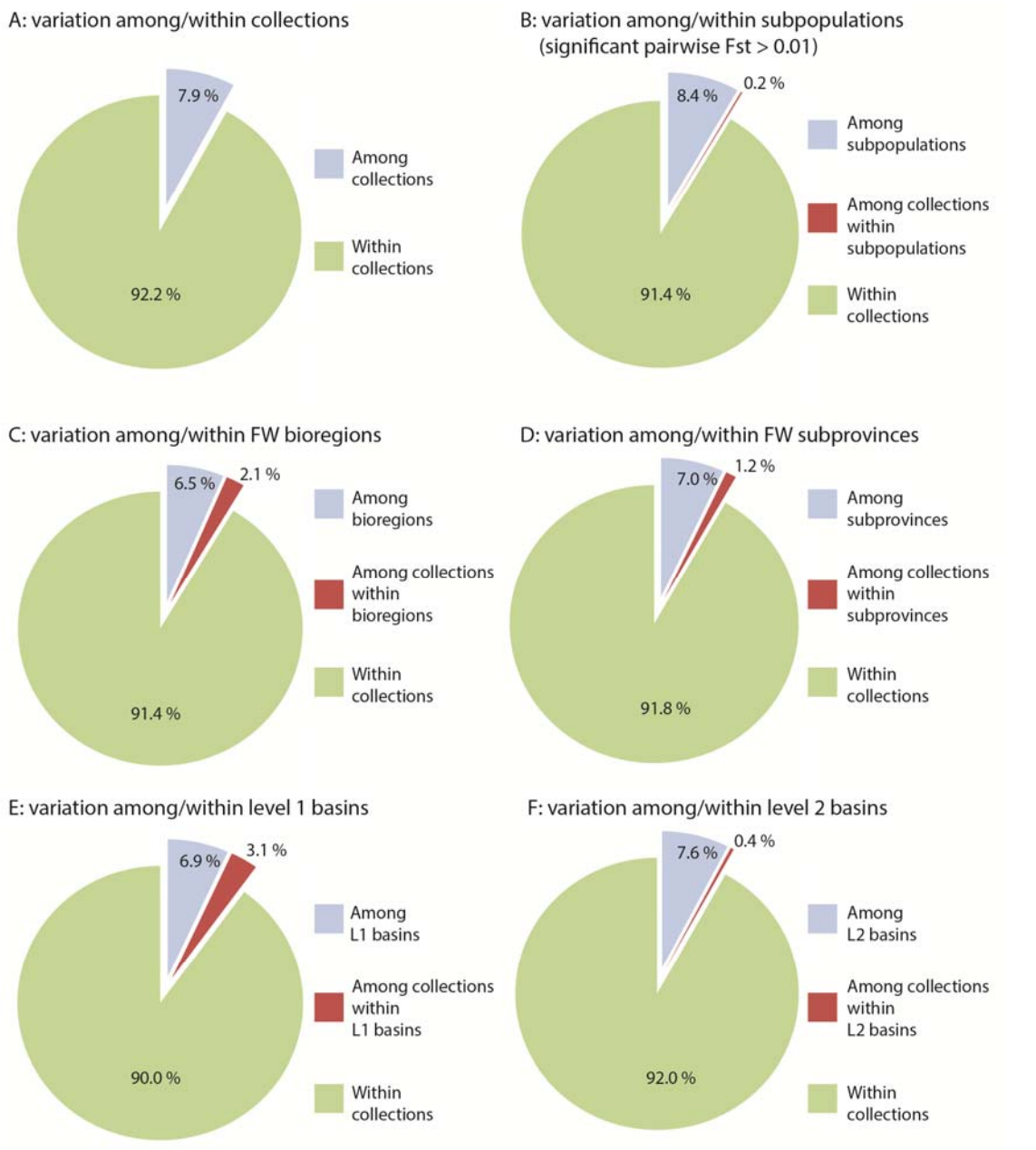

Figure 9: Summary AMOVA pie charts showing the extent of genetic variation attributable to varying hierarchical levels of structure including (A) among 48 wild collections, (B) among and within 21 genetic subpopulations as defined in Figure 5 (C) among and within 9 freshwater fish bioregions as defined by Unmack (2001) or (D) among and within freshwater fish sub-provinces as defined by Unmack (2001). Two additional analyses utilized level 1 and level 2 river basin groups from National Catchment Boundaries v1.1.4 (http://www.ga.gov.au/topographic-mapping/national-surface-water-information.html) these analyses included (E) among and within level 1 drainage divisions (F) among and within level 2 aggregated river basin groups.

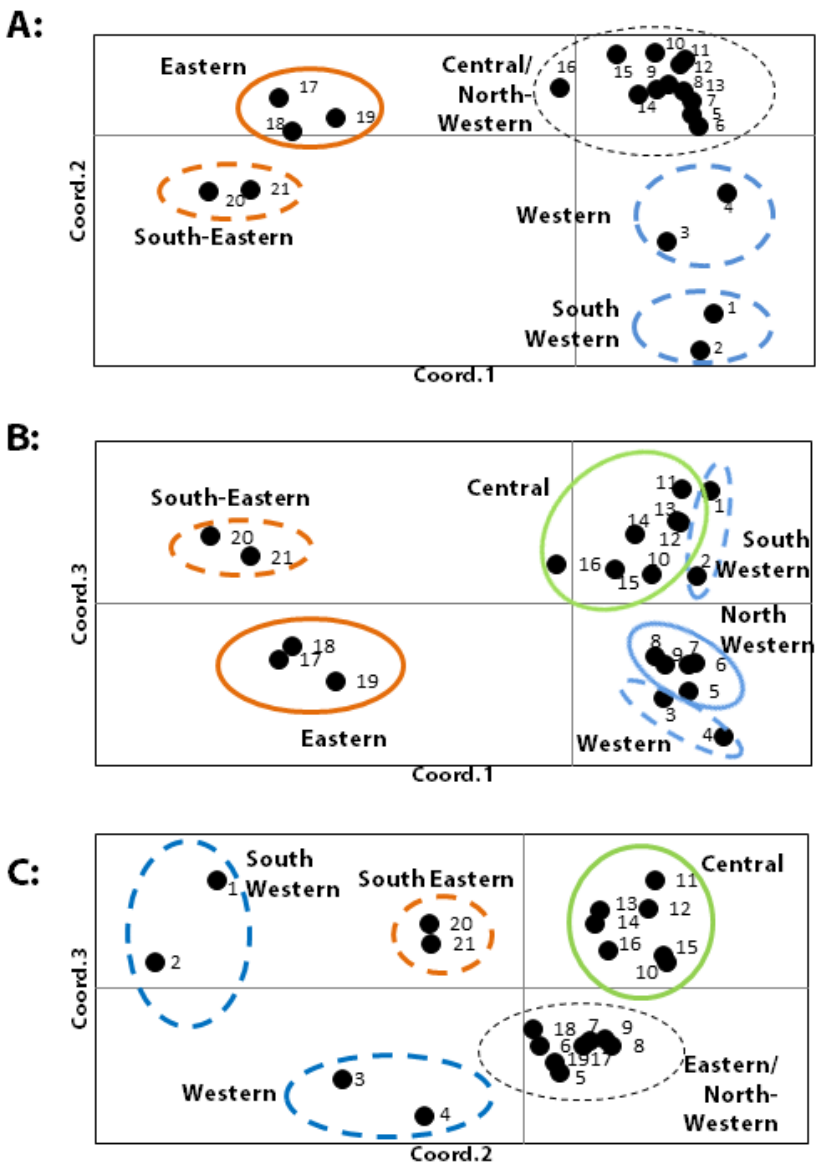

Figure 10: Plots of principle components analysis of Nei's unbiased genetic distance matrix showing main stock groupings (coloured circles) in different dimensions. Plot A shows PCA axes 1 (Coord.1) and 2 (Coord.2) that explain 48% and 21.82% of the variation respectively (cumulative total 69.82%), while plots B and C show additional variation due to the third axis (Coord.3) that explains an additional 12.1% of the variance (cumulative total 81.9%). Blue circles indicate groups within the Western stock, orange circles indicate groups within the Eastern stock, green circles indicate the Central stock while black circles indicate mixed groups not separated on the plotted axes.

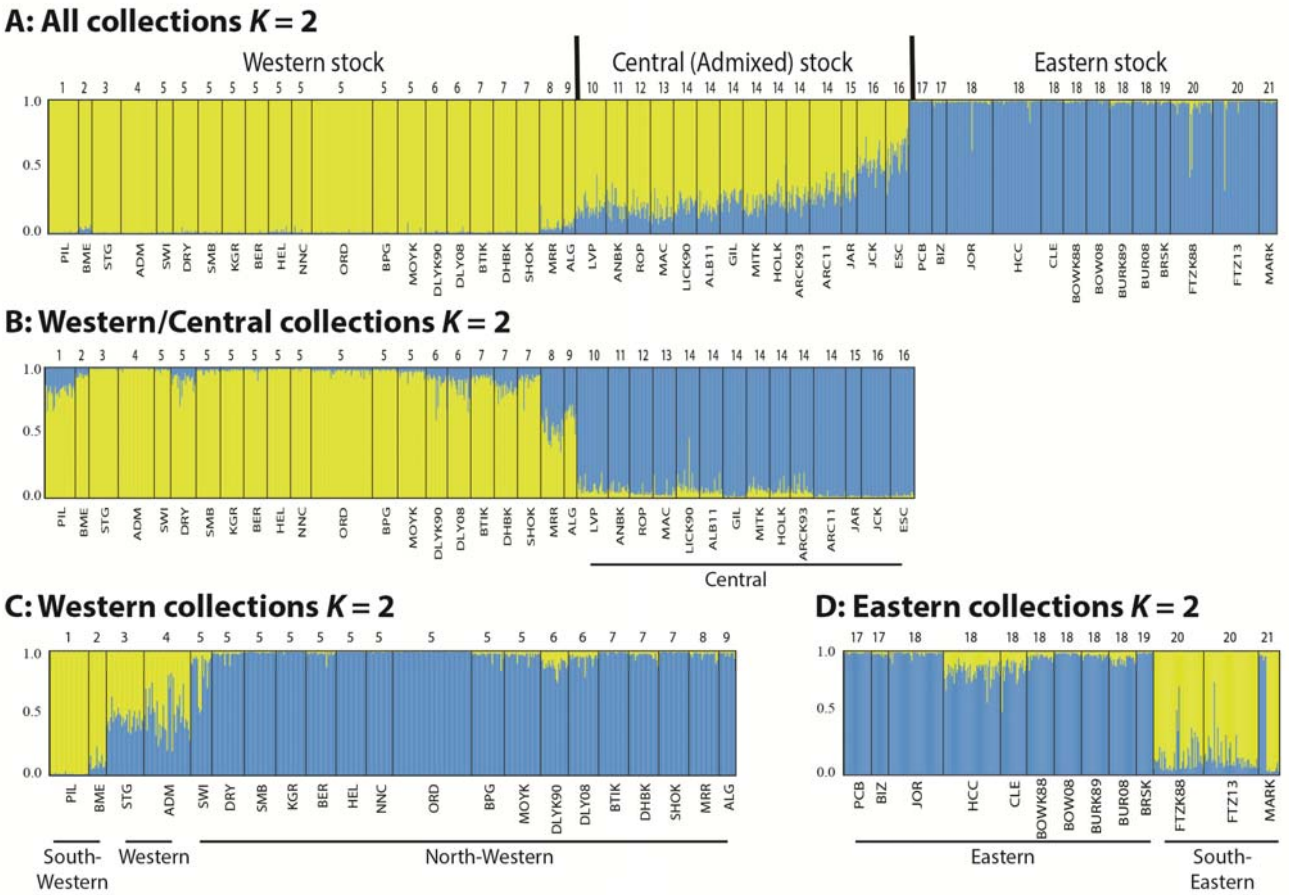

Figure 11: Co-ancestry plots for 1273 individual barramundi sampled from 48 wild collections. Collection codes are given on the x-axis while subpopulation codes are given above each collection. The y-axis shows the average membership coefficients (*q*-values) for each sampled fish indicating level of co-ancestry within two distinct ancestral clusters ($K = 2$) represented by blue and yellow respectively. The analysis was conducted using in a hierarchical fashion and major stock groups are labelled in A, C and D and are indicated by solid lines along the x-axis joining the included collection codes in C and D.

Component B: Physiological testing

i) Live/Dead cell assays

The results from the present investigation confirmed the results of Newton et al., (2010), where it was first demonstrated that populations of barramundi from northern (Darwin) and southern (Gladstone) genetic subpopulations within this species' range exhibited differing tolerances to upper thermal stress, as predicted by both live/dead caudal fin cell analyses and loss of swimming equilibrium tests. In the present study, caudal fin live/dead cell assays were found to likewise differ significantly among the barramundi populations examined (ANOVA; $F_{4,\ 261} = 11.99$; $P < 0.001$), generally corresponding to both genetic stock as defined by molecular genetic approaches (see above), and closely with latitude of fish population origin. Barramundi tested from Broome and Karumba had the highest live/dead cell ratios (4.42 ±0.24 SE and 4.45 ±0.25 SE), indicating these populations to be the most tolerant of the acute heat stress applied, followed by fish from Darwin, Bowen/Townsville and finally Gladstone stocks. The Gladstone population exhibited the lowest ratio of live/dead cells indicating that fish from this stock were the most susceptible to acute upper heat stress (2.87 ±0.14 SE)(Figure 12).

Dissociated caudal fin cell results demonstrated that the populations most tolerant to an acute upper thermal stress were those originating from Broome and Karumba within the South-Western and Central genetic stocks respectively. Based on palaeontology temperature data and computer modelling, coastal areas of the far Kimberley and the Gulf of Carpentaria were historically consistently warmer throughout the last 30,000 years then those along the eastern Australian seaboard (Van Der Wal unpublished simulation). Along the eastern Australian coastline there is also an obvious latitudinal temperature gradient from north towards the south, with the coolest water temperatures experienced historically by barramundi occurring in the south-east region represented here by the Gladstone population. Data obtained through the dissociated caudal fin cell method strongly suggests that this long-term difference in environmental temperature has led to at least some degree of adaptation and/or long-term acclimation among barramundi genetic stocks to local temperature regimes, resulting in biological differences in tolerance to acute high temperature stress events.

Results therefore confirm that thermal tolerance differences exist between populations of Australian barramundi from different genetic stocks across the species' extensive latitudinal range (12°S and 17°S) when tested using the dissociated caudal fin live/dead cell method.

However, it should be noted that the acute temperature shock at 43 °C utilized in these live/dead cell assays was well above that likely to be experienced by fish in their native environments, either currently or under future climate change scenarios (see component D results). Despite this, given the past results of Newton et al., (2010), that were conducted on different batches of fish from the Darwin and Gladstone population and showed similar differences between these two stocks to that of the present study, it is evident that the patterns of acute thermal tolerance revealed are stable and repeatable through time. This provides a strong scientific basis for testing underlying metabolic and physiological differences among barramundi stocks when challenged to more realistic and varying environmental temperatures. This includes temperatures that are likely to be experienced either currently in parts of the species range, or that represents novel temperatures likely to be encountered in the near future.

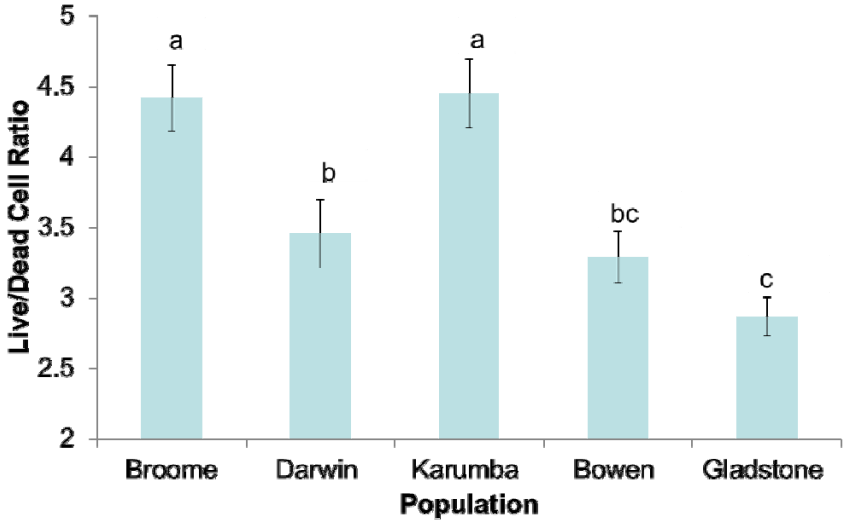

Figure 12: Ratio of mean dissociated live to dead caudal fin cells from five populations of barramundi when subjected to heat stress at 43 °C. Different letters above bars denote populations that were significantly different at $P < 0.05$. Error bars are standard errors of the mean.

ii) Resting metabolic rate, maximum metabolic rate and aerobic scope in populations of barramundi

Validation of open-top respirometry as a method to determine MO_2

The mean mass-specific RMR of barramundi in open-top respirometers was not significantly different when compared to closed-top respirometers at each of the temperature treatments ($P > 0.05$). The mean mass-specific RMR of the Broome barramundi in open-top respirometers was 34.9 ± 0.7 at 18 °C , 58.1 ± 0.8 at 28 °C and 108.1 ± 0.9 mg O_2 $kg^{-0.8}$ h^{-1} at 38 °C and in closed-top respirometers was 33.2 ± 0.6 at 18 °C , 56.2 ± 1.1 at 28 °C and 106.3 ± 0.9 mg O_2 $kg^{-0.8}$ h^{-1} at 38 °C (Figure 13). There were no statistically significant differences among comparative estimates of RMR between the two systems verifying that the open-top respirometer method is an appropriate methodology to estimate MO_2 in barramundi.

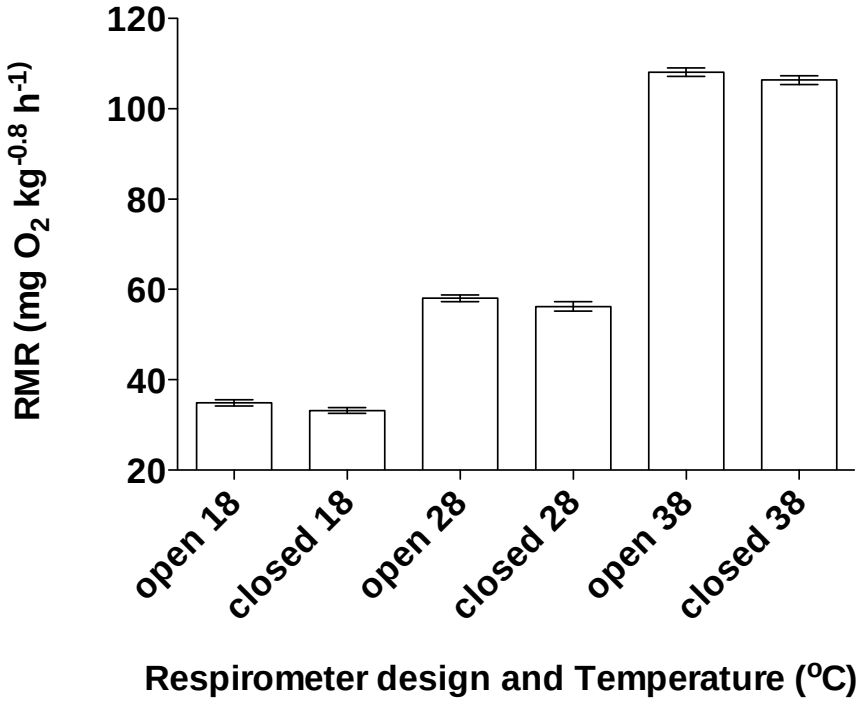

Figure 13. Routine metabolic rate of barramundi (mean ± SE; n = 3) in open and closed-top respirometery systems at 18, 28 and 38 °C.

Determination of routine metabolic rate, maximum metabolic rate and aerobic scope in four genetic stocks of barramundi

With open-top respirometry validated above as an appropriate methodology to measure MO_2 over time, resting metabolic rate (RMR), maximum metabolic rate (MMR), aerobic scope (AS) and F*x* (factorial difference in aerobic metabolic scope) were estimated in four barramundi stocks (Table 15). Surprisingly in contrast to results obtained when caudal fin cells were exposed to an acute heat stress, but consistent with observed tolerances to hypoxia (see below), no significant differences were apparent amongst any of the four barramundi stocks in RMR, MMR or aerobic scope when tested at temperatures up to 38 °C (Table 16). This result shows that these metabolic traits are comparatively conserved among barramundi sub-populations across a broad range of temperatures and there does not appear to be distinctive local adaptation as reflected by metabolic responses of fish from different thermal regimes to varying temperatures. Because no significant differences were found in the assessments of physiological metabolic performance among the barramundi stocks each performance parameter can therefore be expressed in Australian barramundi as a function of temperature (T) per mg O_2 $kg^{-0.8}$ body weight h^{-1} across all stocks tested as follows:

RMR = 3.463T- 27.581 (R^2 = 0.923) (Table 15)

MMR = 7.673T + 0.137 (R^2 = 0.797) (Table 15)

AS = 4.210T + 27.718 (R^2 = 0.55) (Table 15)

F*x* = -0.053T + 4.772 (R^2 = 0.486) (Table 15)

Pooled RMR data of all barramundi sub-populations derived from pilot data, respirometer validation and Table 15 shows that a single global linear equation adequately describes the RMR of all barramundi sub-populations tested as a function of temperature (Figure 14). The RMR of Australian barramundi per mg O_2 $kg^{-0.8}$ body weight h^{-1} can be described as:

RMR = 3.634T – 32.19 (R^2 = 0.920)

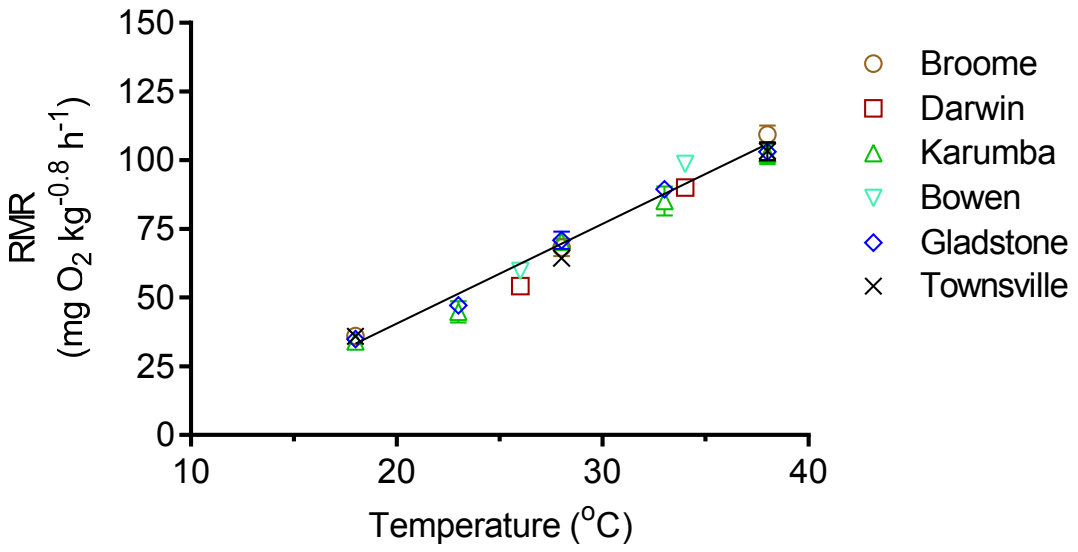

Figure 14: The routine metabolic rate of six subpopulations of barramundi at temperatures ranging from 18 – 38 °C. Data compiled from several respiratory trials: respirometer validation, MMR trial and pilot trials (Bowen and Darwin fish).

Table 15: Mean parameter values (±se; n=10) of each barramundi stock at different temperatures. Routine Metabolic Rate (RMR), Maximum Metabolic Rate (MMR) and Aerobic Scope (AS) expressed as mg O_2 $kg^{-0.8}$ h^{-1}. The factorial difference (F*x*) in aerobic scope was calculated as MMR / RMR.

Temp/Stock	**RMR**	**MMR**	**AS**	**F*x***
18 °C				
Townsville	35.9 (1.33)	134.5 (1.73)	98.6 (1.14)	3.78 (0.10)
Karumba	33.98 (0.72)	133.76 (1.23)	99.78 (1.37)	3.95 (0.09)
Broome	36.06 (0.08)	132.12 (0.94)	96.06 (1.52)	3.68 (0.10)
Gladstone	34.92 (0.98)	132.68 (1.56)	97.76 (1.40)	3.82 (0.10)
28 °C				
Townsville	64.39 (2.24)	227.85 (5.23)	163.46 (4.85)	3.57 (0.13)
Karumba	70.35 (2.47)	220.19 (6.01)	149.84 (6.07)	3.16 (0.13)
Broome	68.17 (3.14)	235.04 (5.98)	166.87 (5.93)	3.51 (0.16
Gladstone	70.86 (3.12)	216.64 (8.64)	145.78 (9.91)	3.12 (0.20)
38 °C				
Townsville	103.28 (3.35)	286.37 (16.00)	183.09 (17.12)	2.81 (0.21)
Karumba	102.32 (3.79)	278.91 (11.28)	176.60 (11.20)	2.75 (0.13)
Broome	109.28 (3.38)	295.09 (16.19)	185.82 (13.68)	2.69 (0.09)
Gladstone	103.01 (3.13)	286.54 (20.17)	183.53 (19.03)	2.78 (0.17)

Table 16: Two-way ANOVA on Routine Metabolic Rate (RMR), Maximum Metabolic Rate (MMR) and Aerobic Scope (AS). ** indicates significantly different at $P < 0.01$, ns indicates not significantly different at $P < 0.05$.

		RMR			MMR			AS		
Term	**DF**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
A: Temperature	2	47988	707.22	**	238461	231.92	**	74456	76.43	**
B: Stock	3	57.94	0.85	ns	602.61	0.59	ns	465.72	0.48	ns
A X B	6	71.12	1.05	ns	259.72	0.25	ns	383.04	0.39	ns
Residual	108	67.86			1028.2			974.21		

iii) Hypoxia trials

Barramundi from five genetic stocks were tested for their tolerance to temperature induced hypoxia by exposing fish to two temperature treatments (26 °C and 36 °C). Initially values of resting oxygen consumption for the various genetic stocks were obtained for each of the temperatures under comparison. Here, within a temperature, no significant differences were apparent among barramundi from the various stocks for resting oxygen consumption. As expected due to well-known effects of increased temperature on metabolism in poikilothermic organisms, resting oxygen consumption rate of barramundi acclimated to 26 °C was significantly lower than resting oxygen consumption rate of fish acclimated to 36 °C (1.47 ± 0.24 mg $O_2.kg^{-1}.min^{-1}$ vs. 3.10 ± 0.43 mg $O2.kg^{-1}.min^{-1}$ for all sub-populations combined), (F_1, 110 = 563.57, $P < 0.001$; Figure 15).

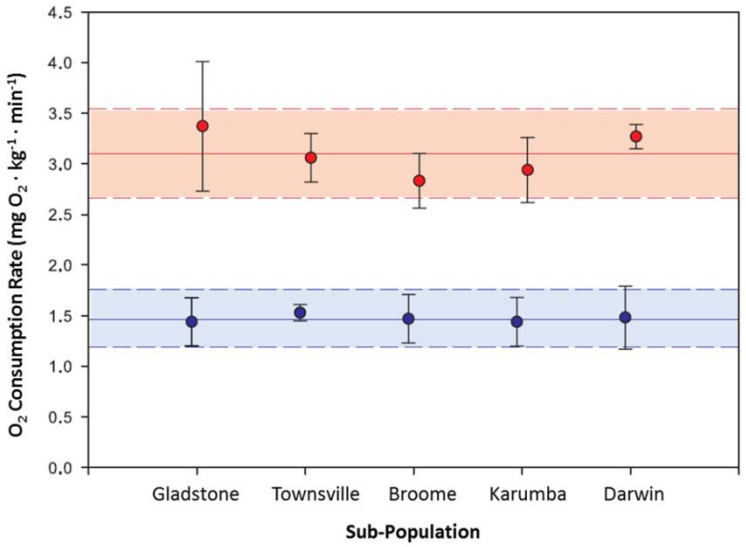

Figure 15: Resting oxygen consumption rates (mg O_2.kg^{-1}.min^{-1}; mean ± standard deviation) for five barramundi populations at 26 °C (blue; n = 48) and 36 °C (red; n = 59). Oxygen consumption rates were calculated from the average slope of the decline in chamber oxygen across a 15 min period during the 'closed' cycle of intermittent flow-through respirometry and was measured every 45 min. The solid and dashed horizontal lines represent the mean and standard deviation for all populations at each temperature.

A small, but nonetheless, significant interaction between population and temperature for resting oxygen consumption, however, was found (P = 0.025). Whilst no differences were observed for resting oxygen consumption of populations at 26 °C (H_5 = 1.446, P = 0.84), at 36 °C resting oxygen consumption was detected to be overall statistically different between populations (H_5 = 11.25, P = 0.0239) (Figure 15), although Dunn's post hoc comparison did not identify differences between individual populations. The average Q_{10} for resting oxygen consumption across all populations was 2.12 ± 0.30.

Barramundi exhibited a common and clear trend in response to decreasing oxygen with fish maintaining a relatively constant rate of oxygen consumption above the O_2 crit, followed by a steep decline (Figure 16). Fish displayed signs of distress such as erratic movements and pale body colour below O_2 crit, however, all fish bar one recovered fully upon returning to 100% saturated conditions.

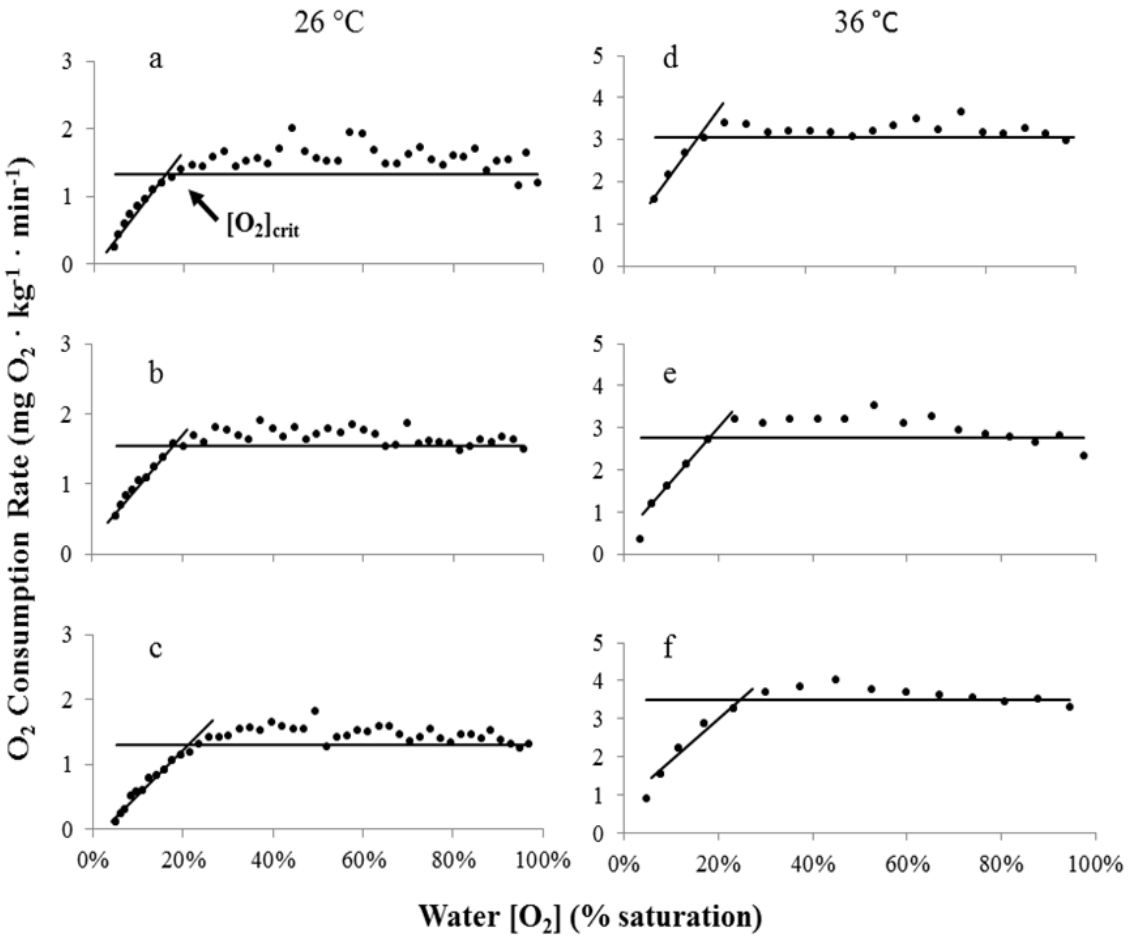

Figure 16: Representative graphs displaying oxygen consumption rate as water oxygen concentration (% saturation) decreases for six individuals from three barramundi populations at 26 °C and 36 °C. Populations are indicated by each panel as follows: Gladstone (a, d), Broome (b, e) and Darwin (c, f). Rate of oxygen consumption was calculated from the average slope of the decline in chamber oxygen for every 5 min period. Fish took ~ 4 h to deplete oxygen within the chambers at 26 °C and ~ 1.5 h at 36 °C. The difference in the vertical scale for 26 °C and 36 °C fish reflects the increased metabolic demands of barramundi at the higher temperature. The O_2 crit is indicated by the arrow in the top left panel.

There was no significant interaction between population and temperature for O_2 crit (P = 0.486). Temperature had a significant effect on mean O_2 crit across all populations, with lower O_2 crit at 26 °C than at 36 °C (F_1, 114 = 69.97; P < 0.001; Figure 17). No significant differences were observed for mean O_2 crit between four of the five populations tested at both 26°C and 36°C (Figure 17). The exception was that mean O_2 crit was highest for fish from

Darwin at both 26 °C and 36 °C (18.91 ± 2.97% and 23.84 ± 3.49% respectively). Critical oxygen saturation measurements varied between individuals, with minimum and maximum values spanning 8.63 to 23.02% saturation at 26 °C (mean = 15.44 ± 3.20% saturation) and 13.47 to 31.17% saturation at 36 °C (mean = 21.07 ± 3.92% saturation).

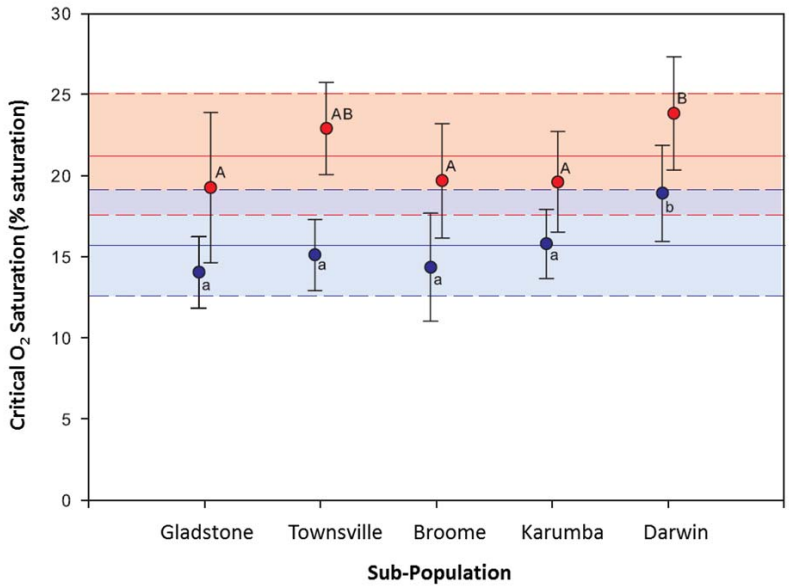

Figure 17. Mean critical oxygen saturation (% saturation; mean ± standard deviation) for five populations of barramundi at 26 °C (blue; n = 50) and 36 °C (red; n = 63). The solid and dashed horizontal lines represent mean and standard deviation respectively of critical oxygen saturation for all populations at each temperature. Letters indicate significant differences ($P < 0.05$; a < b).

In summary, our results from the physiological testing of barramundi from disparate genetic stocks to various temperatures showed that whilst there appears to be an underlying population-specific differential response to acute upper heat stress (dissociated caudal fin cell method), once acclimated within the temperature ranges tested (18 °C to 36/38 °C), barramundi as a species have a nearly uniform response in resting and maximum metabolic rates, as well as tolerances to low oxygen dissolution levels. Moreover in the context of predicted temperature rises due to climate change, barramundi metabolic responses were tested at very high water temperatures (up to 38 °C); temperatures well above that expected to

occur as result of climate change over the next 70 years (current mean water temperatures: Darwin ~32 °C, Gladstone ~28 °C). Tested across a temperature range of 20 °C (18 °C to 36/38 °C) metabolic rate and aerobic scope (F*x*) scaling effects of fish were linear, suggesting that barramundi for short periods of time, at least, can compensate their aerobic metabolism to cope with fluctuations in temperature and to temperatures at or above those predicted to occur in the future (e.g. 2-3 °C rises).

Despite our results detecting no significant differences in the metabolic rates and hypoxia tolerances of barramundi from different populations across a 20 °C temperature range, there is accumulating evidence in the literature for local adaptation of other performance traits to temperature in this species. In perhaps the first study examining the response of barramundi from populations originating from regions with different thermal environments, Rodgers and Bloomfield (1993) co-reared two Queensland strains of barramundi (one from Cairns in northern Queensland (16°S) and the other from Burrum River (25°S) in central Queensland) in open freshwater cage culture on the Atherton Tablelands, a moderately cool tropical environment in the mountains above Cairns. In this study, Rodgers and Bloomfield (1993) found that whilst similar overall growth rates were recorded for both populations, bacterial infections caused greater mortality during cold temperature spells in the more northerly derived Cairns strain (Rogers and Bloomfield 1993). As temperatures cooled with the onset of winter, Burrum River fish were also observed to have comparatively higher feeding rates, whilst Cairns derived fish had lower appetite, lower condition factor, reduced growth during winter and higher mortality rates. These findings were interpreted as symptomatic of the unique adaptation of Cairns and Burrum River strains to local thermal conditions lending initial support to the argument that Australian barramundi may in fact show evidence of local adaptation to temperature. Similarly, more recent research has documented differences in various performance traits due to temperature in barramundi from northern latitudes, compared with those from southern latitudes, through the measurement of critical swimming speed (Edmunds et al. 2010), time to loss of swimming equilibrium when challenged with acute water heating (Newton et al., 2010), growth (Newton 2013) and gene regulation (Newton et al., 2013). Edmunds et. Al., (2012), for example, found a temperature-dependent response in transcript abundance of the glycolytic enzyme lactate dehydrogenase-B (ldh-b) between northern and southern populations of Australian barramundi when fish were swum at different temperatures. However, this temperature-specific response was only observed when fish were swum at cooler temperatures (20 °C), with the southern population tested having higher ldh-b transcript abundance. This suggested an adaptation in the processing of lactic

acid of southern populations after intense bursts of swimming at cooler temperatures. However, at warmer temperatures population-specific trends were not evident. Newton (2013) co-reared barramundi from Gladstone (23°S) and Darwin (12°S) for 106 days at three temperatures (22 °C, 28 °C and 36 °C) in an intensive recirculation system. Here again population-specific differences in growth rate were observed, whereby at a rearing temperature of 22 °C, a higher final weight in Gladstone barramundi was evident compared to the Darwin fish (145.9 ± 11.1 g and 89.9 ± 3.5 g, respectively). However, at 28°C and 36 °C no significant differences were observed in growth of the two populations. This contrast in differences observed among all these experiments and the ones conducted for this report suggest that barramundi may in fact exhibit genetically determined adaptations due to cooler temperatures and extreme acute hot temperatures. In between these cool and high temperature scales, however, there is a common response within the species to temperature changes.

Hypoxia tolerance

Barramundi have featured in mass mortality events in northern Australia (Townsend et al., 1992) and previous reports indicate that barramundi succumb rapidly to hypoxia under severe conditions. Pearson et al., (2003) reported a 'lethal DO concentration' of ~ 15% saturation for barramundi at 28 – 30 °C, however, details of the fish and experimental design were unclear in their study, making the results difficult to interpret in the context of our study. In contrast, Wu (1990) reported no mortality for barramundi (150 – 250 g) held in saltwater after 8 h of exposure to ~ 15% saturation at 25 °C, however, exposure to ~ 8% saturation resulted in 50% mortality after 6.5 h. Butler et al., (2007) reported an 'acute asphyxiation concentration' of 4% saturation for barramundi (200 g) in freshwater at 28 °C. In this study fish were visibly distressed under severe hypoxia, however, there was only one mortality during the O_2 crit tests despite the fact that each individual fish was exposed to DO of ~ 4-5% saturation in order to obtain precise O_2 crit measurements.

As well as measuring 'acute asphyxiation', Butler et al., (2007) investigated the relationship between gill ventilation volume and frequency in response to declining DO for barramundi at 28°C, demonstrating that this species increases ventilation rate down to approximately 15-20% saturation, followed by a steep decline in ventilation rate as DO continued to decline. The maximum ventilation rate observed by Butler et al., (2007) corresponds well with our observed O_2 crit measurements. This suggests increasingly elevated ventilatory requirements to maintain a state of oxygen regulation by the fish under acute hypoxic conditions, that is

followed by a rapid decline in both ventilation rate and oxygen consumption once DO drops below the O_2 crit. It is believed that because of this physiological response barramundi can survive quite low O_2 crit levels for short periods of time, after which they become oxygen conformers and may succumb to low oxygen conditions.

Higher temperatures consistently result in elevated oxygen consumption for teleost fish irrespective of species or life history. This simply reflects increased metabolic requirements at higher temperatures (Fry and Hart 1948; Brett and Groves 1979; Clark et al., 2011). Barramundi in our study elicited a two-fold increase in resting oxygen consumption (Q_{10} = 2.12) over a 10 °C increase in temperature. Higher oxygen consumption at warmer temperatures might be expected to induce a decrease in hypoxia tolerance in all fish species due to the increased oxygen demands of the fish. Results from previous studies on species such as Nile tilapia (*Oreochromis niloticus*; Fernandes and Rantin 1989; Mamun et al., 2013), rainbow trout (*Oncorhynchus mykiss*; Ott et al., 1980) and common carp (*Cyprinus carpio*; Ott et al., 1980), indicate that O_2 crit appears to be less temperature-sensitive than what is typically found for resting oxygen consumption, however, this is not consistent across all teleosts. Species such as Atlantic salmon (*Salmo salar*; Barnes et al., 2011), Doederlein's cardinal fish (*Ostorhinchus doederleini*; Nilsson et al., 2010) and Atlantic cod (*Gadus morhua*; Schurmann and Steffensen 1997), elicit a large increase in O_2 crit with increasing temperature, particularly towards upper thermal tolerance limits. Our results indicate that O_2 crit displays only a small increase for barramundi from typical (26 °C) to high (36 °C) temperatures. This appears to demonstrate the resilient nature of all barramundi sub-populations to the synergistic effects of temperature and environmental hypoxia.

Aside from temperature, a number of other environmental parameters can modify hypoxia tolerance of fish. The ability to adjust to hypoxic conditions through a lowering of O_2 crit following pre-exposure has been reported for the epaulette shark (*Hemiscyllium ocellatum*; Routley et al., 2002) and goldfish (*Carassius auratus*; Fu et al., 2011), although Cook et al., (2011) found no differences in O_2 crit between naïve and hypoxia-conditioned silver sea bream (*Pagrus auratus*). Henriksson et al., (2008) demonstrated that alterations in salinity can increase the O_2 crit by up to 30% in prickly sculpin (*Cottus asper*) acclimated to freshwater, compared with fish adapted to seawater, however, the same trend was not observed in the closely related Pacific staghorn sculpin (*Leptocottus armatus*). Barramundi inhabit environments that are prone to acute and chronic hypoxia, and also experience broad fluctuations in salinity and temperature across their natural range. Currently there is no

understanding of the effects of exposure to repetitive (short term) or chronic (long term) hypoxia on the physiology and consequently performance of barramundi. This topic warrants future research to elucidate more fully the capacity of barramundi to tolerate extreme and variable environments under climate change.

Component C: Parasite surveys and risk analyses

i) Risk assessment to identify the likelihood and consequence of metazoan parasite epizootics in mariculture of barramundi

Parasite species

Metazoan parasites detected on wild and farmed *L. calcarifer* in this study and from previously published records are shown in Table 17. Some parasites could not be identified to species, a result of a combination of factors including limited number of parasite specimens, potentially undescribed parasite species and inability to identify larval parasite life stages definitively. Four new parasite-host records were identified from fish necropsies; *Dollfusiella* sp., *Raphidascaris trichiuri*, *Argulus australiensis*, and Pentastomida (Table 17).

Table 17: Metazoan parasite fauna of wild and farmed marine barramundi *Lates calcarifer* collected in this study and documented in published literature. Museum accession numbers are indicated where known. Abbreviations: SAMA AHC, South Australian Museum Australia, Australian Helminth Collection; C, Crustacean collection; BMNH = British Museum of Natural History; NSMT-Cr, National Museum of Nature and Science, Tokyo; N/g = Not given; N/d = Not determined; NT, Northern Territory; Qld, Queensland; [a]= new host record; [vr] = species validation required; [f] = farmed fish; [w] = wild fish, *= specimen collected by Ben Diggles.

Parasite (Group, Family, *Species*, authority)	**Microhabitat**	**Reference**	**Location**
Myxozoa			
Myxozoa gen et. sp. Indet	Gall bladder	Rückert et al. 2008	Sumatra[f]
Cestoda			
Putative proglotid	Stomach, caeca	Present study Not accessioned	Qld, Australia [w]
Order Tetraphyllidea			
Tetrephyllidea	N/g	Leong and Wong 1992	Sumatra[f]
Tetrephyllidea type 1	Caeca	Present study SAMA AHC 35242	Qld, Australia [w]
Emothrium sp.	N/g	Leong and Wong 1992	Sumatra[f]
Scolex pleuronectis Müller, 1787	Intestine, stomach, pyloric caeca	Rückert et al. 2008	Sumatra[f]
Tentaculariidae			
Nybelinia indica Chandra 1986	Stomach, stomach wall	Rückert et al. 2008	Sumatra[f]
Dasyrhynchidae			
Callitetrarhynchus gracilis (Rudolphi, 1819)	Body cavity, viscera,	Arthur and Ahmed 2002	Bangladesh [w]

	muscle		
	Around intestine and anus	Palm 1995	N/g [w]
Dasyrhynchus indicus Chandra & Rao, 1986	Body cavity, viscera, muscle	Arthur and Ahmed 2002	Bangladesh [w]
Eutetrarhynchidae			
[a]*Dollfusiella* sp.	Intestine	Present study SAMA AHC 35269	Qld, Australia [w]
Gymnorhynchidae			
Gymnorhynchus gigas (Cuvier, 1817)	Body cavity, viscera, muscle	Arthur and Ahmed 2002	Bangladesh [w]
Mustelicolidae			
Bombycirhynchus sphyraenaicum (Pintner, 1930)	Around intestine and gonads	Palm et al. 1998	New Guinea [w]
	Body cavity	Present study SAMA AHC 46153	Qld, Australia [w]
Patellobothrium quinquecatenatum Beveridge & Campbell, 1989	Viscera	Beveridge and Campbell 1989 SAMA AHC18167; BMNH1989.1.24.8-9.	NT, Australia [w]
	Muscle	SAMA collection SAMA AHC26186	NT, Australia [w]
	Around intestine and	Palm 1995	New Guinea [w]

	gonads		
Otobothriidae			
Otobothrium balli Southwell, 1929	N/g	Bilqees 1995	Pakistan [w]
Poecilancistrum caryophyllum (Diesing 1850)	Muscle	Palm 1995	N/g [w]
Pterobothriidae			
Pterobothrium acanthotuncatum Escalente & Carvajal, 1984	Body cavity, mesentery	Campbell and Beveridge 1996	NT, Australia [w]
Pterobothrium lintoni (MacCallum, 1916) (Syn. *Gymnorhynchus malleus* (Linton, 1924)	Body cavity, viscera, muscle	Arthur and Ahmed 2002	Bangladesh [w]
Digenea			
Acanthocolpidae			
Stephanostomum cloacum (Srivastava, 1938)	N/d	Srivastava 1938 (in Bray and Cribb 2003)	Pakistan [w]
	N/d	Saoud et al. 2002	Indian Ocean [w]
Aporocotylidae			
Cruoricola lates Herbert *et al.*, 1994 (Syn. *Cardicola* sp. of Leong & Wong 1986)	Blood vessels	Leong and Wong 1986; 1990	Thailand [f]
	Blood vessels	Herbert et al. 1994	Malaysia [f]
	Blood vessels	Herbert et al. 1994	Qld, Australia [f]
	Blood vessels	Present study Not accessioned	Qld, Australia [w]
Parasanguinicola vastispina Herbert &	Branchial arteries, dorsal	Herbert and Shaharom-	Qld, Australia [f]

Shaharom 1995	aorta, mesenteric venules and renal artery	Harrison 1995	
		Herbert and Shaharom-Harrison 1995	Malaysia[f]
Bucephalidae			
Bucephalus margaritae Ozaki & Ishibashi, 1934	N/g	Leong and Wong 1992	Thailand[f]
		Leong and Wong 1992	Malaysia[f]
Prosorhynchus luzonicus Velasquez, 1959	Intestine	Arthur and Luman-Mayo 1997	Phillipines[w]
Prosorhynchus sp.	Stomach	Rückert et al. 2008	Sumatra [w]
Callodistomidae			
Callodistomum minutus Zaidi & Khan, 1977	N/g	Bilquees 1995	Pakistan[w]
Cryptogonimidae			
Cryptogonimidae gen. sp.	N/g	Leong and Wong 1992	Sumatra[f]
Pseudometadena celebesensis Yamaguti, 1952	Intestine & pyloric caeca	Rückert et al. 2008 ^	Malaysia[f]
		Leong 1997	Indonesia[f]
		Leong and Wong 1990	Thailand[f]
	N/g	Leong and Wong 1992a	Sumatra[f]
		Leong and Wong 1992b	Malaysia[f]
		Present study SAMA AHC 35247	Qld[w]

Pseudometadena sp.	N/d	Arthur and Lumanlan-Mayo 1997	Phillipines[w]
Lecithochirium sp.		Arthur and Lumanlan-Mayo 1997	Phillipines[w]
Hemiuridae			
Hemiuridae gen. sp.	Intestine	Arthur and Ahmed 2002	Bangladesh [w]
Ectenurus sp.	N/g	Leong and Wong 1990	Thailand[f]
	N/g	Leong and Wong 1992b	Malaysia[f]
Erilepturus hamati (Yamaguti, 1934) Manter, 1947	N/g	Bray et al. 1993	Phillipines[N/g]
	Stomach	Bray et al. 1993	NT, Australia[w]
	Stomach, intestine	Present study SAMA AHC 35243-46	Qld, Australia[w]
	Stomach	Velasquez, 1962; Leong and Wong 1990	Phillipines
[vr]*Lecithocladium grandulosum*	N/g	Leong and Wong 1990	Malaysia[f]
[vr]*Lecithocladium neopacificum*	N/g	Leong and Wong 1990	Thailand[f]
Lissorchiidae			
Complexobursa magna Bilqees, 1980	N/g	Bilquees 1995	Pakistan [w]
Psilostomidae			
Psilostomum sp. (metacercaria)	Intestine	Arthur and Ahmed 2002	Bangladesh [w]
Transversotrematidae			
Prototransversotrema steeri (Angel, 1969)	Beneath scales	Cribb et al. 1992	Qld, Australia [w]

	Beneath scales	Rodgers and Burke 1988	Qld, Australia [w]
Transversotrema patialense (Soparkar, 1924) (Syn. *T. laruei* Velasquez)	Beneath scales	Cribb et al. 1992	Phillipines[w]
Monogenea			
Capsalidae			
Benedenia sp.	Body surface	Leong 1997	Indonesia[f]
	Body surface	Leong 1997	Malaysia[f]
		Lim 1998	Indonesia
		Lim 1998	Malaysia
Benedenia epinepheli (Yamaguti, 1937) Meserve, 1938	Gills, body surface	Rückert et al. 2008	Sumatra[f]
Capsalid gen. Et sp. Indet	Gills, body surface	Rückert et al. 2008	Sumatra[f]
Neobenedenia sp.	Body surface	Present study** SAMA AHC 35006–11	Hinchinbrook, Qld, Australia[f]
	Body surface	Hutson et al. 2012** SAMA AHC 35240-41	James Cook University, Qld, Australia[f]
	Body surface	Hutson et al. 2012** SAMA AHC 35461	Bowen, Qld, Australia[f]
Neobenedenia melleni (MacCallum, 1927) Yamaguti, 1963**	Gills, body surface	Deveney et al. 2001 ** QM: G218281–300	Qld, Australia[f]
		Rückert et al. 2008	Sumatra[f]
Diplectanidae			

Diplectanidae gen. sp.	N/g	Leong and Wong 1992a	Sumatra[f]
Diplectanum sp.		Leong and Wong 1990	Thailand[f]
Laticola	Gills	Present study	James Cook University, Qld, Australia[f]
	Gills	Present study	Cleveland Bay
Diplectanum narimeen Unnithan, 1964	Gills	Tingbao et al. 2006	N/g
Diplectanum penangi Laing & Leong, 1991	Gills	Tingbao et al. 2006	China[f]
		Leong and Wong 1992b	Malaysia[f]
			Thailand
Diplectanum setosum Nagibina, 1976	Gills	Tingbao et al. 2006	N/g
Laticola lingaoensis Tingbao *et al.,* 2006	Gills	Tingbao et al. 2006	China[f]
Laticola latesi Tripathi, 1957 (syn. *Diplectanum latesi*;syn. *Pseudorhabdosynochus latesi*)	Gills	Tingbao et al. 2006	China[f]
			India
	N/g	Leong and Wong 1990	Thailand[f]
	N/g	Leong and Wong 1992a	Sumatra[f]
	N/g	Leong and Wong 1992b	Malaysia[f]
Laticola paralatesi Nagibina, 1976 (syn. *Diplectanum latesi*)	Gills	Tingbao et al. 2006	China[f], NT
Pseudorhabdosynochus epinepheli Yamaguti 1938	Gills	Rückert et al. 2008	Sumatra[f]

	Gills	Leong and Wong 1990	Malaysia[f]
	Gills	Leong and Wong 1990	Thailand[f]
	Gills	Leong and Wong 1990	Phillipines[f]
Pseudorhabdosynochus lantauensis Beverley-Burton & Suriano, 1981	Gills	Rückert et al. 2008	Sumatra[f]
Pseudorhabdosynochus monosquamodiscusi Balasuriya & Leong, 1995	Not specified	In : Tingbao et al. 2006	N/g
Nematoda			
Filocapsurine (?) see NHM database			
Anisakidae			
Anisakis sp.	N/g	Leong and Wong 1990	Thailand[f]
Hysterothylacium sp.	Intestine, liver, mesenteries, pyloric caeca	Rückert et al. 2008	Sumatra[f]
Raphidascaris sp.	Stomach wall	Rückert et al. 2008	Sumatra[f]
	N/g	Leong and Wong 1992	Sumatra[f]
	N/g	Leong and Wong 1990	Thailand[f]
	N/g	Leong and Wong 1990	Malaysia[f]
	N/g	Leong and Wong 1992b	Malaysia[f]
Raphidascaris sp. II	Intestine	Rückert et al. 2008	Sumatra[f]
[a]*Raphidascaris trichiuri*		Present study	Qld, Australia [w]
Terranova sp.	Intestine, liver, mesenteries, pyloric	Rückert et al. 2008	Sumatra[f]

	caeca		
Camallanoidea			
Procamallanus sparus Akram, 1975		Akram 1995	Pakistan[w]
Dracunculoidea			
Philometra lateolabracis (Yamaguti, 1935)	Gonads	Moravec et al. 1988	Somalia, Indian Ocean[w]
Gnathostomatoidea			
Echinocephalus sp.		Akram 1995	Pakistan[w]
Echinocephalus uncinatus Molin, 1858		Gibson et al. 2005	Arabian Sea[w]
Seuratoidea			
Cucullanus hians Dujardin, 1845	Intestine	Akram 1994	Pakistan[w]
		Akram 1995	Pakistan[w]
Cucullanus sp.		Akram 1995	Pakistan[w]
Indocucullanus calcariferii Zaidi & Khan,1975		Arya 1991	Indian Ocean[w]
	N/g	Bilquees 1995	Pakistan[w]
Indocucullanus longispiculum Khan, 1969	N/g	Bilquees 1995	Pakistan[w]
Acanthocephala			
Rhadinorhynchidae			
Acanthoocephalus sp.	N/g	Leong and Wong 1992	Malaysia[f]
Serrasentis sagittifer (Linton, 1889)Van Cleave, 1923	Mesenteries	Rückert et al. 2008	Sumatra[f]

	Intestine	Arthur and Ahmed 2002	Bangladesh[N/g]
Tenuiprobsocis sp.	Intestine	Sanil et al. 2011	India[w]
Hirudinea			
Piscicolidae			
Zeylanicobdella arugamensis	Body surface	Kua et al. 2010	Malaysia[f]
Branchiura			
Argulidae			
[a]*Argulus australiensis*	Body surface	Present study SAMA C7644	Qld, Australia [w]
Copepoda			
Caligidae			
Caligus sp.	N/g	Leong and Wong 1992	Sumatra[f]
Caligus sp.	N/g	Muhd-Faizul et al. 2012	Malaysia[f]
Caligus epidemicus Hewitt, 1971	N/g	Johnson et al. 2004b	Thailand[f]
	Body surface, gill cavities	Venmathi Maran et al. 2009 NSMT-Cr 20393-36	Malaysia[f]
	Body surface, gill and oral cavities	Ho and Lin (2004)	Taiwan[f]
	Not specified	Muhd-Faizul et al. 2012	Malaysia[f]
	Body surface	Present study SAMA C7028	Qld, Australia [w]
C. chiastos Lin & Ho, 2003	Not specified	Muhd-Faizul et al. 2012	Malaysia[f]
	Body surface	Present study	Qld, Australia [w]

		SAMA C7028-29	
C. orientalis Gusev, 1951 (syn. *C. communis* and *C. laticorpus*)	Body surface	Ho and Lin 2004	Taiwan[f]
C. pagrosomi Yamaguti 1939	Gills filaments & oral cavity	Ho and Lin 2004	Taiwan[f]
C. punctatus Shiino, 1955	Body surface	Ho and Lin 2004	Taiwan[f]
	Body surface & gill cavities	Venmathi Maran et al. 2009	Malaysia[f]
	N/g	Muhd-Faizul et al. 2012	Malaysia[f]
C. rotundigenitalis Yü, 1933	Gills, gill cavities & body surface	Ho and Lin 2004	Taiwan
	N/g	Muhd-Faizul et al. 2012	Malaysia[f]
Lernanthropidae			
Lernanthropus latis Yamaguti, 1954	Gill filaments	Ho and Kim 2004a	Gulf of Thailand[w]
	Gill filaments	Leong and Wong 1987	Thailand
	Gill filaments	Tripathi 1962	India
	Gill filaments	Pillai 1985	Sri Lanka
	Gill filaments	Kua et al. 2012	Malaysia[f]
	Gill filaments	Small et al. 2009	NT, Australia[f]
	Gill filaments	Brazenor and Hutson 2013 SAMA C7025	WA, Australia[f]
	Gill filaments	Brazenor and Hutson 2013	Qld, Australia[f]

		SAMA C7026	
	Gill filaments	Brazenor and Hutson 2013 SAMA C7027	Qld, Australia [w]
[vr] *Lernanthropus kroyeri*	Gills	Vinoth et al. 2010	India [w]
Isopoda			
Cymothoidae			
Aegathoa sp.	N/g	Leong and Wong 1990	Thailand[f]
Cymothoa sp.	N/g	Leong and Wong 1990	Thailand[f]
Cymothoa indica Schioedte & Meinert, 1884	Body surface	Rajkumar 2005	India[f]
Nerocila barramundae Bruce 1987	Dorsal fin	Bruce 1987 QM WI1008	Qld, Australia [w]
Rocinela latis Southwell 1915	Skin	Southwell 1915	Calcutta, India [w]
Pentostomatoida			
[a]Pentastomida	Encysted, body cavity	Present study SAMA AHC 46154	Qld, Australia [w]

^Incorrect spelling in Rückert et al., 2008 (as *celebensis*); **Likely to be the same taxon, requires further study.

Risk assessment for mariculture of Lates calcarifer

Risk analyses were performed for 61 parasites that infect *L. calcarifer* (Table 18). Monogenean, aporocotylid trematode, copepod and isopod species presented an extreme likelihood of establishment and proliferation (Table 18). The consequence of parasite establishment and proliferation ranged from negligible to high. No taxon was classed as an overall extreme risk. Capsalid monogeneans, *Neobenedenia* spp. and *Benedenia* spp., and the leech, *Zeylanicobdella arugamensis*, were determined to present a high risk for *L. calcarifer* mariculture (Table 18).

Metazoan parasite risks to Lates calcarifer mariculture

This study provides a comprehensive synthesis of marine metazoan parasite infections of *L. calcarifer* and identifies species of negligible to high risk for mariculture. Nevertheless, the rigor of parasite risk analyses is reliant on the accuracy and availability of parasite-host records and knowledge of the potential impact of parasites in culture environments. Four new parasite-host records were determined in this study, which highlights the need for ongoing surveillance of wild and captive fishes. Care should be taken in the interpretation of any risk analysis because low and seasonal sample sizes can underestimate diversity, especially for parasites that exhibit low prevalence. The likelihood of parasite establishment in mariculture is also dependent on the farm location, quarantine protocols, system design and the distribution of parasites (including geographic distribution and host-specificity). As new information on distributions in wild and farmed fish populations becomes available, assessments of risks in specific regions or aquaculture facilities can be better informed.

Information on metazoan parasite species infecting wild organisms that associate with mariculture is largely unavailable. Consequently, this risk assessment was unable to account for risk presented by generalist metazoan parasite species, or species that may be capable of host switches. Information on host-specificity changes rapidly and some parasite species previously considered host-specific have been found to attach to a wide range of hosts under experimental conditions (e.g. King and Cable 2007). Emergence of new parasites in mariculture can also occur from free-living organisms, never previously reported from wild fish (see Nowak 2007). This is of considerable importance for diadromous fishes, including *L. calcarifer*, whose broad environmental tolerance enables them to be cultured in diverse conditions, including polyculture (Muhd-Faizul et al., 2012; Guy et al., 2010), which can create opportunities for host-switching.

High risk

The monogeneans *Neobenedenia melleni* and *Benedenia epinepheli* are notorious, allegedly generalist pathogens of tropical and subtropical fishes in aquaria and aquaculture worldwide. Both species attach to the skin of their host and graze on skin cells, continuously laying eggs into the water which hatch into ciliated larvae that directly re-infect fish. High infection intensities on fish lead to secondary infections by bacteria, ultimately resulting in emaciation and death. In Indonesia and Australia *Neobenedenia* infections have been associated with large and significant fish losses (Rückert et al., 2008; Deveney et al. 2001). Outbreaks of *B. epinepheli* have been recorded in aquaculture on at least six species of fish and on 18 species in aquaria (see Whittington et al., 2001 for review).

Whittington (2004; 2005) has hypothesized that *Neobenedenia* '*melleni*' and *N*. '*girellae*', the two species widely attributed globally to pathogenic infections in aquaria and aquaculture, may each be a complex of several species. Furthermore, the source of *Neobenedenia* outbreaks in maricultured fish is mostly unknown (Whittington and Chisholm 2008) and this applies to the *N*. '*melleni*' infection of barramundi in Queensland, Australia (Deveney et al., 2001; present study, Table 17). Moreover, *B. epinepheli* is only recorded in the wild from two host species (Whittington et al., 2001). These reports emphasize the importance of broader studies to determine specific parasite identities and their host range because it can influence husbandry practices and farm location.

Caligids present a predominant threat to aquaculture due to their broad distribution, direct life cyle and low host specificity. *Caligus epidemicus* has been associated with mass mortality of mullet (Mugilidae) and porgies (Sparidae) in Australia (Hewitt 1971) and Taiwan (Lin 1996). This species is known from sea caged *L. calcarifer* in Malaysia (Venmathi Maran et al., 2009; Muhd-Faizul et al., 2012) and was found infecting wild fish from Queensland in the present study (Table 17). In view of the parasites' direct life cycle and potential for mortality and disease outbreaks, *C. epidemicus* presents a high risk for sea-cage aquaculture of *L. calcarifer* (Table 5). *Caligus chiastos* is also known from from sea-caged *L. calcarifer* in Malaysia (Muhd-Faizul et al., 2012) and was found to infect wild *L. calcarifer* in Queensland (present study, Table 17). This species has been associated with epizootics in *T. maccoyii* sea cage culture with gross corneal damage (Hayward et al., 2008; Table 17).

Three cymothoid isopod parasite species infect wild and/or farmed *Lates calcarifer* (Table 17). *Cymothoa indica* is believed to have been introduced to hatchery reared fish through wild

zooplankton used as feed in India where infections in the branchial and anterodorsal regions resulted in skin lesions and were associated with lowered growth rates and mortality (Rajkumar et al., 2005).

Heavy infestations of the leech *Zeylanicobdella arugamensis* have been reported from moribund *L. calcarifer* fingerlings reared in sea cages (Kua et al., 2010). We propose that severe infestations may render fish unmarketable due to unsightly leeches, frayed fins, haemorrhages and swelling at attachment and feeding sites (Cruz-Lacierda et al., 2000; Kua et al., 2010). Leeches can also serve as a vector for other parasites and pathogens (Burreson, 1995). In view of the parasites' direct life cycle and potential for mortality and disease outbreaks, *Z. arugamensis* presents a high risk for sea-cage aquaculture of *L. calcarifer* (Table 5). Cruz-Lacierda et al., (2000) found a 50 ppm formalin bath treatment effective in managing the leech and suggested drying culture facilities to desiccate leech cocoons.

Moderate risk

At least 10 diplectanid monogenean species (including *Diplectanum* spp., *Laticola* spp. and *Pseudorhabdosynochus* spp.) infect maricultured *Lates calcarifer* (Table 4). Infected fish exhibit a darkened body, pale gills, lethargy, loss of appetite and excess mucus production (Leong et al., 2006). Leong and Wong (1990a) recorded that a large proportion of diseased *L. calcarifer* were infected with *Laticola latesi* (as *Pseudorhabdosynochus latesi*) and *Diplectanum* sp. Until more is known about the specifics of parasite attachment, any associated pathology and fecundity, these diplectanid genera are considered moderate risks.

Blood flukes (Aporocotylids) can be problematic in aquaculture because their intermediate invertebrate host may inhabit areas close to farmed fish, such as on cage structures or nearby sediment (e.g. Cribb et al., 2011), and infection of the definitive host by emerging cercariae is direct (Table 18). *Cruoricola lates* infects farmed *L. calcarifer* in Malaysia, Thailand and Australia (Herbert et al., 1994; present study, Table 4) and *Parasanguinicola vastispina* infects cultured fish in Malaysia (Herbert and Shaharom-Harrison 1995; Table 17). Although these genera have no known pathology, other aporocotylid species have been associated with mortalities of farmed amberjacks (*Seriola dumerili*) in the Spanish Mediterranean (Crespo et al., 1992) and Japan (Ogawa and Fukudome 1994) and in farmed southern bluefin tuna (*Thunnus maccoyii*) in Australia (Hayward et al., 2010).

Argulus spp. are problematic ectoparasites in freshwater and marine fish culture and can cause mortality in farmed salmonids (Boxshall 2005; Schram et al., 2005; Table 17). Argulids attach

to fish skin by means of suckers and spines and feed on blood and external tissues (Boxshall 2005). Infections may enhance severity of bacterial infections (Bandilla et al., 2006; Cusack and Cone 1986). We provide a new host record for *A. australiensis* (Table 17), which has been documented once previously from the sparid, *Acanthopagrus berda* (see Byrnes 1985).

The haematophagous copepod, *Lernanthropus latis*, is of concern in brackish pond culture and sea cage culture (Kua et al., 2012; Brazenor and Hutson 2013; present study, Table 17). *Lernanthropus latis* has not yet been associated with significant fish mortality events, although their presence is usually associated with poor fish health including lacerated tissue, erosion and necrosis of secondary gill lamellae (Kuo and Humphrey 2008; Kua et al., 2012). In the northern hemisphere, *Lernanthropus kroyeri* infections of European seabass (*Dicentrarchus labrax*) and Grey Snapper (*Lutjanus griseus*) have resulted in mortality (Manera and Dezfuli 2003; Henry et al., 2009; Table 18).

Table 18: Likelihood, consequence and risk of parasite species establishment and proliferation in *Lates calcarifer* mariculture. Parasites were scored for four consequence criteria, (denoted with an 'x') including: Previous mortality in aquaculture = Mo; Potential pathology or disease = Pa; Potential negative impact on marketability/consumer acceptance = Ma; and potential negative impact on consumer health = Ch. [vr] = validation required; *See Discussion for comment.

Parasite taxa	Likelihood	Mo	Pa	Ma	Ch	Consequence	Risk
Cestoda							
Scolex pleuronectis	Low	-	-	-	-	Negligible	Negligible
Nybelinia indica	Low	-	-	-	-	Negligible	Negligible
Callitetrarhynchus gracilis	Low	-	-	x	-	Low	Negligible
Dasyrhynchus indicus	Low	-	-	x	-	Low	Negligible
Dollfusiella sp.	Low	-	-	-	-	Negligible	Negligible
Gymnorhynchus gigas	Low	-	-	x	-	Low	Negligible
Bombycirhynchus sphyraenaicum	Low	-	-	-	-	Negligible	Negligible
Patellobothrium quinquecatenatum	Low	-	-	x	-	Low	Negligible
Otobothrium balli	Low	-	-	-	-	Negligible	Negligible
Poecilancistrum	Low	-	-	x	-	Low	Negligible

caryophyllum							
Pterobothrium acanthotuncatum	Low	-	-	-	-	Negligible	Negligible
Pterobothrium lintoni	Low	-	-	x	-	Low	Negligible
Digenea							
Stephanostomum cloacum	Low	-	x	-	-	Low	Negligible
Cruoricola lates	Extreme	x*	x*	-	-	Moderate	Moderate
Parasanguinicola vastispina	Extreme	x*	x*	-	-	Moderate	Moderate
Prosorhynchus sp.	Low	-	-	-	-	Negligible	Negligible
Prosorhynchus luzonicus	Low	-	-	-	-	Negligible	Negligible
Callodistomum minutus	Low	-	-	-	-	Negligible	Negligible
Pseudometadena celebesensis	Low	-	-	-	-	Negligible	Negligible
Pseudometadena sp.	Low	-	-	-	-	Negligible	Negligible
Lecithochirium sp.	Low	-	-	-	-	Negligible	Negligible
Erilepturus hamati	Low	-	-	-	-	Negligible	Negligible
Complexobursa magna	Low	-	-	-	-	Negligible	Negligible
Prototransversotrema steeri	Moderate	-	-	-	-	Negligible	Negligible
Transversotrema patialense	Moderate	-	-	-	-	Negligible	Negligible
Monogenea							
Benedenia sp.	Extreme	x	x	x	-	High	High
Benedenia epinepheli	Extreme	x	x	x	-	High	High
Neobenedenia sp.	Extreme	x	x	x	-	High	High
Neobenedenia melleni	Extreme	x	x	x	-	High	High
Diplectanum sp.	Extreme	-	x	-	-	Low	Moderate
Diplectanum narimeen	Extreme	-	x	-	-	Low	Moderate
D. penangi	Extreme	-	x	-	-	Low	Moderate
D. setosum	Extreme	-	x	-	-	Low	Moderate
Laticola lingaoensis	Extreme	-	x	-	-	Low	Moderate
L. latesi	Extreme	-	x	-	-	Low	Moderate
L. paralatesi	Extreme	-	x	-	-	Low	Moderate
Pseudorhabdosynochus epinepheli	Extreme	-	x	-	-	Low	Moderate

P.lantauensis	Extreme	-	x	-	-	Low	Moderate
P.monosquamodiscusi	Extreme	-	x	-	-	Low	Moderate
Nematoda							
Hysterothylacium sp.	Low	-	-	-	x	Low	Negligible
Raphidascaris sp.	Low	-	-	-	x	Low	Negligible
Raphidascaris sp. II	Low	-	-	-	x	Low	Negligible
Raphidascaris trichiuri	Low	-	-	-	x	Low	Negligible
Terranova sp.	Low	-	-	-	x	Low	Negligible
Acanthocephala							
Serrasentis sagittifer	Low	-	-	-	-	Negligible	Negligible
Branchiura							
Argulus australiensis	High	x	x	-	-	Moderate	Moderate
Copepoda							
Caligus sp.	Extreme	x*	x*	-	-	Moderate	Moderate
Caligus epidemicus	Extreme	x	x*	-	-	Moderate	Moderate
C. chiastos	Extreme	x	x	-	-	Moderate	Moderate
C. orientalis	Extreme	x	x*	-	-	Moderate	Moderate
C. pagrosomi	Extreme	x	-	-	-	Low	Moderate
C. punctatus	Extreme	x	-	-	-	Low	Moderate
C. rotundigenitalis	Extreme	x	-	-	-	Low	Moderate
Lernanthropus latis	Extreme	x*	x	-	-	Moderate	Moderate
Isopoda							
Aegathoa sp.	Extreme	x*	x	-	-	Moderate	Moderate
Cymothoa sp.	Extreme	x*	x	-	-	Moderate	Moderate
Cymothoa indica	Extreme	x	x	-	-	Moderate	Moderate
Nerocila barramundae	High	-	x*	-	-	Low	
Rocinela latis	High	-	x*	-	-	Low	
Pentastomatoida							
Species not identified							
Hirudinella							
Zeylanicobdella arugamensis	Extreme	x	x	x	-	High	High

Parasitic arthropods, monogeneans, aporocotylid trematodes and leeches present the greatest likelihood of establishment and proliferation in mariculture because invasive stages directly infect their host. Ectoparasites *Benedenia epinepheli* and *Neobenedenia melleni* multiply rapidly in high-density aquaculture environments because eggs are often retained in the system, leading to high reinfection rates. These species are the most likely to threaten sustainability of the mariculture industry. Effective parasite management focuses on reducing stress, preventing introduction of pathogens and parasites, and use of effective drugs and vaccines where available. Understanding of the source and transmission of moderate to high-risk parasite species will determine and refine proactive management strategies.

ii) The effects of temperature and salinity on the life cycle of high risk parasites

Time to first and last hatch and hatching success of Neobenedenia

Optimal environmental conditions for *Neobenedenia* hatching success were between 24 and 28 °C at high salinities (40‰) (Figure 18, 19A). These parameters also resulted in rapid hatching completion (Figure 19C). Time to first hatch of *Neobenedenia* eggs was shorter in warmer temperatures (4 days at 24–32 °C) compared to the lowest and highest temperatures tested (7 days at 22 °C in 40‰; 9 days at 34 °C in 22‰) (Figure 19; Table 20). The greatest range of days from first to last hatch was observed at 30 °C in 22‰ salinity (4–9 days) (Figure 19; Table 20). *Neobenedenia* eggs did not hatch in low salinity (0 and 11‰). Hatching success was highest (> 81%) in 26 and 28 °C treatments in seawater and hypersaline solutions (35 and 40‰) (Figure 18A). The highest hatching success was observed at 26 °C in seawater (35‰; 91 ± 3% SE). Hatching was significantly lower (0% and < 5% hatching in 35 and 40‰ respectively) at 34 °C in seawater and hypersaline solutions (35 and 40‰) compared to all other temperatures at those salinities. Hatching was significantly influenced by temperature (PERMANOVA, Pseudo-F6, 175 =39.08, $P < 0.0001$) and salinity (PERMANOVA, Pseudo-F4, 175 =424.87, $P < 0.0001$) (Figure 18A). The interaction between temperature and salinity on the hatching of *Neobenedenia* eggs was also significant (PERMANOVA, Pseudo-F24, 175 =15.92, $P < 0.0001$).

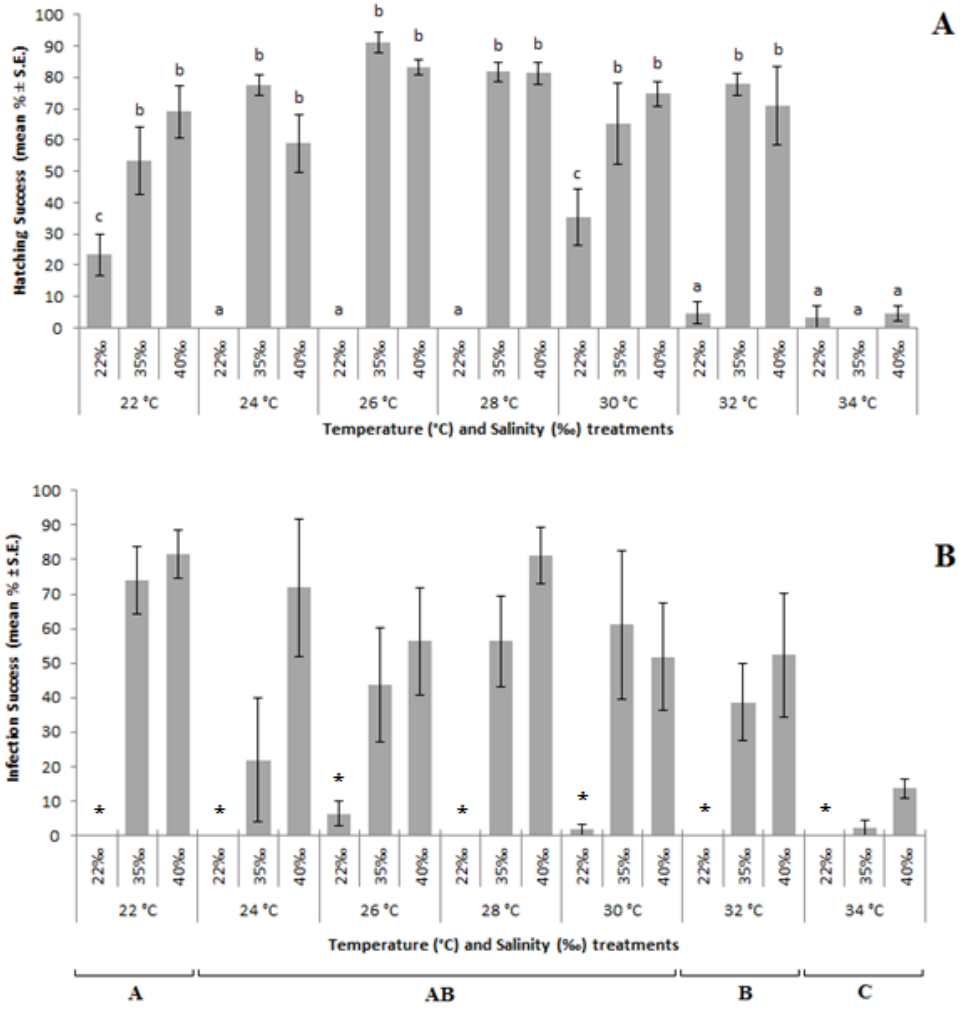

Figure 18: (A) *Neobenedenia* sp. hatching success in temperature and salinity treatments (0 and 11‰ not shown; no hatching observed). 'a', 'b', and 'c' = differences between pairs of means determined using PERMANOVA pairwise comparison test. $P < 0.05$. (B) *Neobenedenia* sp. infection success in temperature and salinity treatments; 'A', 'AB', 'B' and 'C'= differences between temperatures. '*'= differences between pairs of means for salinity determined using PERMANOVA pairwise comparison test $P < 0.05$.

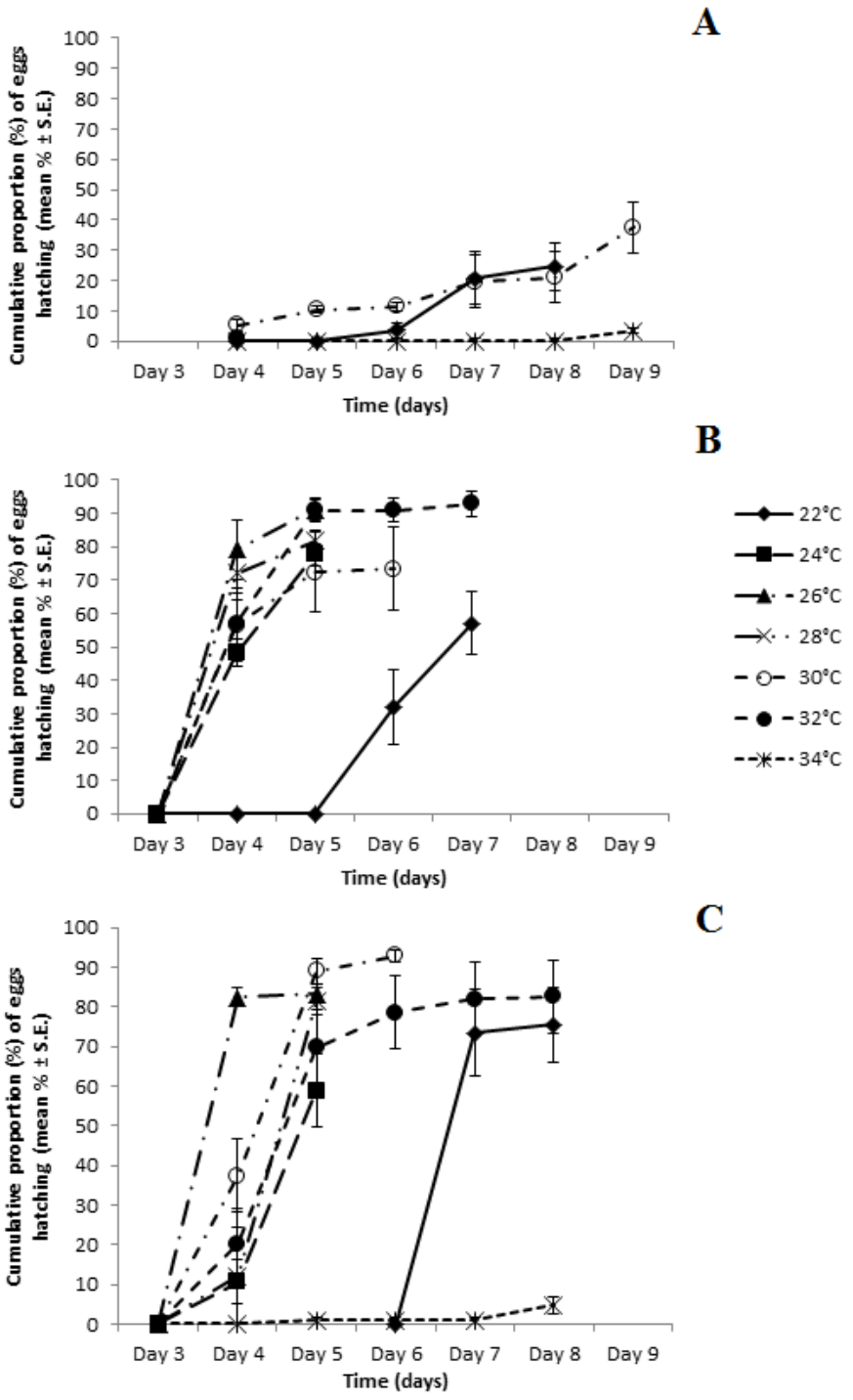

Figure 19: Cumulative proportion of hatched *Neobenedenia* at 22‰ (A), 35‰ (B) and 40‰ (C). Day of last hatch is indicated where data points finish. Error bars indicate SE.

Hatching success and time to first and last hatch of Lernanthropus latis

Eggs hatched in all water temperature treatments (Figure 20). Hatching success was greatest at 30 and 32 °C in 35 ‰ (98 and 92% success, respectively; Figure 20). Hatching did not occur at 0 ‰. Successful hatching only occurred above 22 ‰ with the exception of low survival (1.6%) occurring in one instance in 11 ‰ at 32 °C (Figure 20). Hatching success was greatest at 35 ‰ in all water temperatures with the exception of 34 °C where hatching was highest in the 22 ‰ solution (Figure 20). Hatching began within six hours in all water temperatures with >95% of eggs hatched by 30 h at 30 °C, 32 °C and 34 °C while >95% of eggs hatched by 60 h at 22 °C (Figure 21). There was a significant interaction between water temperature and salinity on the hatching of *Lernanthropus latis* nauplii (pseudo-F[12, 176]=2.13, p=0.02).

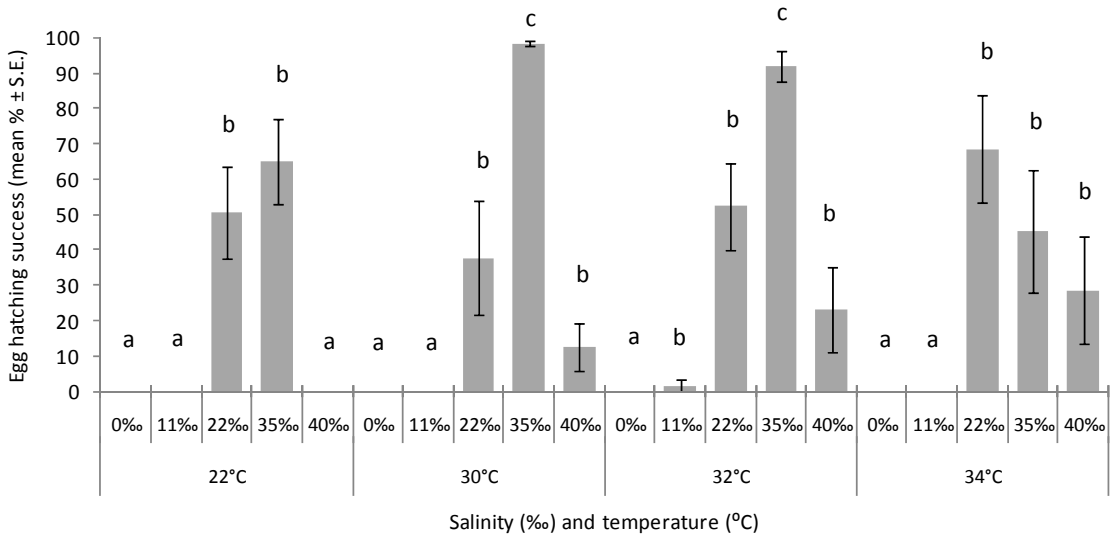

Figure 20: *Lernanthropus latis* egg hatching success in salinity and temperature treatments; 'a', 'b' and 'c' = statistically significant differences between pairs of means determined using PERMANOVA pairwise comparison test $P < 0.05$.

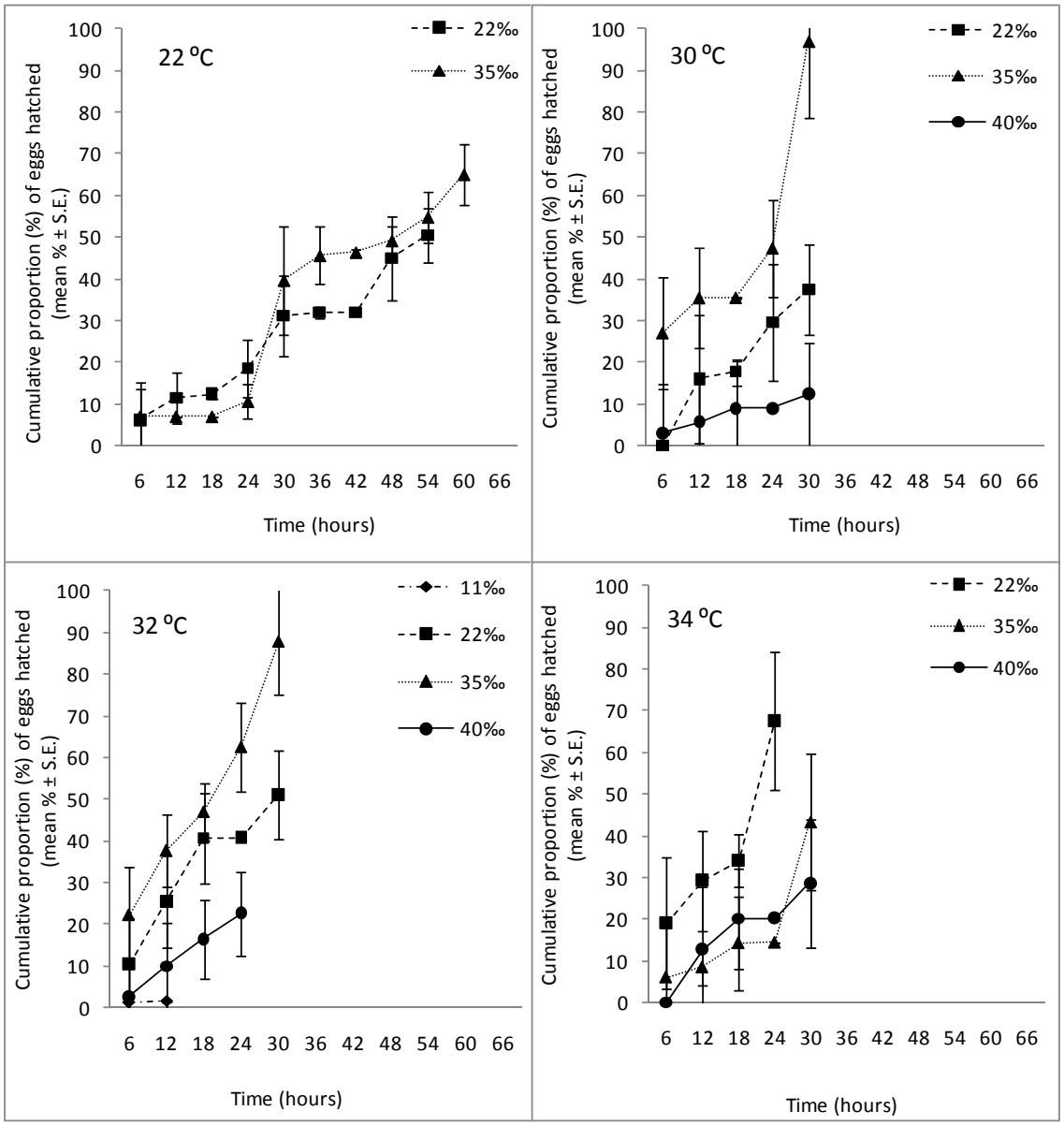

Figure 21: Cumulative mean proportion of successfully hatched *Lernanthropus latis* eggs at temperature and salinity treatments. Lines terminate at the time (h) when 95% of eggs had hatched. (0 ppt and 11ppt at 22 °C, 30 °C and 34 °C not shown as no hatching was observed).

Oncomiracidial longevity

Optimal environmental conditions for maximal longevity of *Neobenedenia* oncomiracidia was 22 °C at high salinities (40‰). Oncomiracidia survived for significantly longer periods of time (49 h) in these conditions (Figure 22). Oncomiracidia did not live longer than 12 hours at temperatures warmer than 28 °C or more than six hours at low salinities (22‰). Longevity

was significantly influenced by temperature (PERMANOVA, Pseudo-F6, 105 =186.24, P < 0.0001) and salinity (PERMANOVA, Pseudo-F2, 105 =391.35, P < 0.0001) (Figure 22). The interaction between temperature and salinity on the longevity of *Neobenedenia* oncomiracidia was also significant (PERMANOVA, Pseudo-F12, 105 =53.57, P < 0.0001).

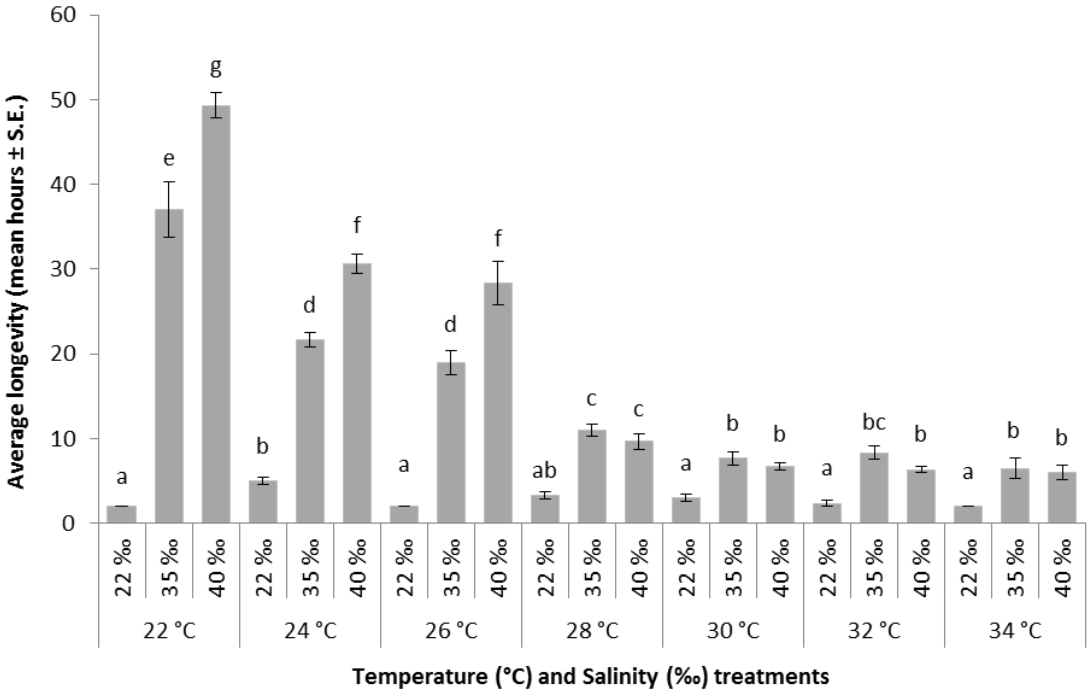

Figure 22: (A) *Neobenedenia* sp. oncomiracidial longevity in temperature and salinity treatments. Letters above columns = differences between pairs of means determined using PERMANOVA pairwise comparison test P < 0.05.

Infection success and time to sexual maturity

Optimal environmental conditions for *Neobenedenia* oncomiracidia infection success was at 22 °C in high salinities (40‰) (Figure 23b). In contrast, warm temperatures (30–34 °C) in seawater (35‰) resulted in the most rapid sexual maturation. Parasites reached sexual maturity in six days at warmer compared to 12 at cooler temperatures (Figure 23b). The highest infection success was observed at 22 °C in 35 and 40‰ solutions (> 73% and > 81% respectively) (Figure 23b). The lowest infection success at each temperature was observed in 22‰. Infection was only detected in this salinity at 26 °C (6.25%) and 30 °C (1.66%) (Figure 23b). Infection success was significantly influenced by temperature (PERMANOVA, Pseudo-

F6, 64 =4.61, P = 0.0006) and salinity (PERMANOVA, Pseudo-F2, 64 =41.34, P < 0.0001). No significant interaction between temperature and salinity on infection success of *Neobenedenia* was identified (PERMANOVA, Pseudo-F6, 64 =1.66, P = 0.103).

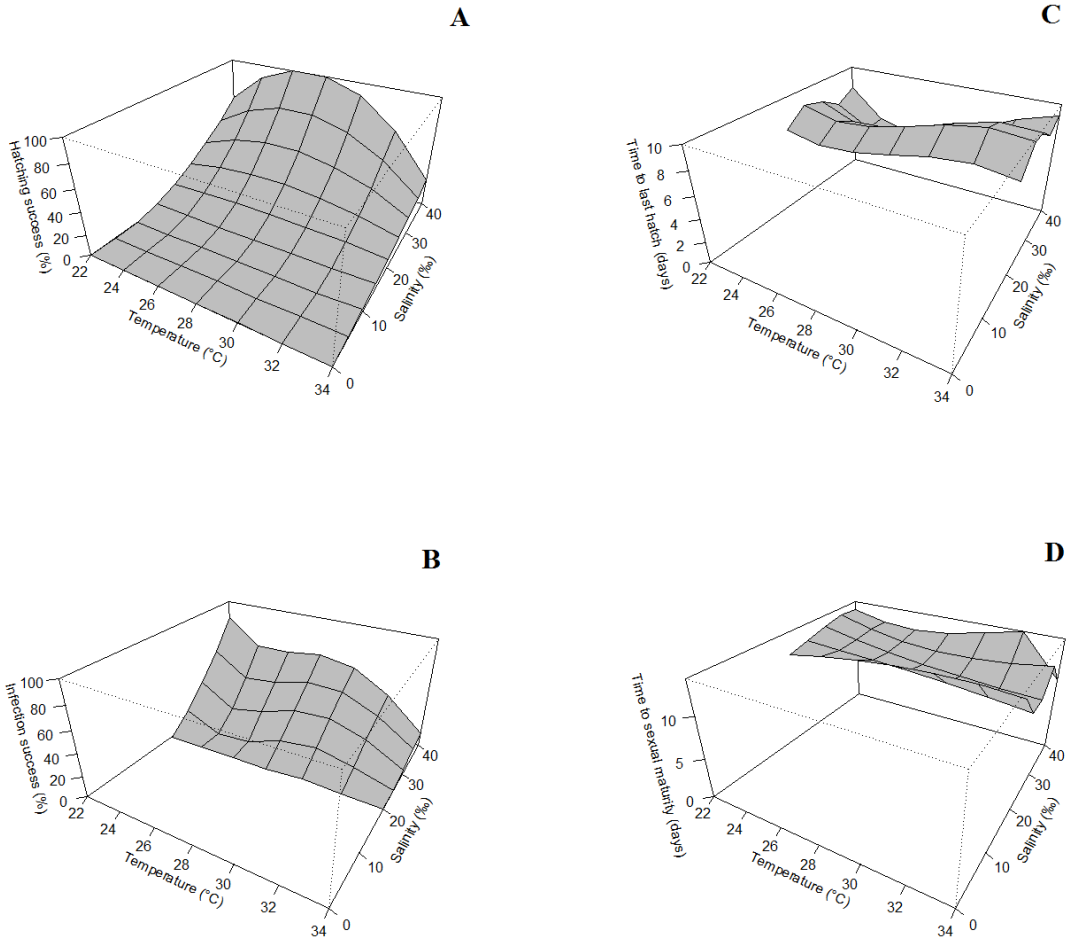

Figure 23: General linear models describing the relationship between temperature, salinity and (a) hatching success, (b) infection success, (c) time to last hatch and (d) time to sexual maturity.

Effect of temperature and salinity on size of Neobenedenia

Parasites were significantly larger at sexual maturity in warmer (30 and 32 °C) compared to cooler temperatures (22 °C) (Table 19). Temperature had a significant effect on all morphological characters measured including total length (ANOVA, F5, 199 =5.119, P < 0.0001), total width (ANOVA, F5, 199 =21.091, P < 0.0001), anterior hamulus length

(ANOVA, F5, 199 =18.358, P < 0.0001) and accessory sclerite length (ANOVA, F5, 199 =35.847, P < 0.0001) (Figure 24a,b). Salinity did not significantly influence size at maturity. No significant interaction was identified between temperature and salinity on morphological characters.

Table 19: Comparative measurements of *Neobenedenia* sp. infecting *Lates calcarifer.* Measurements in micrometres (µm); mean followed by range in parentheses; '-' indicates no measurement given as no infecting parasites were observed.

Temperature (°C)	Salinity (‰)	Sample size (n)	Length (µm)	Width (µm)	Anterior hamulus length (µm)	Accessory sclerite length (µm)
22	22	-	-	-	-	-
	35	6	834 (464-2056)	312 (133-964)	95 (55-198)	49 (23-136)
	40	17	1081 (699-1788)	425 (187-754)	113 (69-167)	56 (41-98)
24	22	-	-	-	-	-
	35	6	1834 (1087-2099)	905 (502-1082)	185 (130-234)	78 (45-97)
	40	23	1899 (1358-2586)	956 (605-1445)	174 (119-233)	81 (53-114)
26	22	2	2804 (2413-3194)	1224 (1188-1260)	181 (155-206)	112 (100-124)
	35	6	1461 (1228-1676)	647 (490-847)	133 (128-142)	65 (47-84)
	40	13	1739 (1278-2170)	777 (553-1028)	162 (118-217)	67 (40-102)
28	22	-	-	-	-	-
	35	9	1591 (1228-1850)	707 (558-847)	147 (126-189)	69 (47-101)
	40	26	1167 (611-1794)	592 (258-876)	122 (53-194)	59 (19-94)
30	22	4	2952 (2793-3277)	1738 (1622-1836)	249 (219-264)	139 (125-160)
	35	18	1715 (1498-2858)	855 (170-1545)	161 (65-266)	101 (39-177)
	40	25	1956 (1065-2625)	917 (397-1587)	174 (115-258)	110 (69-156)
32	22	-	-	-	-	-
	35	16	2013 (999-2672)	1049 (427-1631)	187 (111-254)	116 (46-163)
	40	38	2078 (1079-3124)	1029 (424-2347)	188 (111-340)	114 (59-184)
34	22	-	-	-	-	-
	35	1	1865	914	157	107
	40	1	2147	948	180	126

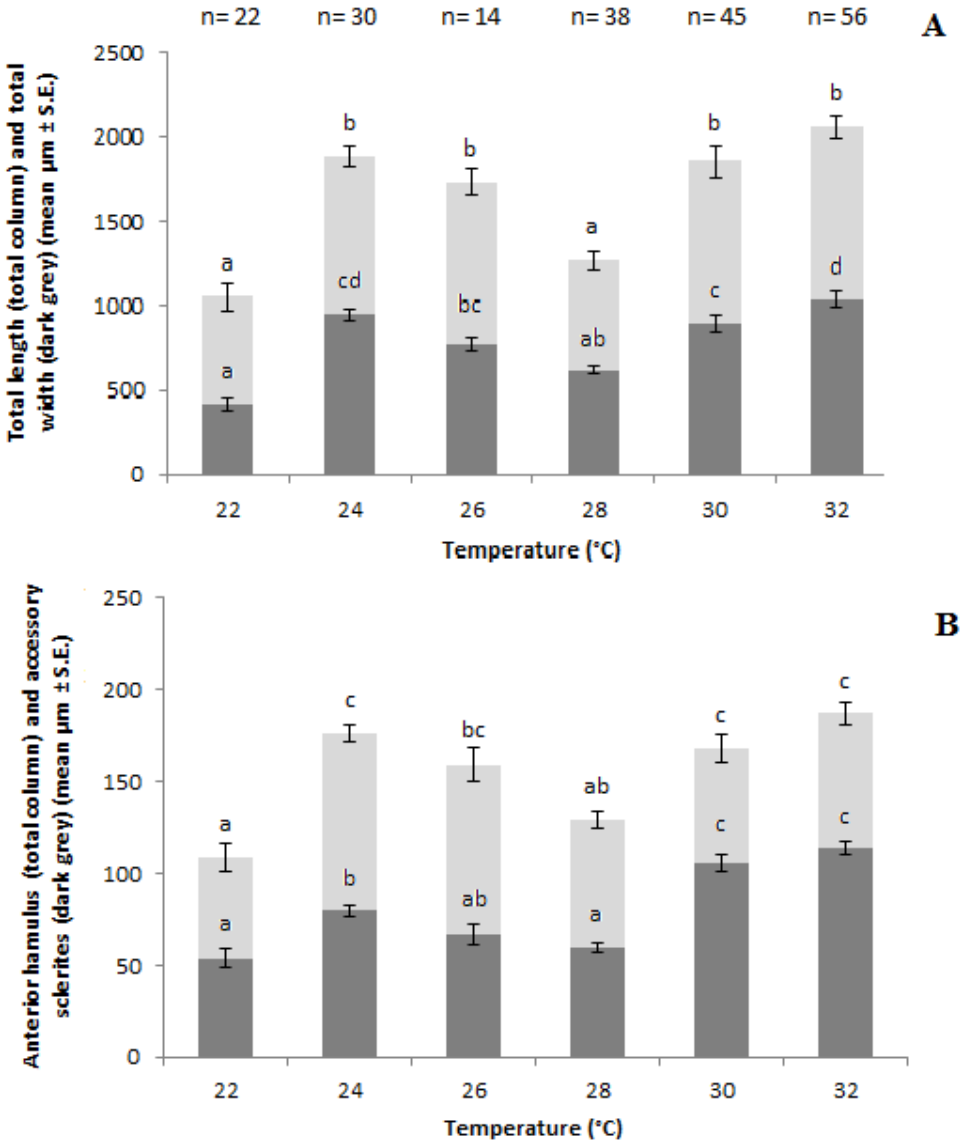

Figure 24: (a) *Neobenedenia* total length (total column height) and total width (dark grey) and (b) anterior hamulus (total column height) and accessory sclerite length (dark grey) with respect to temperature. 'a', 'b' and 'c' = differences between pairs of means determined using Tukey's HSD test. $p<0.05$. Each parameter independently statistically analysed.

Life cycle

The life cycle of *Neobenedenia* was completed faster at warmer temperatures (26, 30 and 32 °C) compared to cooler temperatures (22 and 24 °C). The fastest completion of the life cycle (10 days) was observed at 26, 30 and 32 °C and the slowest (18 days) was observed at the average winter temperature (22 °C). From the time of infection by a newly emerged oncomiracidium at 22–24 °C, a second generation of infective *Neobenedenia* sp. oncomiracidia can emerge between 15–18 days compared to 10–14 days at 26–34 °C. This indicates that at warmer temperatures, *Neobenedenia* has the capacity to produce three consecutive generations within one month. In comparison, two consecutive generations could be achieved within one month at cooler temperatures (Table 20).

Table 20: Treatment timetable for *Neobenedenia* sp. infecting *Lates calcarifer* in various temperature/salinity combinations. Time to first and last hatch (F/LH); average oncomiracidial longevity (OL); minimum time to sexual maturity (SM); minimum time to completion of life cycle (LC); and the days on which subsequent treatments (post an initial treatment at day 0) should be administered ('Second treatment' and 'Third treatment') are indicated. '-' indicates no parasite survival.

Temperature (°C)	Salinity (‰)	F/LH (days)	OL (hours)	SM (days)	LC (days)	Second treatment (days)	Third treatment (days)
22	22	6 – 8	2	-	-	-	-
	35	6 – 7	37	12	18	8 – 11	-
	40	7 – 8	49	9	16	8	11
24	22	-	2	-	-	-	-
	35	4 – 5	22	11	15	7 – 10	-
	40	4 – 5	31	12	15	8 – 11	-
26	22	-	5	12	-	-	-
	35	4 – 5	19	7	11	6	-
	40	4 – 5	28	6	10	5	7
28	22	-	3	-	-	-	-
	35	4 – 5	11	9	13	6 – 8	-
	40	4 – 5	10	9	13	6 – 8	-
30	22	4 – 9	3	8	12	7	10
	35	4 – 6	8	6	10	5	7

	40	4 – 6	7	6	10	5	7
	22	4	2	-	-	-	-
32	35	4 – 8	8	6	10	5	9
	40	4 – 8	6	8	12	7	9
	22	9	2	-	-	-	-
34	35	-	7	12	-	-	-
	40	6 – 9	6	8	14	7	10

iii) Genetic susceptibility of barramundi stocks to *Neobenedenia* infection

All fish became infected with *Neobenedenia* sp., with the exception of one replicate from each treatment. Mean percent infection success was 28 (0-57), 27 (0-64) and 32 (0-52) for fish from Darwin, Bowen/Townsville and Cairns, respectively. There was no correlation between fish length and infection success. The infection experiments indicated there was no difference in susceptibility to *Neobenedenia* sp. infection in *L. calcarifer* from three different genetic stocks. Similarly, Rubio-Godoy et al., (2011) found no difference in susceptibility of two different genetic types of tilapia *Oreochromis* spp. to *Neobenedenia* sp. infection. This suggests there is no particular advantage to culturing different genetic stocks of barramundi with regard to the threat of *Neobenedenia* infection.

iv) Histopathology of *Neobenedenia* infection

Infected *L. calcarifer* exhibited statistically significant greater epidermal thickness on the mid-body and operculum (56 ± 4mm and 66 ± 4mm, respectively) compared to uninfected fish (34 ± 2mm and 50 ± 3mm, respectively). Furthermore, a significantly greater number of epithelial cell layers were observed on the mid-body and operculum (9 ± 0.7mm and 14 ± 0.4, respectively) of infected fish compared to uninfected fish (6 ± 0.4; 7 ± 0.4, respectively). No differences in the number of mucous cells were found in the mid-body and operculum tissue between infected and uninfected fish (Figure 10). Epidermal thickness in the mandible of infected fish (38 ± 3mm; respectively) was reduced compared to uninfected fish (82 ± 3mm). Epithelial layers were also less in the lower jaw of infected fish (5 ± 0.4) when compared to uninfected fish (11 ± 0.6). The number of mucous cells was significantly lower in the lower jaw of infected fish (4 ± 0.7) compared to uninfected fish (20 ± 3, Figure 25).

All infected fish showed signs of variable epidermal damage at the site of parasite attachment compared to uninfected tissue (Figure 26a). The marginal valve and chitinous structures of the haptor (Figure 26b) deformed the host's epidermal tissue at the site of attachment where the

epidermal layer appeared compressed in contact with the marginal valve of the haptor (Figure 26c). The mandibles of infected fish exhibited thin, vacuolated epidermis (Figure 26d, arrow), with loss of intraepithelial attachment and ruptured epithelial/mucous cells at the site parasite attachment. Lifting of the overlying epidermis from the dermis was observed along the basement membrane, as well as scattered inflammatory cells in the dermis (Figure 26d). Cellular debris directly beneath the haptor contained ruptured epithelial and mucous cells (Figure 26d).

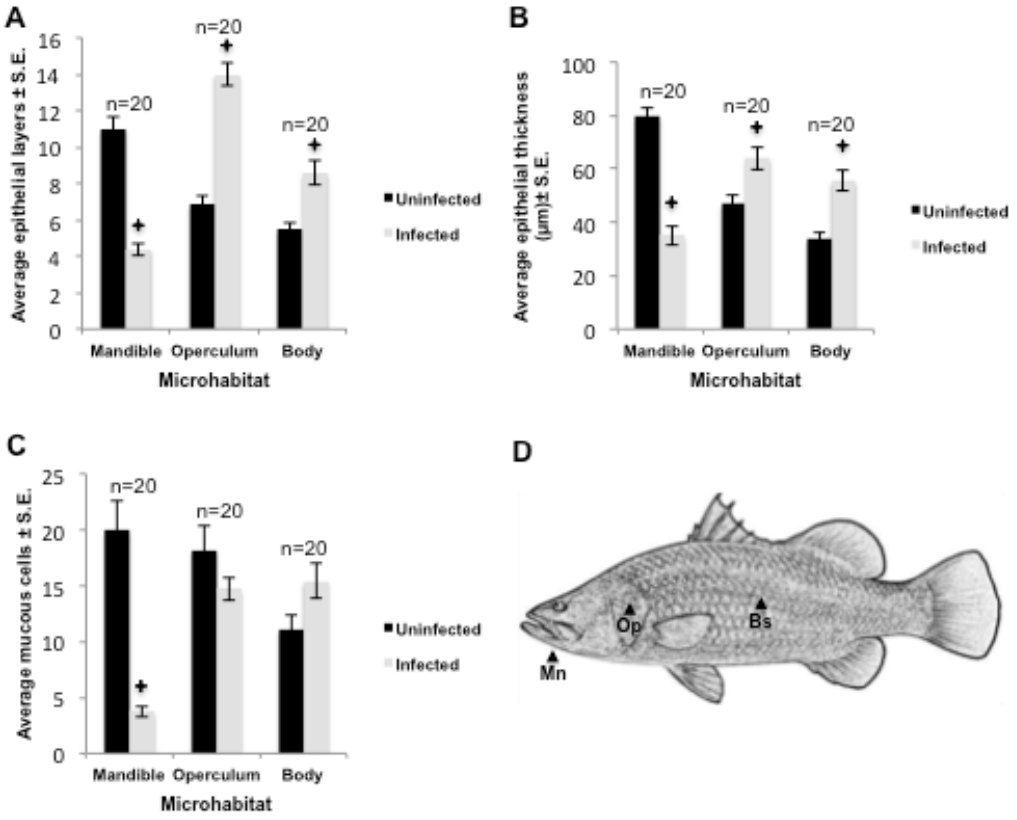

Figure 25. Tissue cell counts and measurements in three microhabitats of uninfected and infected *Lates calcarifer* with *Neobenedenia*. (a) Average number of epithelial layers/mm^2. (b) Average epithelial thickness (µm). (c) Average number of mucous cells/mm^2. (d) Microhabitat sampling locations. *: Two-sample t tests, P<0.001. There were significant interactions between the condition of the fish and the defined microhabitats on the epithelial thickness (Two-way ANOVA, n=20, $F_{3,116}$=31.921, P<0.001), number of epithelial layers (NBR, n=20, $df_{residual}$= 152, Residual: 130, P<0.001) and mucous cells (NBR, n=20, $df_{residual}$= 114, Residual: 130, P<0.001). Bs= body skin tissue, Lj= lower jaw, Op= operculum skin tissue

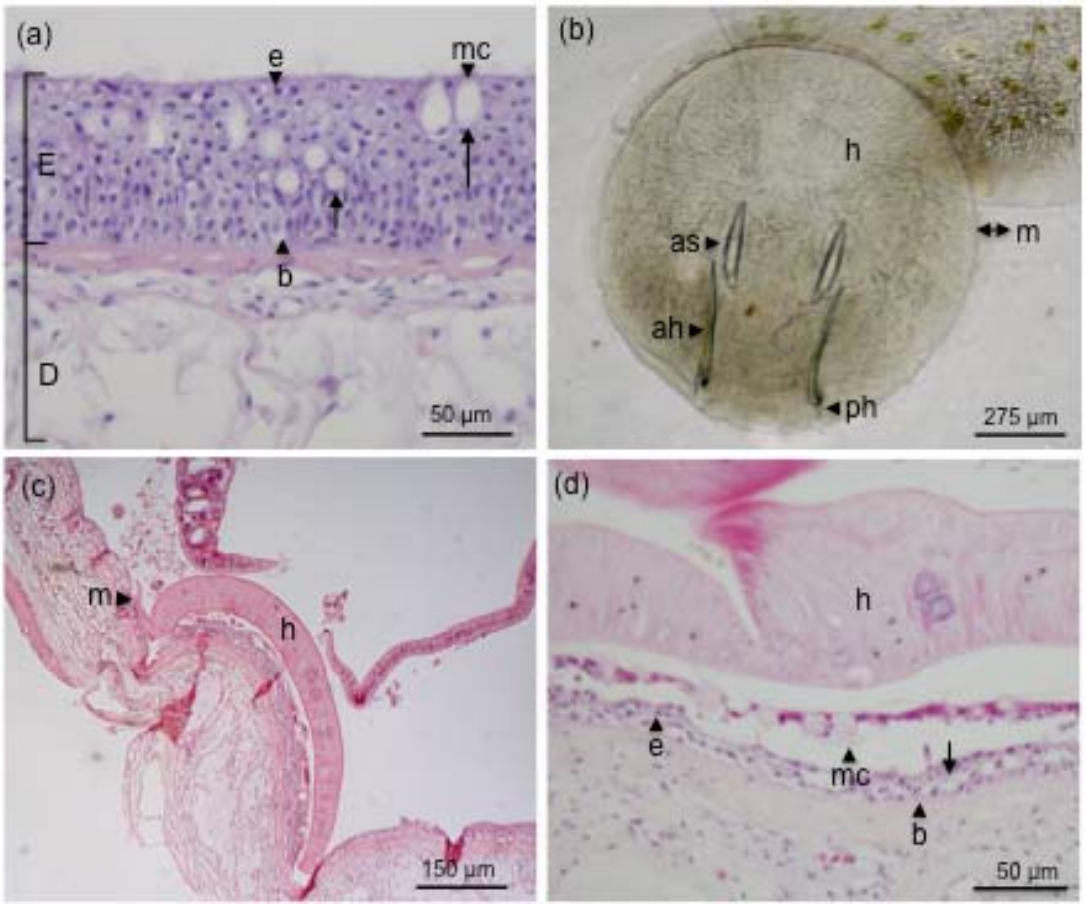

Figure 26. Haptor of *Neobenedenia* and epithelial attachment to infected *Lates calcarifer*. (a) Uninfected mandible host tissue. Micrograph shows two morphologically distinct mucous cells (long and short arrows). (b) Ventral view of haptor showing hamuli, accessory sclerites, hooklets and marginal valve. (c) Bulging of lower jaw epidermis following the contour of the haptor (microhabitat = lower jaw). (d) Epithelial loss under *Neobenedenia* haptor and vacuolated basal epithelial cells (arrow) (microhabitat = mandible). ah = anterior hamulus; as = accessory sclerite; b = basement membrane; cd = cellular debris; e = squamous epithelial cells; h = haptor; ho = hooklet; m = marginal valve; mc = mucous cells ph = posterior hamulus. *Neobenedenia* morphological terms follow Whittington and Horton (1996) and epidermis morphological terms follow Takashima and Hibiya (1995).

v) Adaptable management of parasitic monogeneans

Temperature is a key variable affecting the life cycle parameters of capsalid monogeneans with warmer temperatures correlating with reduced time to life cycle completion (Ernst and Whittington 1996; Tubbs et al., 2005; Lackenby et al., 2007). This is attributed to the increased metabolic and development rate associated with the warmer temperature (Poulin et al., 1989; Conley and Curtis 1993). Time taken to first hatch of *Neobenedenia* eggs in seawater (35‰) decreased with increasing temperature (22–32 °C). Similar trends were found by Bondad-Reantaso et al., (1995) who observed that *Neobenedenia* girellae eggs take fewer days to hatch at high temperatures. Temperature is considered the most important abiotic factor regulating larval longevity (Gannicott and Tinsely 1998) and significantly longer life span was observed at cooler (22–28 °C) compared to warmer (30–34 °C) temperatures, varying from 49 hours at 22 °C to 6 hours at 34 °C in 40‰. A similar reduction in longevity was documented by Gannicott and Tinsely (1998) for oncomiracidia of *Discocotyle sagittata*, a gill monogenean of rainbow trout (*Oncorhynchus mykiss*) where longevity decreased from 96 hours at 6 °C to 26 hours at 22 °C.

Neobenedenia reached sexual maturity more quickly at warmer temperatures. Lackenby et al., (2007) and Hirazawa et al., (2010) documented similar findings for *Benedenia seriolae* and *N. girellae* where the time taken to reach sexual maturity was inversely correlated to water temperature. Parasites were found to be smaller at sexual maturity in cooler temperatures both in the present study and that by Hirazawa et al., (2010). Conversely, Lackenby et al., (2007) observed *B. seriolae* attained sexual maturity at similar mean total lengths at all temperatures (Lackenby et al., 2007). The mean total length of mature parasites measured in the present study were consistently smaller at comparable temperatures and maturation times to those observed by Hirazawa et al., (2010) for *N. girellae* infecting *S. dumerili*. At 30 °C after 8 days, *N. girellae* parasites were > 3.0mm whereas parasites in this study were < 2.0mm.

The life cycle of *Neobenedenia* was rapid (10 days) in warmer temperatures (26, 30 and 32 °C) compared to cooler temperatures examined (15 days; 22 and 24 °C). Hirazawa et al. (2010) similarly observed that the life cycle of *N. girellae* was completed faster with increasing water temperatures ranging from 20 °C to 30 °C at 33‰ salinity where the second generation of *N. girellae* presented 16 days at 20 °C, 12 days at 25 °C and 8 days at 30 °C after oncomiracidial attachment. Environmental conditions at warmer temperatures (30–32 °C at high salinities) could result in 3 generations a month by this parasite, resulting in faster population growth in open and semi-closed aquaculture systems. This may require more

vigilant and frequent stock monitoring in warmer years as infections may be able to build more rapidly (Marcogliese 2001). However, although warmer temperatures speed up hatching and time to sexual maturity, the benefits of the increased rate of life cycle completion could be mitigated by significantly shorter oncomiracidial longevity and significantly reduced infection success. If this is the case, cooler temperatures would facilitate the increase in *Neobenedenia* infections on farmed fish. Indeed, a prolonged period of unseasonably low water temperatures was hypothesised to be the cause for why *N. melleni* presented on sea caged barramundi in Hinchinbrook Channel in July, 2000 in epidemic proportions (Deveney et al., 2001). This was thought to have compromised the immune systems of the barramundi, a well-documented effect of cold temperature in teleosts (reviewed by Bly and Clem 1992), inhibiting their capacity to deal with parasite infection. Although the causes behind mass infections of *Neobenedenia* spp. are still unknown, it may be that increased oncomiracidial longevity and infection success observed at cold temperatures (as demonstrated in this study) may, together with immune-suppressed hosts, contribute to epidemic infection levels on farms.

Low salinity was found to significantly inhibit both hatching and infection of *Neobenedenia* across all temperature treatments with no hatching observed in 0 and 11‰ salinities. Similarly, Müeller et al. (1992) observed < 12% hatching success for *N. melleni* eggs incubated at salinities of ≤ 18‰ for 4 days while Ellis and Watanabe (1993) found *N. melleni* eggs did not hatch when exposed to salinities ≤ 18‰ for 7 days under ambient temperature conditions. Infection success of *Neobenedenia* sp. also decreased from >80% at optimal salinities (35 and 40‰) to < 7% at 22‰. Similarly, Ellis and Watanabe (1993) observed a considerable reduction in *N. melleni* infection after treatment of fish with ≤18‰ saline resulting in a 100% reduction in infection after a four day treatment. These results confirm that low salinity and freshwater are viable therapeutants and can effectively be used in the management of *Neobenedenia* infections in aquaculture.

The life cycle of *Neobenedenia* was extensively investigated and quantified in this study. Understanding parasite life cycle parameters and how they are affected by environmental variables enables more effective parasite management through precise timing of treatments and the data collected in this study was used to formulate a strategic management timetable to maximise treatment efficacy and limit *Neobenedenia* epizootics in aquaculture. Recommended subsequent treatments occur earlier at warmer (26–34 °C) compared to cooler temperatures (22 and 24 °C)(Table 20). Newly recruited *Neobenedenia* reach sexual maturity faster than eggs hatch in most of the temperature and salinity combinations examined. The

rate at which the life cycle is completed in these environments is too fast to ensure elimination of infection within a system with only one subsequent follow up treatment. Two subsequent treatments are therefore recommended; one 24 hours prior to parasites reaching sexual maturity and the second 24 hours after all oncomiracidia have either infected fish or died. The timing of these subsequent treatments varies between temperature/salinity combinations.

As has been identified in this study, freshwater represents an effective therapeutant in managing *Neobenedenia* infections on fish. Freshwater treatments cause little stress to euryhaline fish and are completely harmless to the culturist, environment and consumer of the product (Hoshina 1968; Kaneko et al., 1988; Müeller et al., 1992). Results from this study indicate prolonged exposure to brackish and hyposaline solution could be used to reduce egg hatching of *Neobenedenia* on farms. The long-term treatment of stenohaline stock with brackish and hyposaline water may be impractical and it may be prudent to use acute freshwater treatments in order to kill adult and juvenile parasites. Many marine fish can tolerate short-term immersion in fresh water and dips of 2–3 minutes are effective in killing adult monogeneans (Hoshina 1968; Kaneko et al., 1988; Ohno et al., 2009; Seng 1997).

The data collected in this study has been used to predict how quickly this parasite is able to complete its life cycle at a variety of temperatures and salinities influencing how often parasite management needs to be conducted. This research enhances our understanding of the influence that temperature and salinity have on the life cycle parameters of *Neobenedenia* sp. and resulted in the proposal of a strategic management timetable formulated from the times taken for *Neobenedenia* sp. to hatch and to reach sexual maturity and dictates a 'window of opportunity' to break the life cycle by a timed treatment following the first treatment of infected stock. As treating fish is a costly exercise in terms of labour, stress to stock and cost of chemicals/treatment apparatus, accurate treatment timetables reduce the cost incurred by parasite management. The timetable provides a simple tool to assist implementation of a strategic management of *Neobenedenia* sp. infections in commercial *Lates calcarifer* farms across Australasia where similar temperature and salinity combinations are experienced.

vi) Adaptable management of parasitic copepods

Lernanthropus latis exhibits broad environmental tolerance. Parasite eggs hatched successfully in a range of water temperatures (22–34 °C) and salinities (22–40 ‰) representing current and predicted water temperature and salinity scenarios. The thermal and salinity tolerance of ectoparasite fauna is likely to be directly associated with the

environmental tolerance exhibited by the host fish species. *Lates calcarifer* is a coastal, euryhaline species that occupies tropical monsoonal climates where seasonal rainfall and runoff can lead to dramatic drops in salinity in coastal areas, while dry seasons can result in warm, hypersaline environments in shallow waters. In the open ocean, where salinity is almost constant, copepod parasites of oceanic fishes are not adapted to cope with large salinity changes. Cressey and Collette (1970) noted that *Lernanthropus tylosuri* infect oceanic needlefish species which avoided estuarine areas and Bricknell et al. (2006) found survival of free-swimming copepodids of the sea-louse *Lepeophtheirus salmonis* was severely compromised at salinity levels below 29 ‰. In contrast, *Lernanthropus latis* tolerates various water parameters that may be encountered by the host species in coastal environments.

Warmer temperatures can increase metabolic rates and reduce development times. The time taken for the majority of *Lernanthropus latis* nauplii to hatch was quicker in the 30 °C+ water temperatures compared to 22 °C. This suggests that winter water temperatures can prolong *L. latis* egg hatching in wild and cultured *Lates calcarifer*. Similarly, Conley and Curtis (1993) and Poulin et al., (1989) observed earlier onset of hatching of the parasitic copepod *Salmincola edwardsii* and a greater hatching rate at warmer water temperatures. Likewise, Johnson and Albright (1991) observed an earlier onset of hatching of the parasitic copepod *Lepeophtheirus salmonis* at warmer water temperatures.

Freshwater can be used to kill *L. latis* in aquaculture as it completely inhibits egg hatching. The use of freshwater to manage marine ectoparasites is not a new concept for copepods. Freshwater offers an alternative management strategy to chemical use, causes little stress to fish and is completely harmless to the culturist, environment and consumer of the product. Parasites can also be excluded from cultured fishes by filtering intake water at land-based facilities.

Component D: Modelled effects of future climate change on wild fisheries and aquaculture production

i) Modelling climate change impacts on the wild barramundi fishery

Current climate and future exposure of distinct genetic stocks to climate changes

Genetic sampling sites covered the full spatial range of barramundi in Australia with 21 genetically distinct management units (subpopulations) identified within 6 major stocks. These subpopulations persist across a broad range of temperature, rainfall and evaporative regimes (Figure 27, Appendix 5). With respect to temperature, most subpopulations currently experience mean temperatures in the coldest month of 22.3 (±2.6) °C and mean air temperatures in the hottest month of 29.6 (±1.5) °C (Figure 27a, Appendix 5). However, the temperature range experienced by barramundi in Australia is considerably broader, with the Pilbara subpopulation in Western Australian (subpopulation 1) persisting where the mean air temperature of the hottest month remains at 32.4 °C and the monthly maximum reaches 36.7 °C. Subpopulations along the east coast of Australia experience the steepest gradient in environmental temperature exposure (Figure 27a) with those located in SE Queensland experiencing mean air temperatures in the coldest month of as low as 16.3 °C and the monthly minimum drops below 10.7 °C (Subpopulation 21 in the Mary River). The barramundi fishery in this area of SE Qld is the focus of substantial stocking efforts, particularly in heavily impounded river systems, and extreme cold-weather events in this region have been responsible for recent and extensive fish-kills including large numbers of stocked barramundi in shallow impoundments (Sawynok et al., 2009b).

Future projections of climate change scenarios (18 GCMs) indicate that by 2085 all subpopulations will experience similar increases in minimum mean and minimum monthly temperatures which may lead to a decrease in the frequency or severity of cold-weather die offs. In addition, similar rises in the maximum monthly air-temperatures are projected for all subpopulations under the climate change scenarios examined. By 2035 both maximum mean and monthly maximum temperatures increase by 1.2 degrees and all subpopulations remain below known physiological thermal maximum. By 2085 all subpopulations are likely to be exposed to ambient temperatures 3.6 (± 0.7) °C above current mean monthly conditions in both the warmest and coolest months. Maximum monthly ambient temperatures across the range are expected to average 38.1 °C, but eleven subpopulations within the South-Western, Western and broader North-Western/Central stocks are likely to reach maximum ambient temperatures above 39 °C in the hottest month (Appendix 5). Four subpopulations may see

rises in air temperature to greater than 40 °C (See Figure 27a, Appendix 5) including subpopulation 1 in the Pilbara region of WA which may see rises in maximum monthly temperature in the hottest month to as high as 42.9 °C. Average water temperatures, particularly in deeper flowing water bodies, are expected to remain below the current 40 °C threshold considered as suitable for barramundi (see also discussion on habitat suitability models below). Short-term heat wave events may, however, start to affect some shallow water bodies containing barramundi by 2085 (Hamilton 2010), but data suitable for model extreme weather events at a continental scale has yet to be generated, and as such both the likelihood and impact of such events remains difficult to predict.

Substantial differences in current temperature seasonality are evident for the 21 barramundi subpopulations examined with little change in seasonality expected for most subpopulations by 2085 (Figure 27a). The highest temperature seasonality is experienced by subpopulations at the southern extremes of both the east and west coast including subpopulations 1 and 2 in the Pilbara and Broome areas of Western Australia, respectively, and in subpopulations 19, 20 and 21 in the Broad Sound, Fitzroy River and Mary River regions of Queensland, respectively. Subpopulations 12 and 13 in the Gulf of Carpentaria (including the Roper River and Mcarthur River respectively) also experience high temperature seasonality. The extensive subpopulation which extends from the Albert River in the southern Gulf to the Archer River on the Eastern side of the Gulf (subpopulation 14) experiences the widest range of seasonality in part due to it's extensive geographic coverage (see Figure 6, component A). In contrast, relatively low temperature seasonality (i.e. greater annual temperature stability) is experienced by the most northern subpopulations sampled including subpopulation 7 in the NT (Darwin Harbour, Shoal Bay and Bathurst Island in the NT) and subpopulations 15 and 16 in QLD (from the Jardine River to the Escape River, Cape York). Barramundi subpopulations currently persist across a wide range of rainfall regimes from across the highly seasonal monsoonal wet-dry tropics and down the less-seasonal east coast (Figure 27b). Currently the average dry season rainfall experienced by the 21 subpopulations identified is 28 ml, while average wet-season rainfall is 776 ml (Appendix 5). Notable, however, east coast subpopulations (subpopulations 18 – 21) from the Johnstone River to the Mary River receive more than twice the 28 ml average rainfall in the dry season with between 75 - 141 ml (Appendix 5). Subpopulations in the Gulf of Carpentaria (subpopulation 12-14) currently receive the lowest dry season falls with just 4 – 6 ml (Figure 27b, Appendix 5). The Pilbara region in the south-west of the species range (subpopulation 1) currently receives the lowest wet-season rainfall with as little as 168 ml in the wet-season and average rainfalls (22 ml) in

the dry-season (Figure 27b, Appendix 5). Although rainfall is one of the harder variables to predict under climate change, it appears likely that by 2085 the majority of subpopulations will experience similar seasonal rainfalls (at the 50th percentile of 18 GCMs) to that currently experienced (Figure 27b). The east-coast subpopulations from Princess Charlotte Bay to the Mary River (subpopulations 19 – 21) are likely to experience the greatest increases in rainfall seasonality (Figure 27b).

Mean monthly potential evaporation is currently 134 (±13) ml, but is noticeably variable through space and typically lower in east coast and northern Queensland populations (subpopulations 15 – 21 Figure 27b, Appendix 5). Potential monthly evaporation is likely to increase by 2085 (at the 50th percentile of 18 GCMs) across the species range although with some variability in the extent of change (Figure 27b, Appendix 5). Little to no change in mean monthly potential evaporation is projected for subpopulation 12 in the Roper River (NT), while modest increases of between 9 – 28 ml are predicted for all other subpopulations with the greatest difference to be experienced by subpopulation 4 in the Admirality Gulf in the Kimbeley region of Western Australia (Figure 27b, Appendix 5). A similar increase in potential monthly evaporation (an increase of ~26 ml) is projected for subpopulation 11 in the Arnhem Bay region of the NT. The Arnhem Bay subpopulation is also projected to see the greatest rise in maximum monthly mean temperatures (a rise of 5.9 °C from 29.3C to 35.2 °C) and maximum monthly temperature (a rise of 7.9 °C from 33.5 °C to 40.9 °C) and is further projected to experience the largest decreases in rainfall in the two periods encompassing the wet-season from October-December and January - March (Figure 27b, Appendix 5). Future monitoring of recruitment strength and population abundance in this region is recommended. Similarly, the barramundi subpopulation in the Pilbara region may warrant ongoing monitoring due to it's relative geographic isolation, genetic distinctiveness and reduced genetic diversity (component A), and the potential for ambient temperatures to reach maximums of as high as 42.9 °C by 2085 (Figure 27a, Appendix 5).

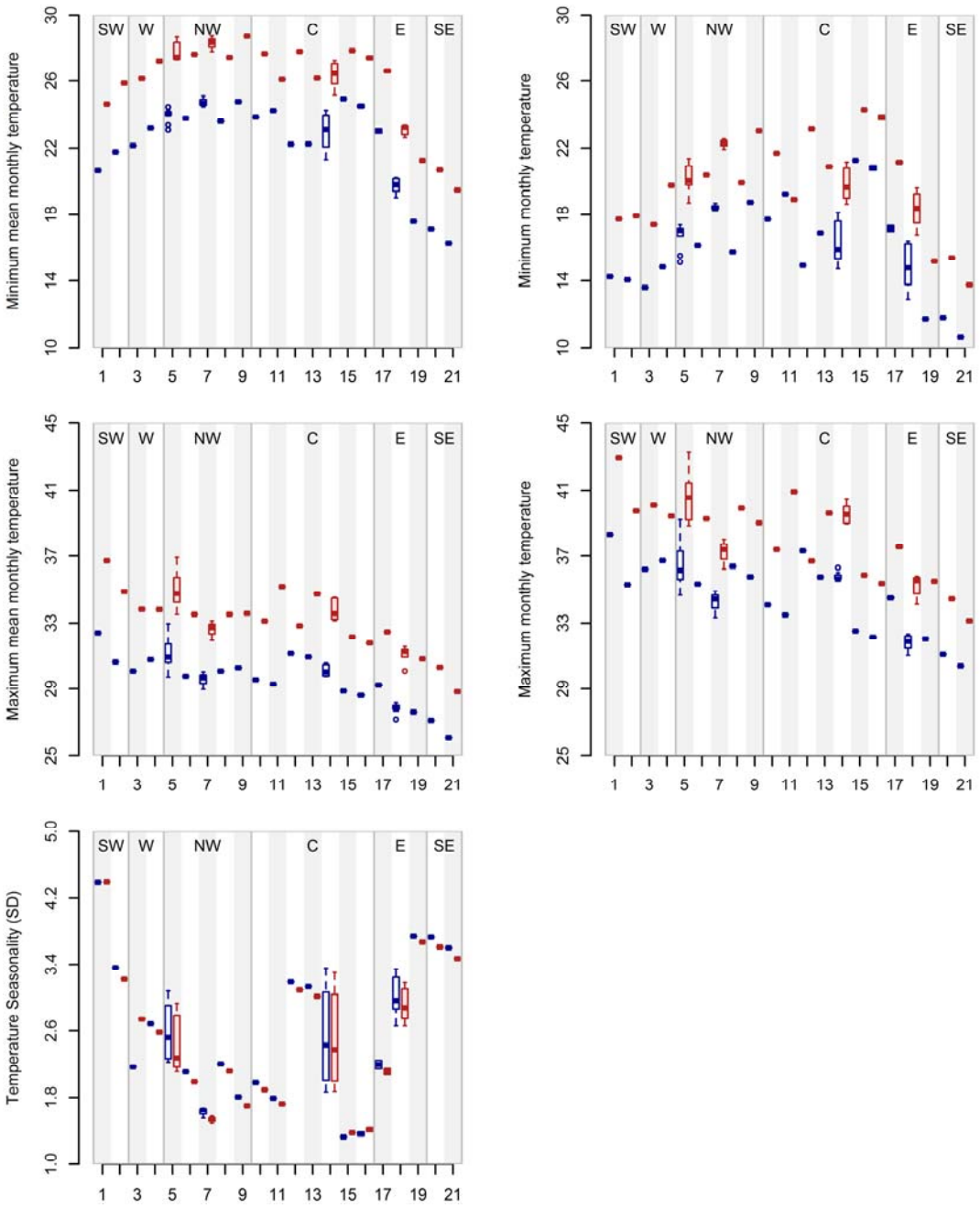

Figure 27a. Boxplots showing variation in exposure to temperature , rainfall and evaporative conditions for each of the 21 genetically distinct subpopulations identified in the genetic analyses of Component A. Subpopulations belonging to each of the six major stocks identified are boxed and labelled as follows SW = South-Western Stock, W = Western Stock, NW = North-Western Stock, C = Central Stock, E = Eastern Stock and SE = South-Eastern Stock. Blue represents current exposure and red represents future exposure for the 50th percentile GCM of RCP 8.5 in 2085.

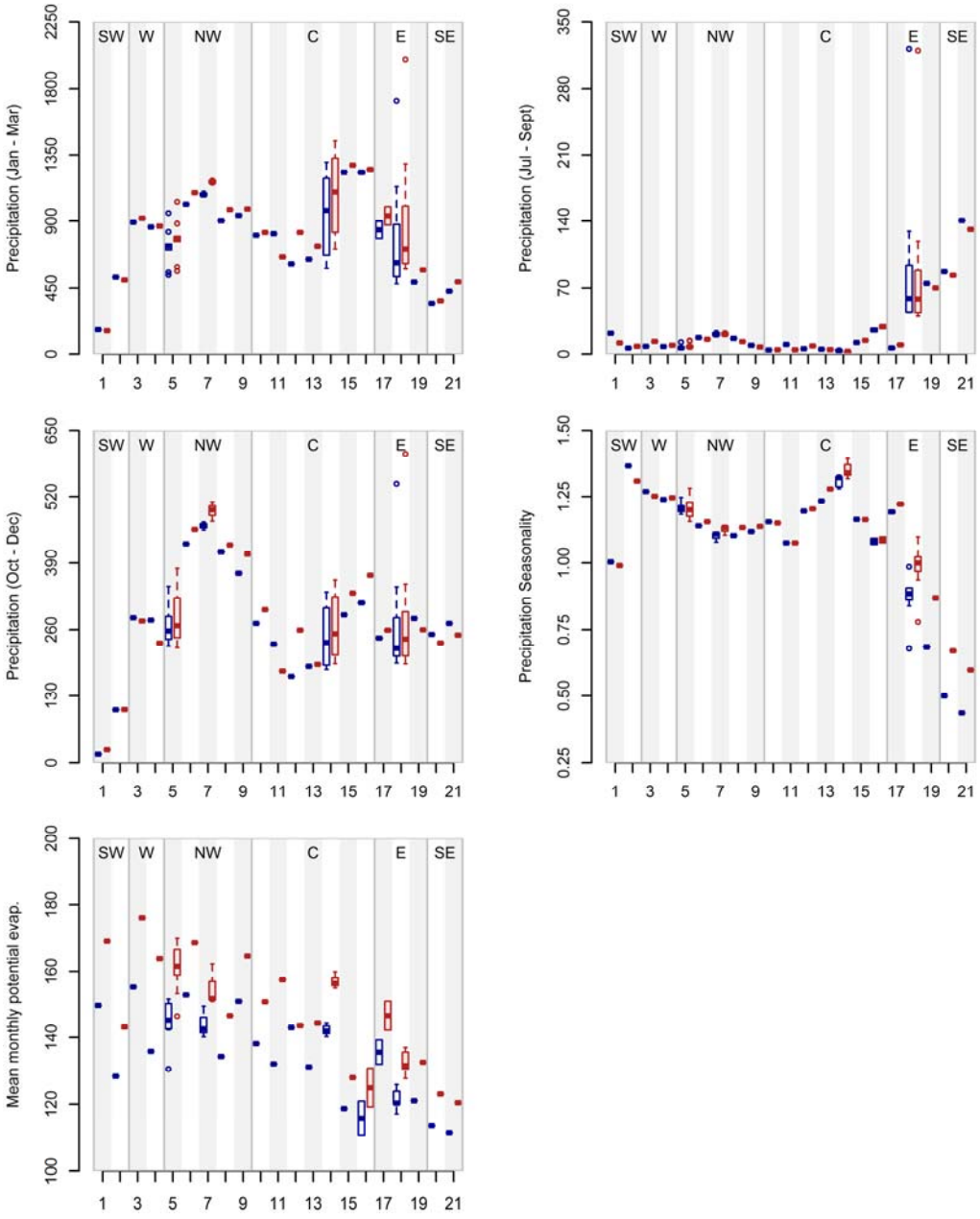

Figure 27b. Boxplots showing variation in exposure to rainfall and evaporative conditions for each of the 21 genetically distinct subpopulations identified in the genetic analyses of Component A. Subpopulations belonging to each of the six major stocks identified are boxed and labelled as follows SW = South-Western Stock, W = Western Stock, NW = North-Western Stock, C = Central Stock, E = Eastern Stock and SE = South-Eastern Stock. Blue represents current exposure and red represents future exposure for the 50th percentile GCM of RCP 8.5 in 2085.

Modelling climate change impacts on the wild barramundi fishery (CPUE)

The 10-fold cross-validated presence-only species distribution modelling was shown to accurately represent the current distribution of barramundi in Australia (Figure 28). Climate change, however, is predicted to vary the current distribution, extending the range south on both the east and west coasts for the median (50^{th} percentile) future distribution calculated across distributions modelled using 18 GCMs for the 'business-as-usual' RCP 8.5 emissions scenario (Figure 29). We consider this median distribution our 'best estimate' model. Some trends, such as the southerly improvement in climate, hold across all 18 future distributions (the variation in which is represented here by the 10^{th} and 90^{th} percentiles), but there is considerable variation in area suitability between the 10^{th} and 90^{th} percentile of future species distribution models which is driven by the broad variation in predicted rainfall.

The optimal model for assessing climate-derived correlates of catch per unit effort (CPUE) explained 59.2% of the variance (adjusted R^2) across Queensland (Eqn 3, Table 21, Figure 30). The key variables included dry season (July–September) rainfall, annual evaporation, and minimum temperature during the summer wet season. The best equation explaining CPUE was:

$$\text{Log } N(\text{CPUE}) = -2.583 - 0.211(RJS) - 0.00003983(E^2) + 2.489(TJM) \qquad (3)$$

where RJS is Log N(total July–September rainfall), E is mean monthly Potential Evaporation (mm) squared, and TJM is Log N(mean minimum January-March temperature). The adjusted R^2 takes into account collinearity between variables and the degrees of freedom in the model. Predicted *v.* observed values of catch are plotted in Figure 31.

The future median CPUE is expected to increase in key areas with climate change (Figure 32). Areas that currently yield the highest CPUE are expected to remain high into the future and all areas are expected to show some increase in CPUE. Outside Queensland (area of training data), areas of highest estimated CPUE (Figure 32) correspond to areas of highest modelled suitability (Figure 29). Increased CPUE is expected along the northern coastline into the future, with a possible southerly expansion of the fishery as climate warms towards 2085 (Figure 32).

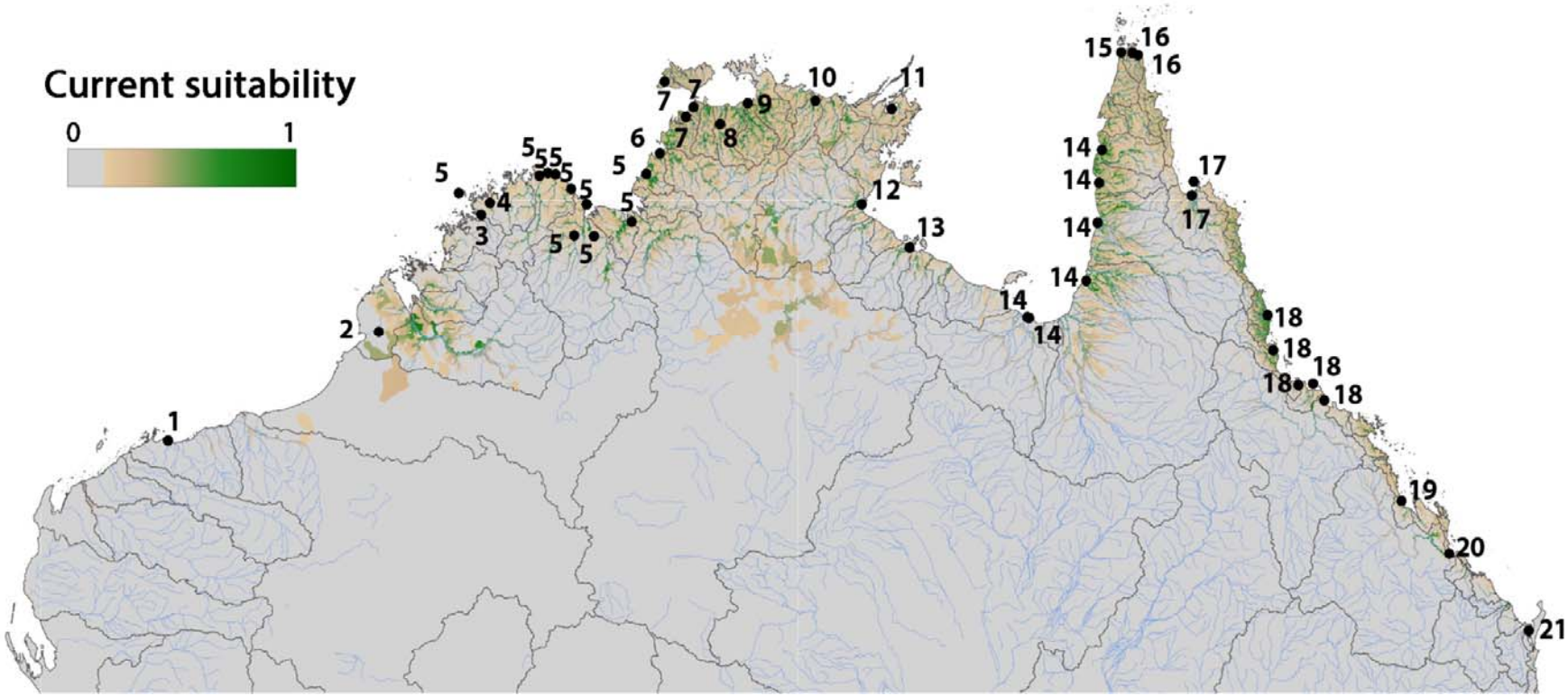

Figure 28: Current Species Distribution Model of Barramundi, where grey represents areas beneath the threshold (climatically unsuitable) and green represents the most suitable areas. Black polygons represent level 2 basin denominations from National Catchment Boundaries v1.1.4 (http://www.ga.gov.au/topographic-mapping/national-surface-water-information.html). The location of genetic sample collections used for the microsatellite analysis (Component A) are indicated as black circles and labels indicate the genetic subpopulation number as per Figure 5/Table 13.

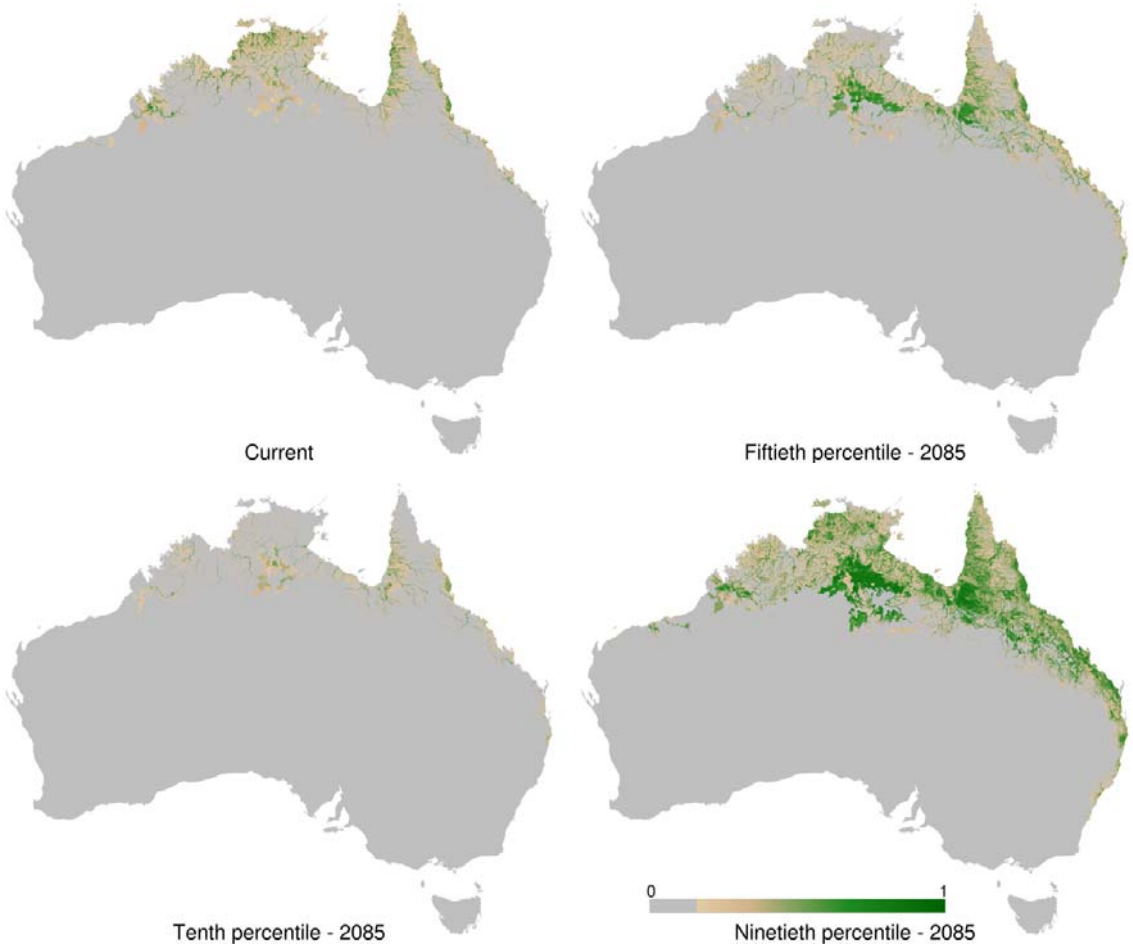

Figure 29: Future species distribution model of barramundi for RCP85 in 2085 (and variability using 10th, 50th and 90th percentiles), where grey represents area beneath the threshold (climatically unsuitable); tan represents marginal suitability; and green represents the most climatically suitable areas. The scale of 0 to 1 is drawn from the MaxEnt probability of occurrence. The distribution was clipped to connected streams in which barramundi have been observed as per records in the ALA database.

Table 21: Model developed to provide an estimate of future Queensland barramundi catch per unit effort (CPUE) from climate variables. The adjusted R^2 takes into account collinearity between variables and the degrees of freedom in the model.

	Estimate	**Std Error**	**t value**	**Pr(>|t|)[1]**
Intercept	2.583	2.713	-0.952	0.34457
Log *N*(RJS)	-2.583	2.713	-0.952	0.34457
E^2	$-3.983e^{-5}$	$1.967e^{-5}$	-2.024	0.04693 *
Log *N*(TJM)	2.489	$8.706e^{-1}$	2.859	0.00567 **

[1]Sign. Codes: *** 0.001 ** 0.01 * 0.05 . 0.1

Residual standard error: 0.2398 on 67 degrees of freedom

Multiple R^2: 0.6093, Adjusted R^2: 0.5918

F-statistic: 34.83 on 3 and 67 DF, p-value: $1.104e^{-13}$

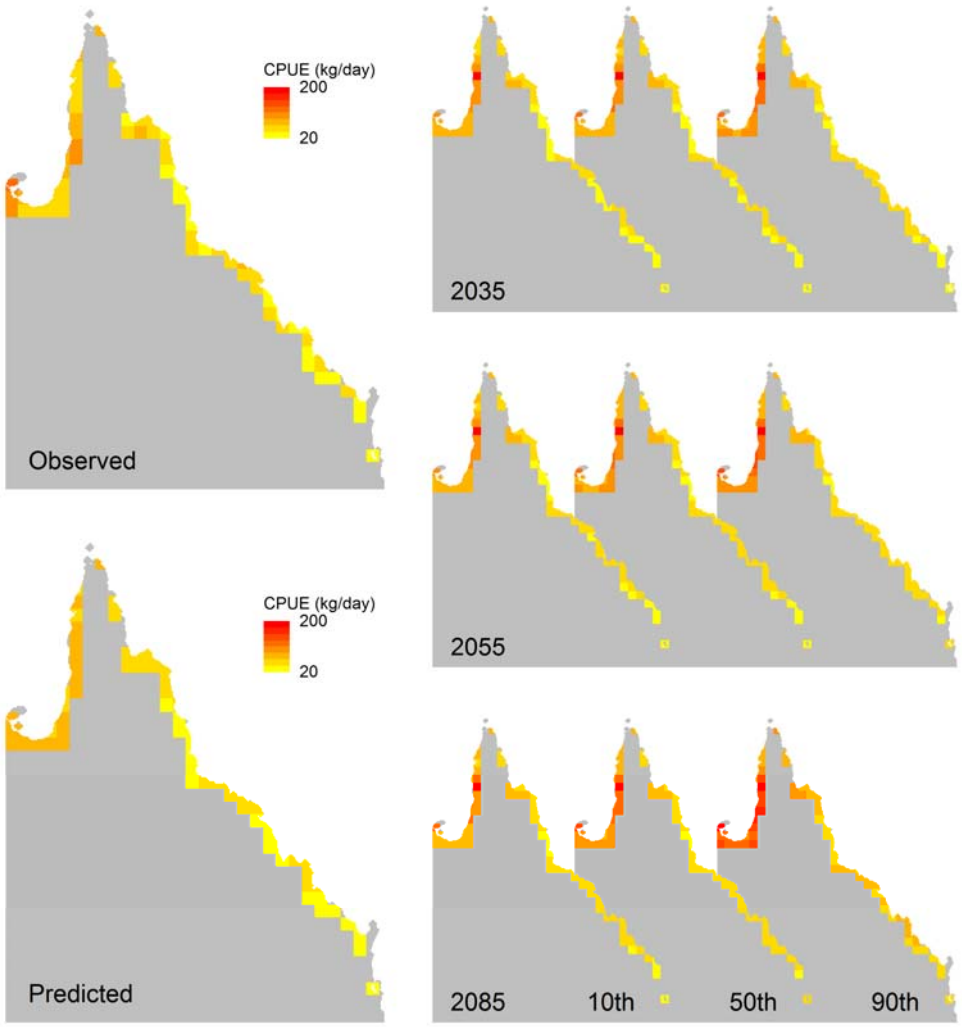

Figure 30: Current observed and current predicted catch per unit effort (CPUE) for Queensland shown on the left. The right panels show future predictions (and variability using 10^{th}, 50^{th} & 90^{th} percentiles) for a high emission scenario (RCP 8.5) for 2035, 2055 and 2085.

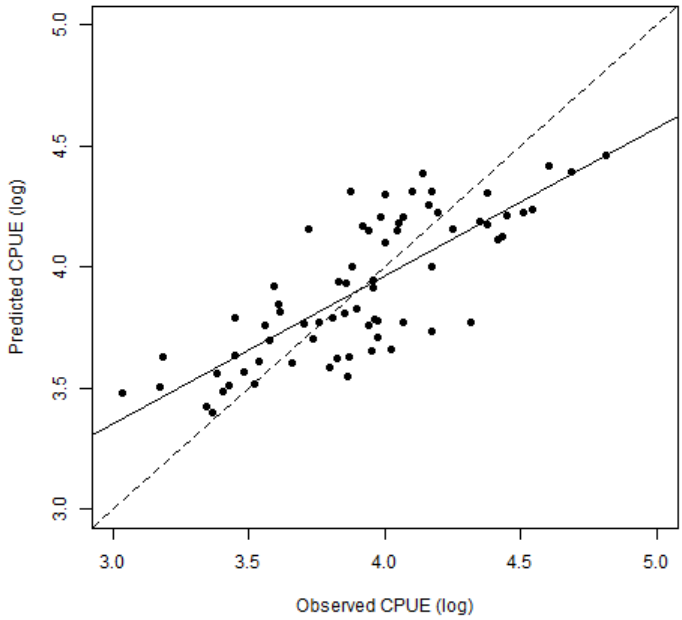

Figure 31: Predicted values of Queensland barramundi catch per unit effort (CPUE) from the predictive model *v.* observed CPUE. Variables in the model are dry season (July–September) rainfall, mean annual evaporation, and maximum temperature of the wet season (January-March). Linear fit plotted, adjusted R^2= 0.59.

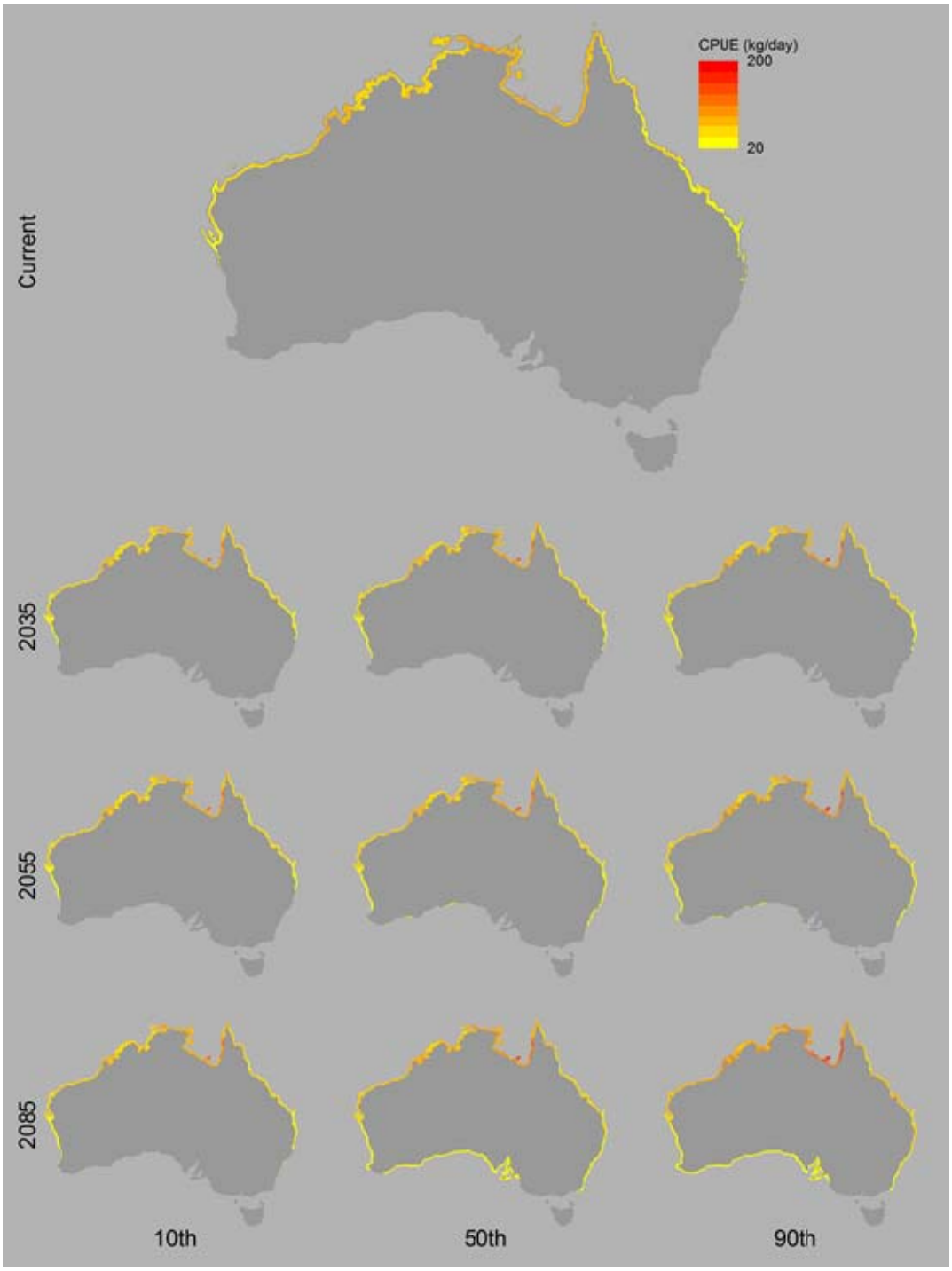

Figure 32: Current predicted catch per unit effort (CPUE) for Australia shown on the top using training data from Queensland. The bottom panels show future predictions (and variability using 10th, 50th & 90th percentiles) for a high emission scenario (RCP 8.5) for 2035, 2055 and 2085. Both current and future have been clipped to areas that are never exposed to monthly mean temperatures below 15 degrees.

Species Distribution Model (SDM)

The current species distribution model generated closely matches that of the present barramundi range, though due to our conservative methods of clipping the distribution to river reaches connected to waterways in which barramundi have been recorded, the realised range is likely to be larger than estimated. Observation records are highly limited by the inaccessible coastline of the remote monsoonal north of Australia. Gaps in the distribution are likely to be artefacts of remoteness and rugged terrain and lack of reliable reporting. Interestingly, the coastal lowlands of the Queensland Wet Tropics bioregion appear to be very climatically suitable for barramundi. However, this suitability is not reflected by the relatively low commercial catch rates evident from the region. This discrepancy may be explained in several ways. Firstly, the waterways in this region are characterised by very short catchment lengths, steep slopes, and constant flow, meaning that total area capable of supporting males in the freshwater stage is actually relatively low. Coupled with this is the fact that large swathes of the fresh to brackish wetlands on floodplains have been drained and developed for sugarcane and other agricultural activities, as well as urban development. Thus the recent loss of habitat in this bioregion of the species' distribution might not have been adequately captured by the model and these two factors may explain the discrepancy between measured CPUE and estimated suitability, which is predictive of abundance (VanDerWal et al., 2009). Additionally, barramundi is a prized sport fish and as such is further subject to observer bias driven by remoteness. The Queensland Wet Tropics has both a higher population density than the remote north and has been developed for tourism, including a focus on sport fishing. Despite the limitations of the observation data for this species, the SDM was proven to be a useful indicator of climate suitability and current species range from which projections into the future could be benchmarked against.

The future distribution of climate suitable for barramundi, as predicted by MaxEnt (Figure 29), contracts in the northern-most range of the species across all GCMs (represented here by the 10^{th}, 50^{th}, and 90^{th} percentiles of distributions estimated under RCP8.5). Suitable climate increased in inland parts of the Northern Territory and the Gulf of Carpentaria. While all areas are predicted to be exposed to warmer temperatures between 1.7 and 5.8 °C above current temperatures for measured distinct subpopulations (Figure 27, Appendix 5), the tip of Cape York and the Top End are projected to experience entirely novel average temperatures to which Australian barramundi have not yet been exposed. However, because northern Australian populations of barramundi perform well at high temperatures above their current

exposure (Newton et al., 2010; Component C) , some adaptability and even improvements in abundance with populations in northern regions is still possible although monitoring in this area is advised. Our future SDMs are useful in that they show that the northernmost range of barramundi may be exposed to average temperatures that are entirely novel to Australian populations of the species (Figure 29).

The variation between the GCMs in the future is mostly driven by broadly variable estimates of future rainfall, which most models project to change little, but few project to either increase or decrease dramatically. It is this increase in rainfall and therefore flow that drives the unrealistic projected inland improvement of suitability of climate for the species at the wettest 90th percentile of GCMs. Additionally, James at al., (2013) flagged slight overestimates of runoff in inland Australia as a known issue with their method of modelling runoff. While it represented an overestimate of less than a millimetre when compared with other runoff models for current conditions, this accumulates over the large reaches of inland Australia. Inland expansion is also limited by the catadromous breeding structure of the species and their requirement for perennially flowing waterways.

Regression model of CPUE

We found that 59.2% of spatial variation in CPUE could be described by three long-term climate variables: rainfall in the dry season (July – September); mean monthly evaporation; and minimum temperature in the summer wet-season (January-March). Our results indicate that abundance of barramundi is highest in areas with low rainfall in the dry season, high mean monthly evaporation, and high minimum temperature in summer. These findings at first seem to contradict findings of temporal models (which we expand upon below), but are not unexpected given that barramundi catch is highest in the north of the wet-dry tropics which is hotter and more monsoonal (distinct wet-dry monsoon) (Figure 32).

Based on current CPUE, our model underestimated highest levels of catch and overestimates lower levels of catch. Despite the limitation of our model around the extremes in CPUE, however, barramundi CPUE is expected to increase under all future scenarios in a pattern consistent with current measured mean CPUE.

Temperature

Temperature is not known to directly affect fecundity in barramundi and is therefore not directly related to changes in abundance. However, it can indirectly affect recruitment success

by influencing species range, growth, prey availability, parasite load, and mortality. Temperature is a major driver of species distribution ranges in terrestrial (Hughes 2003), marine (Perry et al., 2005) and freshwater species (Oberdorf 1995). In particular, the influence of temperature on distributions of ectotherm species is well established and recent shifts in distribution have already been observed to track climate change velocity among marine fishes (Pinksy et al, 2013). Our result showed that expansion in suitable range of the fishery is expected towards 2085, with barramundi expected to naturally colonise habitat south of their current geographical limits into the future. This expansion will occur both along the eastern and western Australian coastlines where suitable habitat to complete this species' lifecycle permits. The realisation of this expansion, however, may not be solely determined by environmental factors and habitat suitability factors, but also by the relatively low rates of inter-estuary migration of barramundi (Moore et al., 1982; Keenan 1994). It suggests that translocation, or stocking of fish from adjacent genetic stocks, may be a successful strategy to expedite the expansion of this species' range, particularly if there is a desire to expand the size of the fishery south of the current species geographical limits. Of course ecological considerations will need to be considered under any such strategy.

The modelled increase in abundance with higher minimum summer temperatures could be explained by increases in growth and prey availability and changes to parasite load and mortality. Given a well-balanced diet, barramundi exhibit a clear growth curve at all life stages, with growth increasing up to an optimum temperature of 31 °C, before dropping off upon nearing the thermal maximum of 40 °C (Katersky et al., 2007). Higher growth efficiency is expected where temperatures deviate least from the optimum growth range, thus we expect areas with the highest minimum temperatures to exhibit the greatest growth. Accordingly, it is not surprising to find that highest catch occurs in the regions with the highest minimum temperatures under our models. This effect may be compounded by increased prey availability. Heterotrophs at all levels of the food chain are influenced by autotroph primary productivity (Power 1992), which is closely coupled to temperature (Edwards et al., 2006). Hence, resource restriction and prey density of herbivorous zooplankton determines growth and recruitment success of many juvenile fish (Fermin and Seronay 1997). Barramundi prey availability may be higher in regions with hotter minimum summer temperatures during periods of optimum growth. Additionally, increases in feeding intensity can also be induced by higher temperature (Katersky 2007). Whilst higher minimum summer temperatures may drive an increase in productivity of the barramundi- specific food web, it also has to be noted that if water temperatures reach outside the range optimum for

lower trophic members of that food-web there may actually be a decrease in productivity. Obviously more research is needed to examine the effect of climate change on the productivity of tropical freshwater, estuarine and near-shore aquatic foodwebs.

Rainfall and flow

A number of studies have found rainfall and total river and stream flow to correlate with temporal abundance (Meynecke et al., 2006; Staunton-Smith et al., 2004, Robins et al., 2005). Flow is known to influence changes in population dynamics such as recruitment, abundance, survival, and migration in many estuarine fisheries (Drinkwater and Frank 1994; Gillanders and Kingsford 2002; Whitfield 2005). There is some evidence that this may be linked to changes to primary and secondary production due to addition of terrestrial nutrients (Salen-Picard et al., 2002), though the physical effects of strong flow may be more important to dry-tropics fish species because it can scour away sediment and aquatic vegetation (Rayner et al., 2008) leading to a negative correlation with plankton (Kimmerer 2002). Warm regions with low (but constant) dry season flow may facilitate higher prey availability and therefore higher abundance, though this hypothesis needs validation. Flow is well known to affect distribution of abundance by expanding, reducing, or connecting habitats suitable to barramundi (Loneragan and Bunn 1999). High flow in the wet season may fill and expand nursery habitat and decreased flow in the dry may reduce disturbance to the nursery, thereby allowing higher recruitment of juveniles. Year class strength is linked to higher flow temporally (Quinones and Montes 2001; Staunton-Smith et al., 2004), so areas with high flow in the wet and low disturbance in the dry may support higher abundance. High wet season flow also increases catchability (Balston 2009a,b), which might confound its use as an indicator of abundance.

Evaporation

Evaporation is most likely to affect barramundi abundance in the nursery habitat, and indeed has been shown to have a positive relationship with catch. Balston (2009b) found catch increased with decreased evaporation through time at the study location. This was most likely attributed to higher nursery habitat availability and therefore juvenile recruitment success during years with high dry season rainfall and low evaporation. Juvenile barramundi require connectivity between the mangrove zone and coastal swamp systems (Moore 1982) and higher dry season rainfall and lower evaporation were hypothesised to facilitate this connectivity (Balston 2009b). This result seems to be in conflict with our finding that high evaporation and low dry season rainfall contribute to higher abundance when considered across space rather than through time, ie. abundance is higher in the hotter, dry, highly seasonal gulf compared to the wetter, cooler east coast. However, the location of the Balston (2009b) study fell among sample grids with the lowest rainfall, highest evaporation and highest CPUE. This suggests that low dry season rainfall and high evaporation might be

spatially descriptive of broader habitat availability or area. Barramundi abundance has been linked to extent of saltmarshes, flats and mangroves (Meynecke et al., 2007) which co-vary with evaporation, rainfall and temperature. Determining the mechanism of influence of evaporation on barramundi recruitment requires further examination of links between habitat extent, availability and climate.

Future CPUE

Our results showed future catch improves under all GCMs in the same pattern as observed CPUE (Figure 32). This improvement is seen across all GCMs in spite of wide variation in rainfall between the models. This is due to the uniform prediction of higher temperature and evaporation across all GCMs. Unlike the MaxEnt Species Distribution Model, our regression model enabled us to project changes in CPUE outside the known exposure of the species, so we did not see the same decrease in suitability of the north to the species as the north became hotter and drier. It's important to note that CPUE will not increase indefinitely with hotter, drier conditions due to thermal growth and mortality limits and barramundi's requirement of interconnected waterflow between freshwater and estuaries during its life-cycle. Future mean temperature in the north does not reach above the range experienced by the species as a whole, or the known physiological limits (Katersky and Carter 2007), but it is not known whether the northern areas may become too dry as the model's training populations in Queensland are exposed to slightly wetter conditions than the populations in the Northern Territory. It is also not known what impact the increase in daily maximum temperatures will have on CPUE. Monthly maximum air temperatures will reach above the thermal limit of the species in some populations by 2085, but the relationship between air temperature and water temperature is complex. In large or flowing bodies of water, the relationship between air temperature and water temperature has been described as sinusoidal (Mohseni et al., 1998); water temperature is higher than low air temperatures and lower than high air temperatures. As such, we expect large and flowing bodies of water to remain well below the thermal threshold of the species. This assumption requires verification, but there is a paucity of available water temperature measurement data in the tropics. In the case of shallow water bodies, like lagoons and billabongs, they track diurnal temperature fluctuations much more closely (Hamilton 2010) and may therefore reach temperatures above thermal maximum, which may affect recruitment and therefore CPUE by 2085. We need to monitor the influence of present-day heat waves on shallow water bodies in the north to better understand how recruitment of the species is affected by extremes.

Like the SDMs, the regression model suggests a range expansion of the species due to a southerly shift of exposure to minimum temperatures of above 15 °C, though a realistic expansion is dependent upon the ability of barramundi to migrate south in numbers great enough to establish more southerly populations. Extreme cold events would also impact significantly on the viability of barramundi in southern regions of range expansion.

Overall, we are most likely to see the fishery remain stable or improve into the future, but this result should be considered with caution in the hottest and driest parts of the species range given that the CPUE model extrapolates slightly outside current exposure.

Modelling limitations

In any modelling exercise it is important to recognise limitations due to input parameters that are available and/or not available. Limitations of the modelling methods used include observer bias; a lack of robust availability of spatially representative training data; projecting into novel space; projecting into novel climates; complexity of predicting factors like perenniality or incorporating barriers; difficulty predicting influence of extreme temperatures; and the possibility that climate variables act as surrogates for habitat types. Further, both methods used are subject to inability to prove causality (Quiñones and Montes 2001), confounding factors such as habitat use limitations, habitat loss, and pollution (Meynecke et al., 2006), recent climate change, in this case experienced during the 25 years of data collection, improvements in CPUE with fishing experience, the confounding effects of stock size and fishing pressure (Walters and Collie 1988); and a lack of consideration of species interaction such as prey availability. While these issues can pose serious limitations to the application of correlative models for fisheries species under high harvest pressure, or persistence in highly modified or impacted environments, the ability of our training models to predict current CPUE and species distribution demonstrates the overall reliability of our approach to forecast future responses of barramundi under the 18 GCMs.

Conclusion

Two correlative methods of modelling reveal that the barramundi fishery is likely to remain stable or see improved CPUE across much of northern Australia. Both also found the climatically suitable range of the species to spread further south if temperature increases as predicted by GCM models. The novel and exploratory nature of these methods have revealed future areas of study for the species, such as more detailed analysis of the interaction between climate and habitat extent on recruitment. Our regression results are largely consistent with

the patterns of abundance already shown by the species, but must be interpreted with caution at the extremes of high temperature and aridity predicted in the future as these climatic variables are projected to extend beyond the range to which the species is currently exposed.

ii) Modelling climate change impacts on barramundi aquaculture

Currently, 38.2% of continental Australia is thermally suitable for pond-based aquaculture of barramundi (Figure 33). Thermally suitable area covers much of northern Australia (Figure 33). Within this suitable area, weight gain per fish per year (Jan-Dec) ranges between 296-2989 g, with the most productive areas in the North. Most of Australia (27.5% of Australia or 71.9% of area suitable for barramundi aquaculture) could theoretically produce fish between 1500-2500 g within one year; only 5.7% of Australia produces fish over 2500 g. This closely matches growth rates reported at the four farms (Figure 33).

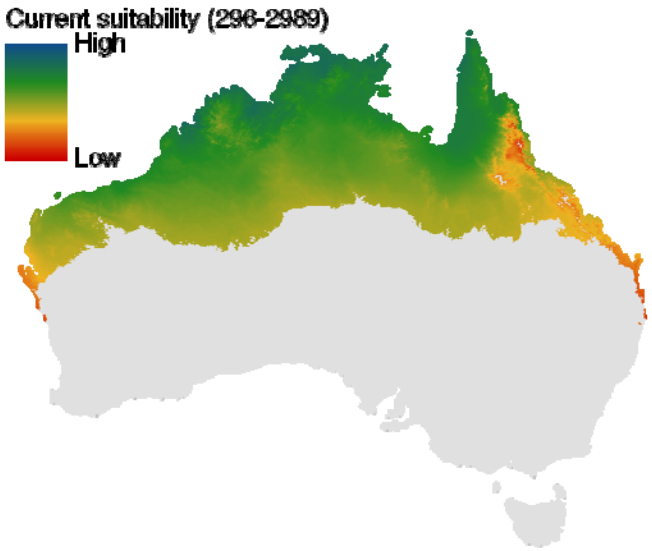

Figure 33: Current suitability of Australia for barramundi aquaculture as predicted by temperature and a factorial growth model (Bermudes et al., 2010). Numbers indicate growth in grams over one year, but should be interpreted as suitability for growth.

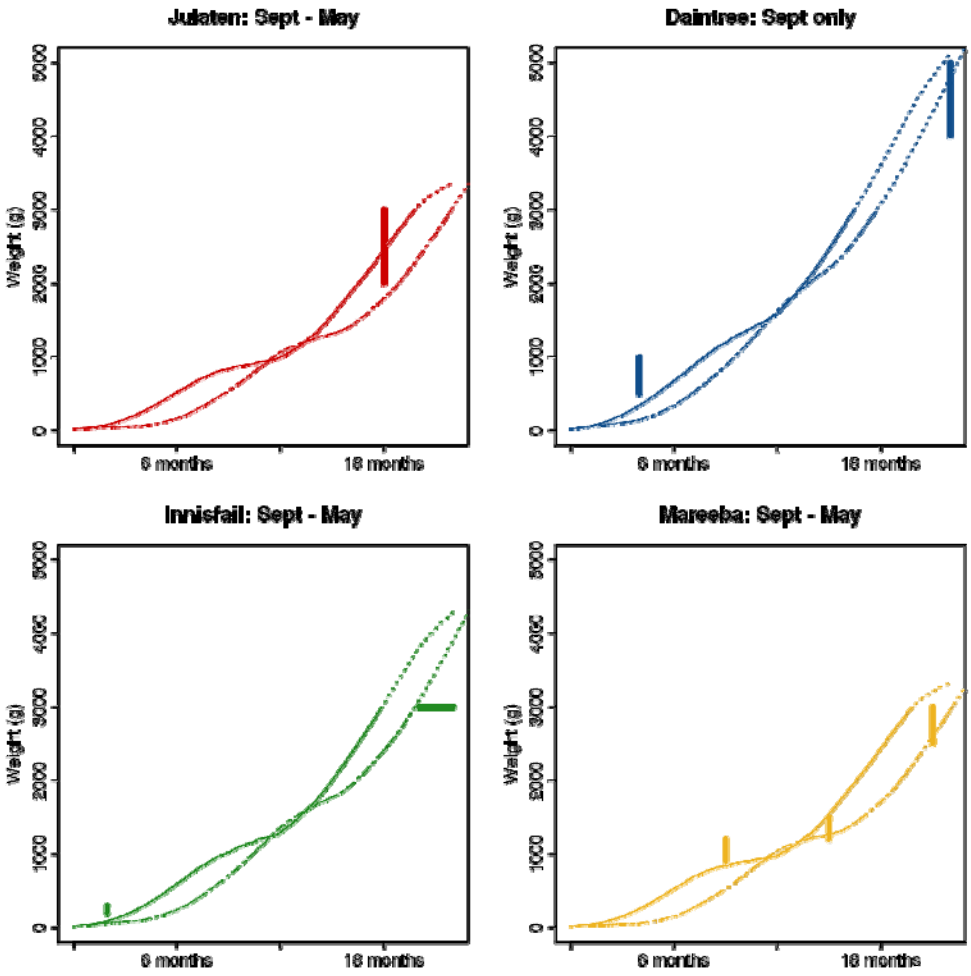

Figure 34: Validation of the performance of the Bermudes et al., (2010) growth model. Reported size ranges at a given age from four different spatially distant farms were compared with the growth curve at the earliest stocking month (September – solid line) and the latest stocking month (May – dot-dash line). Dotted lines represent the growth range beyond which the Bermudes et al. (2010) equation was tested (3000 g).

All future scenarios showed that a) there will be a significant southward expansion of regions suitable for farming barramundi; and b) areas currently suitable will see increased weight gain in the future (Figure 35). The total suitable area for pond-based barramundi aquaculture increases in the future. Currently, 38% of continental Australia is thermally suitable for barramundi aquaculture. Suitable area increases to 47.5% by 2030; 54.5% by 2050; and 66% by 2080. This represents an expansion of thermally suitable area of 173% of current area by 2080. Barramundi will grow faster in the future due to rising temperature. In 2030,

barramundi grown within current suitable areas will gain a further 238.3 g per year, but may increase as much as 484 g. By 2080, the average and maximum gain per year increase to 354.5 and 1000 g more than current, respectively. Areas not currently suitable for barramundi aquaculture may support fish that grow up to 1754.7 g by 2030, and up to 1936.2 g by 2080. Currently suitable areas will produce larger fish, and some previously unviable areas will produce fish of a size that is on par with current viable areas.

This will lead to larger fish growing across more of Australia. The actual extent of increased growth will be obviously limited by the biological and farm management capacity for barramundi to achieve these growth levels and we do not imply from our models that the true biological capacity of barramundi to grow to the size predicted by the model may be actually achieved. Growth expectations under our models are indicative only, especially outside of the range of weights not encapsulated by the Bermudes et al., (2010) growth model. However, by 2080, 42.4% of Australia will be thermally capable of producing barramundi between 1500-2500 g within one year. This is 154% of current area producing fish of this size range. Furthermore, 13.1% of Australia will produce fish > 2500 g, which is 230% of current area. Of this area, 16.8% (or 2.2% of Australia) will produce fish above 3000 g, which is larger (and therefore faster-growing) than any current area can produce.

The majority of inland Australia experiences, on average, more potential evaporation than precipitation throughout all months (shown in tan, Figure 36). Figure 36 also shows the number of months, current and future, where precipitation is greater than evaporation. Only a very small area in Tasmania receives more precipitation than can potentially evaporate every month. Within the range thermally suitable to barramundi, the Wet Tropics experiences rainfall that is most consistently higher than evaporation. This area, therefore, requires the least water supplementation. Much of the east coast of Queensland, the tip of Cape York, and Arnhem Land are unlikely to require water supplementation for at least six months of the year, while the north of the continent broadly receives a more rain than can evaporate for during at least 3 months of the year. This general pattern remains the same across all GCMs in 2085 (represented by the 10^{th}, 50^{th} and 90^{th} percentile), with a trend toward drying at the 10^{th} percentile and more rainfall than evaporation during more months at the 90^{th} percentile. Areas that are most likely to remain the most suitable are those that are bluest on the 10^{th} percentile model in 2085.

Although there is significant uncertainty in the exact nature of future climate change, there was consensus across all future scenarios examined for several important factors. Where

winter temperatures drop below optimal growth levels, but do not reach the lower thermal tolerance limit, temperature can greatly reduce the annual mean weight gain of barramundi in southern suitable regions due to low weight gain during these months. Therefore, two factors limit productivity in southern barramundi aquaculture, 1) the hard southern line where the monthly mean temperature drops below the minimum thermal tolerance limit, and 2) the sub-optimal winter temperatures that prevent quick weight gain. We confirm that in a warmer climate, a southern expansion of pond-based aquaculture will be possible; higher annual weight gain and productivity can be expected much further south.

Barramundi both grow and feed optimally between 26 and 35 °C before feeding declines steeply at 38 °C (Bermudes et al., 2010). Barrmundi growth continues to increase into the future because air temperature is not predicted to go above this growth limit before 2085. The relationship between water temperature and air temperature deviates at both high and low temperatures (Mohenesi et al., 2002) at high temperatures water temperature is lower and at low temperatures it is higher. This means that it is likely that use of air temperature will over-predict actual size at higher temperatures, but while it is not likely that we will see barramundi growing to the sizes predicted here, the pattern of suitability stands.

Model limitations

The factorial growth model developed by Bermudes et al., (2010) does not incorporate variability of growth between individual fish, and as such only represents median possible growth. Some differences in performance of fish from different genetic stocks under different temperatures are known (Newton et al., 2010) and may account for some of the variability in the model as the data used in its development were collected over a wide area and therefore a wide range of climate (Glencross 2008).

The relationship between monthly average air temperature and water temperature has long been known and used to determine water temperature (e.g. Mackey and Berrie 1991; Stefan and Preud'homme 1993; Imholt et al., 2012), but it is not directly attributable to radiation of energy from the air to water. Instead, this relationship is driven by correlations between air temperature and solar radiation and evaporation. Development and validation of continental-scale energetic heat flux models is complex and some necessary data inputs defined by Bechet et al., (2011), such as wind velocity and pan evaporation have not yet been developed for the future. However, given the close relationship between air temperature and water temperature show by Johnson et al., (2013), we considered use of air temperature in conjunction with a

factorial growth model able to robustly project patterns of growth across space and therefore predict the most suitable areas for barramundi aquaculture.

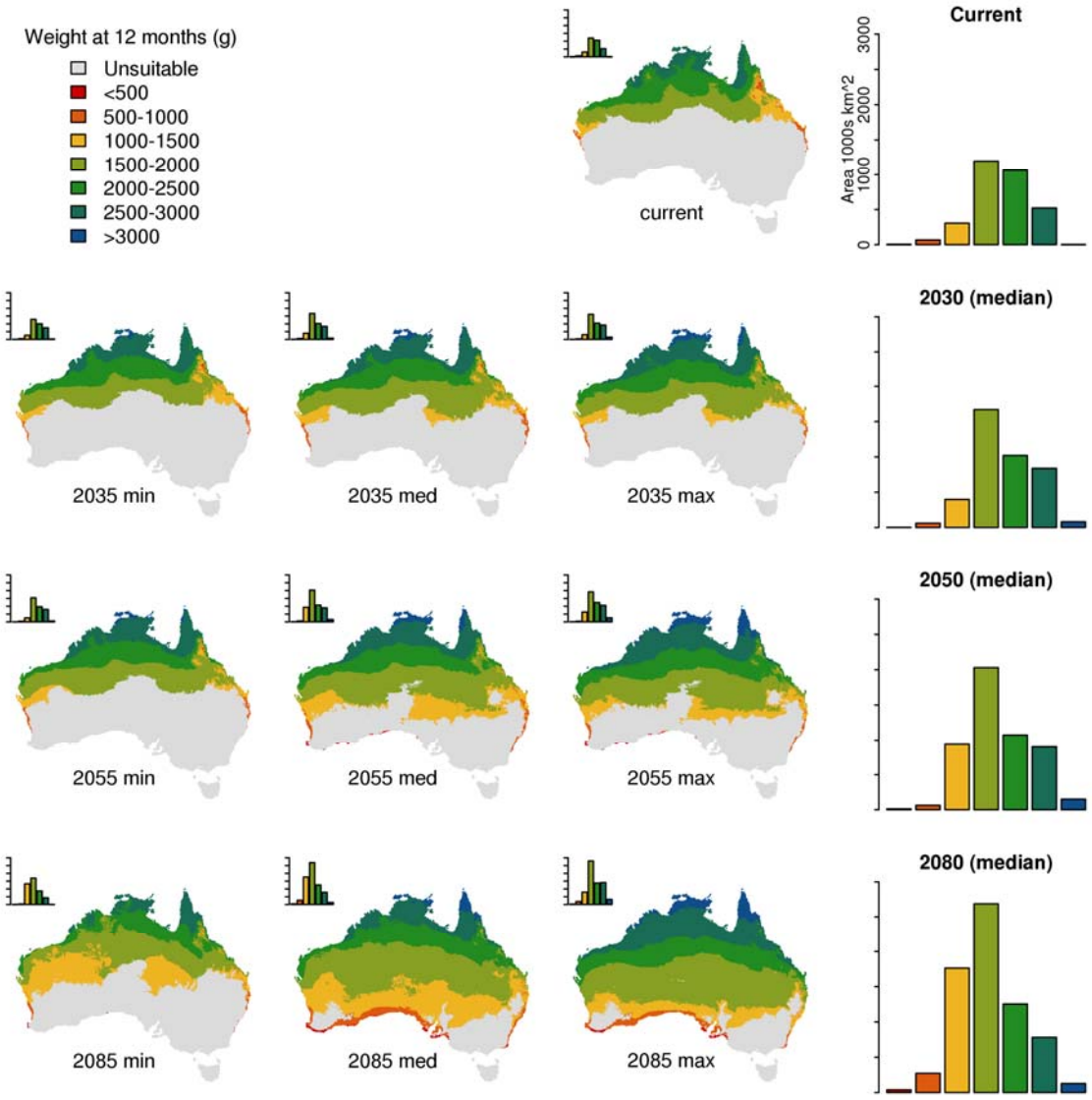

Figure 35. Current and future suitability (estimated as gain in grams per day for one year) for barramundi aquaculture. Where monthly mean temperature during the coldest month reached 15°C these areas were considered unsuitable and were marked in grey. Future scenarios show SRES a1b, weighted mean of 30 runs of 8 GCMS. Area within each growth category shown on barplots in 1000s km^2.

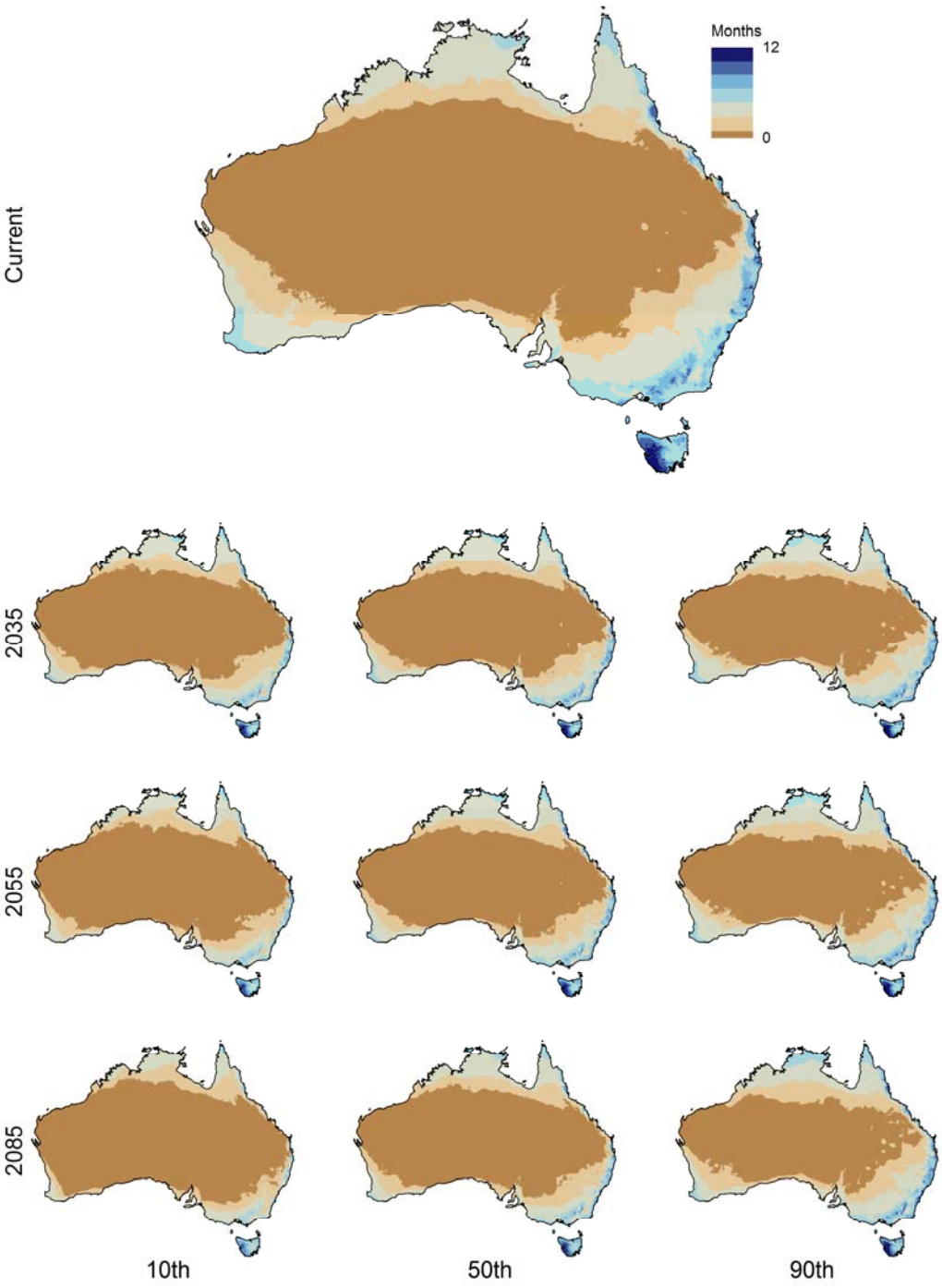

Figure 36: Number of months for which total precipitation is greater than total potential evaporation. Tan areas indicate areas where, on average, potential evaporation is higher than precipitation for all months, and darkest blue indicate areas where precipitation is greater than potential evaporation in all months.

Future work

Given the limitations and assumptions involved in developing a novel continental-scale model for fin-fish aquaculture, we focussed on the impact of temperature on production. This is because the IPCC can project the likely range of temperature increase with confidence, making temperature the most certain to impact aquaculture production. However, assessing the suitability of areas for pond-based aquaculture is a complex process of a) integrating information on environmental parameters (including temperature, dissolved oxygen, and evaporation), b) determining how these impact the biology of the fish (ie. growth rate, vulnerability to parasites and disease), and c) translating these parameters to industry costs (ie. impact on quantity of feed required, water replacement, and aeration). Sufficient datasets for these variables do not as yet exist across a broad spatial scale, but future work should seek to gather such data and incorporate it into a predictive model.

Biological interactions with growth should also be considered. Currently, temperatures restrict the range of many fish pests and parasites, and warmer waters are likely to lead to an increase in the incidence of outbreaks of unwelcome infections (Lafferty and Kuris, 1999; Marcogliese 2001). Temperature increases of only a few degrees could have indirect implications for aquaculture facilities, e.g. increased incidence of harmful algal blooms that release toxins into the water and generate fish kills (Shumway 1990).

The validation of the capacity of the Bermudes et al., (2010) equation to predict growth could be improved upon through sourcing further real growth data from ponds outside the size ranges they examined, through a wider range of space and therefore climate, as well as using fish from different genetic stocks which may exhibit differing growth trajectories. Construction of stock-specific factorial growth models would allow us to assess the potential for maximising production conditions for each stock, both currently and under a warming climate.

Implications for farmers

Continued expansion of the barramundi aquaculture industry should be possible well into the future even under the hottest 'business-as-usual' emissions scenario. Importantly, the higher temperatures experienced further south may open up possibility of novel farming locations closer to the southern markets. For example, south-east Queensland is projected in 2030 to experience the same weight gain currently experienced by the lucrative northern east coast farms. Meanwhile, north-eastern coast farms can expect increased weight of up to 500 g

annually by 2030, and weight gain equivalent to current Northern Territory rates by 2080. The east coast of Australia requires some of the lowest levels of water supplementation (Figure 36), so farms located in this region are likely to improve biomass yield on their current water supplementation regimes. The greatest southerly expansion occurs in coastal Western Australia, and some of New South Wales becomes thermally viable for aquaculture. Most importantly, annual temperatures are not projected to reach averages greater than 40 °C (although maximum temperatures within a month may – Figure 27), so even the barramundi aquaculture industry in the far north of the NT is expected to remain viable with potential for expansion.

BENEFITS AND ADOPTION

Scientific explorations undertaken in this project have provided important data on the genetic stock structure, physiological tolerances and parasitic risks of Australian barramundi populations, along with predictions on future resilience of both the wild barramundi fishery and aquaculture production to climate change. Therefore the beneficiaries of knowledge disseminating from this project are multifaceted and include resource-conservation managers, commercial and recreational fishers, aquaculturists, development planners and scientists.

The most comprehensive and definitive study to date on the genetic population structure of Australian barramundi now provides clear delineation of important genetic stock boundaries across the entire northern distribution of this species. This information is of value to fisheries managers in the formulation of translocation policies and fishery management of individual stocks. Requests for up to date information on genetic stock boundaries have already been received from fisheries policy makers developing translocation policies for this species in Queensland. The current genetic data for the Ord River region has also already been provided and used by a Western Australian hatchery involved in stocking barramundi into Lake Kununurra in Western Australia. Furthermore the wild-population genetic dataset has also been utilized by the current Seafood CRC in an Industry-wide genetic audit of aquaculture brood-stock to assess the extent to which current cultured brood-stock populations capture available genetic diversity within the species in Australia, as well as to determine the broad geographic region of origin for wild-caught brood-stock currently being held by the aquaculture industry. It has also been successfully used within the current project to confirm the wild-stock ancestry of hatchery produced fingerlings used in physiological experiments. Genetic data show that genetic stock structure of barramundi has been remarkably stable over the last quarter of a century, indicating that the stock boundaries defined herein will be likely indicative of Australian barramundi stock structure throughout the critical period defined by climate change simulations. One thing for fishery managers to take notice of, however, is the possibility for short-term perturbations in genetic stock structure due to heavy restocking and widespread flooding, as was observed in the 2008 samples from the Fitzroy River, Queensland.

The parasite risk assessment undertaken demonstrates that salt water barramundi aquaculture managers need to be aware of metazoan parasites that have the potential to inflict losses under

current and future climate conditions. Experiments with two of these parasite species demonstrate that the various genetic stocks of barramundi are equally susceptible to infection and that parasite life histories may speed up under warmer conditions leading to more frequent and severe disease outbreaks. Therefore aquaculture farm managers should begin to implement more routine parasite screening procedures and consideration of how strategic parasite management needs to be conducted. To aid farmers to begin to develop these strategic management plans, results from this project have been widely disseminated to the aquaculture industry and government through direct communication and the CI Hutson has already begun to work closely with key representatives of the Australian Barramundi Farmers Association to develop a protocol that can be used by farmers to enable strategic parasite management responses in variable climates.

Our modelling of projections of CPUE indicate that the barramundi fishery, as predicted under the various IPCC "business as usual" greenhouse gas emission scenarios, should respond quite positively to changes in climate over the time frames examined. The fishery is observed to expand and increase in productivity and this information will be vital for resource managers when assigning commercial fishery "catch allocations" into the future. Similarly, pond-based barramundi aquaculture is predicted to benefit with an expansion in the land area possessing thermal profiles suitable for barramundi culture, both inland in northern regions, and towards the south of the continent, as well as overall increases in productivity primarily as a consequence of increased annualised growth rates. This opportunity for expansion should be incorporated into future aquaculture development plans that are currently underway by several state governments that account for the possibility of barramundi aquaculture in regions where viability is presently marginal. Barramundi is rapidly becoming a global aquaculture fish commodity and in Australia there is enormous potential to capitalise on the culture of this species by having in place development plans and aquaculture precincts that enable quick establishment of barramundi aquaculture farms.

Barramundi from multiple genetic stocks were also shown to be quite resilient to high water temperatures and correlated hypoxic oxygen conditions. This, coupled with increases in farming area, may suggest that the barramundi aquaculture industry will not be dramatically affected by future climate scenarios. However, we caution against this perception as aquaculturists need to be mindful of extreme "cold spells" in the south of the distribution which over a sharp period may reduce water temperatures close to that considered lethal to the species (<15 °C). Conversely, extreme heatwave weather events may rapidly lower oxygen

levels inducing stress on farmed stocks that may impact both on growth and immune functioning. These stress events, coupled with speeding up the life cycle of several important barramundi parasites, would need to be implemented into production management.

PLANNED OUTCOMES

This project has delivered on the principal planned outcomes by providing managers, commercial fishers, aquaculturists and policy planners with new quantitative and modelling data to facilitate the development of adaptation strategies for the multi-faceted barramundi fishery. Specific outputs that have contributed to this key outcome are:

- an expanded knowledge on the population genetic structure of barramundi across northern Australia that has recognised five new genetic subpopulations (to bring the total to 21 genetically distinct subpopulations) and defined the species into six major stocks. It was also shown that the majority of the current genetic stock structure was relatively stable over a 25 year period from that of previous genetic studies.
- Tolerance to acute thermal stresses, as determined by disassociated caudal fin cells as an indicator of loss of swimming equilibrium, showed differences among populations from five of these genetic stocks. Using this approach, barramundi from northern regions of the distribution were shown to have higher tolerance to acute warm temperature stresses.
- Physiological testing of barramundi from 4-5 genetic stocks showed that while there are fine scale differences amongst populations, collectively Australian barramundi show similar responses in their resting metabolic rate, maximum metabolic rate, aerobic scope and hypoxia tolerance when subjected to various environmental temperatures up to 38 °C. Despite barramundi being exposed to high water temperatures examination of the factorial difference in aerobic scope showed a 280% increase from resting to maximum metabolic rate at 38 °C, indicating that barramundi may be able to tolerate even higher temperatures before severe physiological effects on energy metabolism are seen.
- A parasite survey and risk assessment identified over 80 metazoan parasites known to affect barramundi of which the monogeneans *Neobenedenia melleni* and *Benedenia epinepheli,* among others, were identified as high risk parasites. Experiments manipulating temperature and salinity on *Neobenedenia* and the parasite *Lernanthropus latis* showed that the life history dynamics of these two important parasites changes under different environmental backgrounds which results in these parasites completing their life cycles quicker. Also barramundi from the different

genetic stocks did not exhibit differences in infection when challenged by the east coast strain of *Neobenedenia.*

- Modelling of the wild fishery under different Global Climate Models that make up the IPCC "business as usual" greenhouse gas emissions scenario predicted that climate suitable for barramundi in Australia will be extended in a southerly direction on both the eastern and western coasts due to increasing average and minimum temperatures. The optimal model incorporating climate derived correlates of catch per unit effort (CPUE) predicts that future medium CPUE will increase in key areas with climate change. Areas that currently yield the highest CPUE are expected to remain high into the future and all areas are expected to show some increase in CPUE. Potential for new fisheries may arise as suitable climate for this species moves southward.
- As with the wild barramundi fishery, simulations predicted an increase in land area with thermal profiles suitable for barramundi pond-based aquaculture, as well as increased productivity, particularly in northern regions of the distribution. Thus barramundi aquaculture may be able to expand as an industry south as climate change proceeds towards 2080.

CONCLUSIONS

Barramundi, *Lates calcarifer*, is an iconic fish species in northern Australia which has high socio-economic importance. Barramundi, as a species, supports both wild harvest and recreational fisheries, as well as being the major finfish species farmed in northern Australia. Due to the importance of barramundi, this project aimed to identify potential vulnerabilities for this species that may impact on aligned stakeholders as a result of future climate change. As a result, the most comprehensive study to date that incorporates new data on genetics, physiology, parasites and modelling to determine the vulnerability of Australian barramundi to future climate, was conducted. The project commenced addressing four major objectives;

- define the current genetic stock structure of barramundi so that a rigourous understanding of neutral genetic variability across the whole distribution of the species could be known. This study confirmed the existence of 21 genetic subpopulations of barramundi which could be defined to six major genetic stocks. Despite sustained periods of commercial and recreational harvest, along with large restocking programs in many areas, barramundi genetic stock structure was found to be remarkably conserved over 25 years from original genetic surveys conducted. Definition of the genetic stock structure of barramundi for the first time across its entire species range will be valuable information for those addressing translocation and fishery stock management purposes, as well as identification of possible genetic sources for future barramundi genetic improvement programs.

  The confirmation that Australian barramundi comprise six major genetic stocks was used as the basis in later experiments to examine whether these differences in population genetics translated into any physiological differences in metabolism and hypoxia tolerance when exposed to higher water temperatures than the species is commonly exposed, as well as to examine environmental parameters currently and predicted to be experienced by the various subpopulations into the future.
- Undertake physiological testing of barramundi from the various major genetic stocks to examine if there was evidence for differences in energy metabolism due to temperature. The series of experiments in this component identified differences in the response of different genetic barramundi strains stocks to acute temperature stresses, but no major significant differences in their resting and maximum metabolic rates, or tolerance to hypoxia. When barramundi caudal fin cells were challenged to higher

temperatures as an indicator of loss of swimming equilibrium due to an acute temperature stress (40 °C) northern populations were observed to be more tolerant to this thermal stress than southern latitudinal barramundi populations. These acute temperatures are unlikely to be experienced routinely by barramundi under future climate change, however, the responses seen do indicate that an underlying genetic difference in tolerance to upper thermal stress exists among genetic stocks.

When barramundi from the various genetic stocks were exposed to temperatures between 18° in and 38°C no significant differences were seen in their resting and maximum metabolic rates, aerobic scope, or tolerance to low oxygen conditions. These results show that energetic aspects of the metabolism of this species are similar among the genetic stocks. Even when fish were pushed to temperatures as high as 38° C they possess the capacity to cope with these temperatures for a short time at least. Given that the water temperatures we challenged barramundi to are above those expected by climate models to the year 2080, our scientific tests suggest that barramundi will be able to cope with several degrees Celsius rise across the majority of the current distribution.

- Before this project there had not been a comprehensive survey of metazoan parasites infecting barramundi, nor a risk assessment of their potential impact for barramundi aquaculture. The tolerance of the various genetic stocks of barramundi to parasite infection, as well as the predicted response of important parasites themselves to future climate scenarios had never been examined. Parasite surveys from the field and literature identified greater than 80 metazoan parasite species infecting barramundi. Risk assessments have been conducted for these parasites to identify their potential impact for Australian aquaculture, with several parasites being ranked as being high risk species (ie *Neobenedenia, Benedenia* spp).

  Barramundi from the various genetic stocks were shown to exhibit similar susceptibility to the East coast strain of *Neobenedenia*. Additionally, life cycles of these important parasites were shown to speed up under increased temperature. However, a limit was reached where increased temperature and decreased salinities negatively impact on these marine parasites.

- Predict climate impacts on the barramundi species range, catch per unit effort (CPUE) across Australia, and aquaculture production through a modelling approach. Climate models showed that an average increase of 3.6 °C in air temperature is expected across

the current distribution of Australian barramundi by 2085, whilst rainfall may change little (as predicted by the 50th percentile of 18 GCMs). Evaporation is expected to increase. Some subpopulations are expected to be exposed to higher levels of change than others, however, monthly averages show that air temperatures (and thus water temperatures) should not reach the limits of barramundi thermal tolerance of 40 °C. The species distribution model of barramundi showed the northernmost range of the species contracts across all GCMs in the situation where the northern part of the continent became drier (10th percentile of GCMs), and to expand inland under the expectations of a wetter climate (90th percentile of GCMs). With little change in rainfall and hotter temperatures (50th percentile), the northernmost populations at the tip of Cape York and the Top End may be exposed to average temperatures that are novel to Australia barramundi, but which remain below the thermal maximum. The wild barramundi fishery has the climatic potential to expand in distribution southward as mean monthly temperatures reach that optimal to their survival. Our results indicate that abundance of barramundi is currently highest in areas with low rainfall in the dry season, high mean monthly evaporation, and high minimum temperatures in summer, and as such barramundi CPUE was modelled to increase under all future Global Climate Models that comprise the "business as usual" IPCC scenario.

We also modelled the impact future climate changes may have on barramundi aquaculture. Similarly, as observed for the wild fishery, aquaculture production is predicted to increase driven primarily by increased growth rates no longer impeded by low minimum monthly temperatures in winter. The thermal environmental profile suitable for barramundi aquaculture was also observed to increase, primarily in a southward manner.

In conclusion, the scientific research conducted throughout this project suggests that given an average 3.6 °C rise in temperature over the next several decades that wild barramundi and aquaculture populations may see increased productivity. As coastal and inland areas become warmer over time the climatic suitability across the continent for barramundi is expected to create the opportunity for them to expand their southerly distribution which will provide new opportunities for both the wild fishery and aquaculture industries. Implementation of this knowledge into future fishery management and aquaculture development planning should be considered as a priority to ensure that rapid adaptation of these industries can occur as these new opportunities for fishing and farming arise.

Furthermore, some distinct subpopulations such as that found in the Pilbara region are currently exposed to very hot, dry, seasonal conditions along the range edge and as such are already adapted to conditions that the more easterly populations are projected to be exposed to in the future. Similarly, along the east coast, southerly subpopulations will in the future be exposed to the same temperature conditions as more northerly subpopulations. Some assisted migration or stocking along the west-to-east and north-to-south gradient could increase resilience of subpopulations exposed to new climates through introduction of genes from subpopulations already adapted to cope with higher temperatures.

REFERENCES

Adamkewicz, S. L. and Harasewych, M. G. 1996. Systematics and biogeography of the genus *Donax* (Bivalvia: Donacidae) in Eastern North America. *American Malacalogical Bulletin,* 13, 97-103.

Adjei, E.L., Barnes, A. and Lester, R.J.G. 1986. A method for estimating possible parasite-related host mortality illustrated using data from *Callitetrarhynchus gracilis* (Cestoda: Trypanorhyncha) in lizardfish (*Saurida* spp.). *Parasitology,* 92, 227–243.

AIMS, 2008. In-situ sea temperature data.

AIMS 2012. AIMS Data Centre,. Townsville, Australia

Alberto, F. 2009. MsatAllele_1.0: An R package to visualize the binning of microsatellite alleles. *Journal of Heredity,* 100, 394-397.

Akram, M. 1994. Studies on host parasite relationships between marine food fishes of Sindh creeks and cucllanid nematode parasites (Cucullanidae: Nematoda) in Pakistan. *Pakistan Journal of Zoology,* 26, 182–185.

Akram, M. 1995. Occurrence of nematode parasites in marine food fishes of Sindh Creeks in Pakistan. *Biologia*, 41, 117–127.

Amin, O. M. and Christison, K. W. 2005. *Neoechinorhynchus* (*Neoechinorhynchus*) *dorsovaginatus* n. sp. (Acanthocephala: Neoechinorhynchidae) from the dusky kob *Argyrosomus japonicus* (Sciaenidae) on the southern coast of South Africa. *Systematic Parasitology,* 61, 173–179.

Amos, W., Hoffman, J. I., Frodsham, A., Zhang, L., Best, S. and Hill, A. V. S. 2007. Automated binning of microsatellite alleles: problems and solutions. *Molecular Ecology Notes,* 7, 10-14.

Andre, C., Larsson, L. C., Laikre, L., Bekkevold, D., Brigham, J., Carvalho, G. R., Dahlgren, T. G., Hutchinson, W. F., Mariani, S., Mudde, K., Ruzzante, D. E. and Ryman, N. 2010. Detecting population structure in a high gene-flow species, Atlantic herring (*Clupea harengus*): direct, simultaneous evaluation of neutral vs putatively selected loci. *Heredity*, 106, 270-280.

Australian Quarantine and Inspection Service, 1999. Final reports of import risk analyses on non-viable salmonid products, non-viable marine finfish products and live ornamental finfish and adoption of new policies. AusInfo, Canberra.

Arthur, J. R. and Ahmed, A. T. A. 2002. Checklist of the parasites of fishes from Bangladesh. *FAO Fisheries Technical Paper. No. 369/1. Rome*, pp 77. viewed online: http://www.fao.org/DOCREP/005/Y4350E/y4350e04.htm#bm4

Arthur, J. R. and Lumanlan-Mayo, S. 1997. Checklist of the parasites of fishes of the Philippines. *FAO Fisheries Technical Paper. Rome,* 369, 1-102.

Arya, S. N. 1991. Synonymy of the genus *Oceanocucullanus* Schmidt and Kuntz, 1969 with the genus *Indocucullanus* Ali, 1956 with the proposal of two new subgenera. *Rivista di Parassitologia* 7, 179-182. Not sighted, In Gibson, D. I., Bray, R. A., and Harris, E. A. (Eds.) 2005. *Host-Parasite Database of the Natural History Museum*, London. URL http://www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/.

Baker, R. L. 1996. Characterisation and utilisation of sheep and goat breeds that are resistant to helminths. In: LeJambre, L. F. and Knox, M. R. (Eds.), Sustainable Control of Parasites in Small Ruminants pp 172–177. ACIAR, Canberra.

Balston, J.M. 2007. An examination of the impacts of climate variability and climate change on the wild barramundi (*Lates calcarifer*): A tropical estuarine fishery of north-eastern Queensland, Australia. Ph.D. Thesis, James Cook University, Queensland.

Balston, J.M. 2009a. Short-term climate variability and the commercial barramundi (*Lates calcarifer*) fishery of north-east Queensland, Australia. *Marine and Freshwater Research,* 60, 912–923.

Balston, J. 2009b. An analysis of the impacts of long-term climate variability on the commercial barramundi (*Lates calcarifer*) fishery of north-east Queensland, Australia. *Fisheries Research,* 99, 83-89.

Bandilla, M., Valtonen, E. T., Suomalainen, L. R., Aphalo, P. J. and Hakalahti, T. 2006. A link between ectoparasite infection and susceptibility to bacterial disease in rainbow trout. *International Journal for Parasitology,* 36, 987–991.

Barger, I. 1997. Control by management. *Veterinary Parasitology*, 72, 493–506.

Barger, I. A. and Southcott, W. H. 1978. Parasitism and production in weaner sheep grazing alternately with cattle. *Australian Journal of Experimental Agriculture and Animal Husbandry*, 18, 340–346.

Barlow, C. G., M. G. Pearce, L. J. Rodgers, and Clayton, P. 1995. Effects of photoperiod on growth, survival and feeding periodicity of larval and juvenile barramundi *Lates calcarifer* (Bloch). *Aquaculture* 138, 159-168.

Barnes, R., King, H. and Carter, C. G. 2011. Hypoxia tolerance and oxygen regulation in Atlantic salmon, *Salmo salar* from a Tasmanian population. *Aquaculture,* 318, 397-401

Bauer, O. N. and Hoffman, G. L. 1976. Helminth range extension by translocation of fish. In: *Wildlife Diseases* Page, L. A. (Eds.). Plenum, New York. pp 163–172

Béchet, Q., A. Shilton, J. B. K. Park, R. J. Craggs, and Guieysse, B. 2011. Universal Temperature Model for Shallow Algal Ponds Provides Improved Accuracy. *Environmental Science & Technology* 45, 3702-3709.

Benjamini, Y. and Hochberg, Y. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological),* 57, 289-300.

Benzie, J. A. H. and Smith-Keune, C. 2006. Microsatellite variation in Australian and Indonensian pearl oyster *Pinctada maxima* populations. *Marine Ecology Progress Series,* 314, 197-211.

Bermudes, M., Glencross, B., Austen, K. and Hawkins, W. 2010. The effects of temperature and size on the growth, energy budget and waste outputs of barramundi (*Lates calcarifer*). *Aquaculture*, 306, 160-166

Beveridge, I. and Campbell, R. A. 1989. *Chimaerarhynchus* n. g. and *Patellobothrium* n. g., two new genera of trypanorhynch cestodes with unique poeciloacanthous armatures, and a reorganisation of the poeciloacanthons trypanorhynch families. *Systematic Parasitology* 14, 209–225.

Bilqees, F. M. 1995. Teaching and research in marine parasitology - problems and solutions. *Proceedings of Parasitology. Karachi*, 20, 63–89. Not sighted, In Gibson, D. I., Bray, R. A., and Harris, E. A. (Eds.) 2005. *Host-Parasite Database of the Natural History Museum*, London. URL http://www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/

Bly, J. E. and Clem, L. W. 1992. Temperature and teleost immune functions. *Fish and Shellfish Immunology,* 2,159–171.

BOM 2012. Bureau Of Meteorology - Commonwealth of Australia. Canberra, Australia

Bondad-Reantaso, M. G., Ogawa, K., Fukudome, M. and Wakabayashi, H. 1995. Reproduction and growth of *Neobenedenia girellae* (Monogenea: Capsalidae), a skin parasite of cultured marine fishes of Japan. *Fish Pathology,* 30, 227–231.

Boxshall, G. A., 2005. Crustacean parasites: *Branchiura* (fish lice). In: Rohde, K. (Ed.), *Marine Parasitology*. CSIRO publishing, Collingwood, Australia, pp 145–147.

Bray, R. A. and Cribb, T. H. 2003. Species of *Stephanostomum* Looss, 1899 (Digenea: Acanthocolpidae) from fishes of Australian and South Pacific waters, including five new species. *Systematic Parasitology,* 55, 159–197.

Bray, R. A., Cribb, T. H. and Barker, S. C. 1993. Hemiuridae (Digenea) from marine fishes of the Great Barrier Reef, Queensland, Australia. *Systematic Parasitology,* 25, 37–62.

Brazenor, A. K. and Hutson, K. S. (2013). Effect of temperature and salinity on egg hatching and description of the life cycle of *Lernanthropus latis* (Copepoda: Lernanthropidae) infecting barramundi, *Lates calcarifer*. *Parasitology International,* 62, 437-447.

Brett, J. R. and Groves, T. D. D. 1979. Physiological energetics, In Hoar, W. S., Randall, D. J. and Brett, J. R. (Eds.), *Fish Physiology: Bioenergetics and Growth*, Academic Press, New York press, pp74

Bricknell I.R., Dalesman S.J., O'Shea B., Pert C.C., Luntz J.M. 2006. Effect of environmental salinity on sea lice *Lepeophtheirus salmonis* settlement success. *Diseases of Aquatic Organisms*, 71, 201–12.

Brownstein, M. J., Carpten, D. and Smith, J. R. 1996. Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. *BioTechniques,* 20, 1004-1010.

Bruce, N. L., 1987. Australian species of *Nerocila* Leach, 1818, and *Creniola* n.gen. (Isopoda: Cymothoidae), crustacean parasites of marine fishes. *Records of the Australian Museum,* 39, 355–412.

Bruce, N. L. and Harrison-Nelson, E. B. 1988. New records of fish parasitic marine isopod crustaceans (Cymothoidae, subfamily Anilocrinae) from the Indo-west pacific. *Proceedings of the Biological Society of Washington*, 101, 585-602.

Burke, J. B. 1994. Temperature and salinity requirements in the early life-history of two catadromous fishes, Barramundi, *Lates calcarifer* (Bloch), and Australia Bass, *Macquaria novemaculeata* (Steindachner) in Eastern Australia. unpubl. Masters Thesis. University of New South Wales, Sydney

Burreson, E. M., 1995. Phylum Annelida: Hirudinea as vectors and disease agents. In: Woo, P. T. K. (Eds.), *Fish Diseases and Disorders: Vol. 1. Protozoan and Metazoan Infections*. CAB International, UK, pp 599–629.

Butler, B., Burrows, D. and Pearson, R. G. 2007. Testing the Hypoxia Tolerance of Tropical Freshwater Fishes, Australian Centre for Tropical Freshwater Research: James Cook University, Townsville, QLD, Australia

Byrnes, T. 1985. Two new *Argulus* species (Branchiura: Argulidae) found on Australian Bream (*Acanthopagrus* spp.). *Australian Journal of Zoology,* 21, 579–586.

Campbell, R. A. and Beveridge, I. 1996. Revision of The Family Pterobothriidae Pintner, 1931 (Cestoda : Trypanorhyncha). *Invertebrate Taxonomy,* 10, 617–62.

Cannon, L. R. G. 1977. Some larval ascaridoids from south-eastern Queensland marine fishes. *International Journal for Parasitology,* 7, 233–243.

Chai, J. Y., Murrel, K. D. and Lymbery, A. J. 2005. Fish-borne parasitic zoonoses: Status and issues. *International Journal for Parasitology,* 35, 1233–1254.

Chenoweth, S.F., Hughes, J.M., Keenan, C.P. and Lavery, S. 1998. Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer* (Australian barramundi). *Heredity,* 80, 187-197.

Chambers, C. B. and Ernst, I. 2005. Dispersal of the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for sea-cage farming of *Seriola* spp.. *Aquaculture,* 250, 60–69.

Chen, C., Durand, E., Forbes, F. and François, O. 2007. Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes,* 7, 747-756.

Chenoweth, S. F., Hughes, J. M., Keenan, C. P. and Lavery, S. 1998a. Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer* (Australian barramundi). *Heredity,* 80, 187-197.

Chenoweth, S. F., Hughes, J. M., Keenan, C. P. and Lavery, S. 1998b. When oceans meet: a teleost shows secondary intergradation at an Indian-Pacific interface. *Proceedings of the Royal Society London Series B - Biological Sciences,* 265, 415-420.

Cheung, W. W. L., V. W. Y. Lam, J. L. Sarmiento, K. Kearney, R. Watson, D. Zeller, and D. Pauly. 2010. Large-scale redistribution of maximum fisheries catch potential in the global ocean under climate change. Global Change Biology 16:24-35.

Clark, T. D., Jeffries, K. M., Hinch, S. G. and Farrell, A. P. 2011. Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *Journal of Experimental Biology,* 214, 3074-3081

Conley, D. C. and Curtis, M. A. 1993. Effects of temperature and photoperiod on the duration of hatching, swimming, and copepodid survival of the parasitic copepod *Salmincola edwardsii. Canadian Journal of Zoology*, 71, 972–976.

Cook, D. G., Wells, R. M. G. and Herbert, N. A. 2011. Anaemia adjusts the aerobic physiology of snapper (*Pagrus auratus*) and modulates hypoxia avoidance behaviour during oxygen choice presentations. *Journal of Experimental Biology*, 214, 2927-2934

Corkum, C. P. and Gamperl, A. K. 2009. Does the ability to metabolically downregulate alter the hypoxia tolerance of fishes?: a comparative study using cunner (*T. adspersus*) and Greenland cod (*G. ogac*). *Journal of Experimental Zoology: Part A*, 311A, 231-239

Crespo, S., Grau, A., and Padros, F. 1992. Sanguinicoliasis in the cultured amberjack *Seriola dumerili* Risso, from the Spanish Mediterranean area. *Bulletin of the European Association for Fish Pathology*, 12, 157–159.

Cressey, D. 2009. Future Fish. *Nature,* 458, 398–400.

Cressey R,F., and Collette B.B. 1971. Copepods and needlefishes: a study in host–parasite relationships. *Fishery Bulletin,* 68, 347–432.

Cribb, T. H., Bray, R. A. and Barker, S. C. 1992. A review of the Family Transversotrematidae (Trematoda: Digenea) with the description of a new genus, *Crusziella*, 6, 909–935.

Cribb, T. H., Adlard, R. D., Hayward, C. J., Bott, N. J., Ellis, D., Evans, D. and Nowak, B. F. 2011. The life cycle of *Cardicola forsteri* (Trematoda: Aporocotylidae), a pathogen of ranched southern bluefin tuna, *Thunnus maccoyi*. *International Journal for Parasitology,* 41, 861–870.

Cruz-Lacierda, E. R., Toledo, J. D., Tan-Fermin, J. D. and Burreson, E. M. 2000. Marine leech (*Zeylanicobdella arugamensis*) infestation in cultured orange-spotted grouper, *Epinephelus coioides*. *Aquaculture,* 185, 191–196.

Cusack, R. and Cone, D. K., 1986. A review of parasites as vectors of viral and bacterial diseases of fish. *Journal of Fish Diseases,* 9, 169–171.

Davis, T. L. O. 1986. Migration patterns in barramundi, *Lates-calcarifer* (bloch), in Vandiemen Gulf, Australia, with estimates of fishing mortality in specific areas. *Fisheries Research,* 4, 243-258.

Davison, A. and Chiba, S. 2003. Laboratory temperature variation is a previously unrecognized source of genotyping error during capillary electrophoesis. *Molecular Ecology Notes,* 3, 321-323.

De Silva, S. S. a. S., D. 2009. Climate change and aquaculture: potential impacts, adaptation and mitigation. In K. Cochrane, C. De Young, D. Soto and T. Bahri (eds). Climate change

implications for fisheries and aquaculture: overview of current scientific knowledge. FAO Fisheries and Aquaculture Technical Paper. No. 530. Rome, FAO.:pp. 151-212.

Deveney, M. R. Chisholm, L. A. and Whittington, I. D. 2001. First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia. *Diseases of Aquatic Organisms,* 46, 79–82.

Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak and Martin P. R. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences* 105, 6668-6672.

Deveney, M., Chisholm, L. and Whittington, I. 2001. First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia. *Diseases of Aquatic Organisms,* 46, 79–82.

Dewoody, J., Nason, J. D. and Hipkins, V. D. 2006. Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes,* 6, 951-957.

Dhillon, R. S. and Schulte, P. M. 2011. Intraspecific variation in the thermal plasticity of mitochondria in killifish. *Journal of Experimental Biology*, 214, 3639-3648

Diaz, R. J. and Breitburg, D. L. 2009. Chapter 1 The Hypoxic Environment, In Jeffrey, G., Richards, A. P. F. and Brauner, C. J. (Eds.), *Fish Physiology*,. Academic Press, pp 1-23

Diggles, B. K., 2003. Important risk assessment: juvenile yellowtail kingfish (Seriola lalandi) from Spencer Gulf Aquaculture, South Australia. National Institute of Water and Atmospheric Research, Wellington. pp 54

Doupe, R.G., P. Horwitz and Lymbery, A.J. 1999. Mitochondrial genealogy of Western Australian barramundi: applications of inbreeding coefficients and coalescent analysis for separating temporal population processes. *Journal of Fish Biology,* 54, 1197-1209.

Drinkwater, K. F. and Frank, K. T. 1994. Effects of river regulation and diversion on marine fish and invertebrates. *Aquatic Conservation: Marine and Freshwater Ecosystems* 4, 135-151.

Duarte, C. M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J., Black, K. and Karakassis, I., 2009. Will the oceans help feed humanity? *BioScience,* 59, 967–976.
Dunstan, D. J. 1959. The barramundi *Lates calcarifer* (Bloch) in Queensland waters. Technical Paper, Division of Fisheries and Oceanography. CSIRO Australia.

Earl, D. A. and Vonholdt, B. M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources,* 4, 359-361.

Edmunds, R.C., van Herwerden, L. Smith-Keune, C. and Jerry, D.R. 2009. Comparative characterization of a temperature responsive gene (lactate dehydrogenase-B, ldh-b) in two congeneric tropical fish, *Lates calcarifer* and *Lates niloticus*. *International Journal of Biological Sciences,* 5, 558-569.

Edwards, M., D. Johns, S. Leterme, E. Svendsen, and Richardson A. 2006. Regional climate change and harmful algal blooms in the northeast Atlantic. *Limnology and Oceanography* 51, 820-829.

Egusa, S. 1983. Disease problems in Japanese yellowtail, *Seriola quinqueradiata*, culture: a review. *Rapports et procès-verbaux des réunions*. , 182, 10–18.

Elith, J., C. H. Graham, R. P. Anderson, M. Dudík, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. McC. M. Overton, A. Townsend Peterson, S. J. Phillips, K. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberón, S. Williams, M. S. Wisz, and Zimmermann N. E. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29, 129-151.

Elith, J. and Leathwick, J. R. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics* 40, 677-697.

Elith, J., S. J. Phillips, T. Hastie, M. Dudík, Y. E. Chee, and Yates C. J. 2011. A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions* 17,43-57.

Elliott, J.M., Davison, W., 1975. Energy equivalents of oxygen-consumption in animal energetics. *Oecologia* 19, 195-201.

Ellis, J., Gilbey, J., Armstrong, A., Balstad, T., Cauwelier, E., Cherbonnel, C., Consuegra, S., Coughlan, J., Cross, T., Crozier, W., Dillane, E., Ensing, D., García De Leániz, C., García-Vázquez, E., Griffiths, A., Hindar, K., Hjorleifsdottir, S., Knox, D., Machado-Schiaffino, G., Mcginnity, P., Meldrup, D., Nielsen, E., Olafsson, K., Primmer, C., Prodohl, P., Stradmeyer, L., Vähä, J. P., Verspoor, E., Wennevik, V. and Stevens, J. 2011. Microsatellite standardization and evaluation of genotyping error in a large multi-partner research programme for conservation of Atlantic salmon (*Salmo salar*). *Genetica,* 139, 353-367.

Ellis, E. P. and Watanabe, W. O. 1993. The effects of hyposalinity on eggs, juveniles and adults of the marine monogenean, *Neobenedenia melleni* Treatment of ecto-parasitosis in seawater-cultured tilapia. *Aquaculture,* 117, 15–27.

Ernst, I. and Whittington, I. D. 1996. Hatching rhythms in the Capsalid Monogeneans *Benedenia lutjani* from the skin and *B. rohdei* from the gills of *Lutjanus carponotatus* at Heron Island, Queensland, Australia. *International Journal for Parasitology,* 26,1191–1204.

Ernst, I., Whittington, I. D., Corneillie, S. and Talbot, C. 2002. Monogenean parasites in sea-cage aquaculture. *Austasia Aquaculture,* 22, 46–48.

Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology,* 14, 2611-2620.

Excoffier, L., Hofer, T. and Foll, M. 2009. Detecting loci under selection in a hierarchically structured population. *Heredity,* 103, 285-298.

Excoffier, L., Laval, L. G. and Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online,* 1, 47-50.

Fajer-Ávila, E. J., Martínez-Rodríguez, I., Isabel Abdo De La Parra, M. I., Álvarez-Lajonchere, L. and Betancourt-Lozano, M. 2008. Effectiveness of freshwater treatment against *Lepeophtheirus simplex* (Copepoda: Caligidae) and *Neobenedenia* sp. (Monogenea:

Capsalidae), skin parasites of bullseye puffer fish, *Sphoeroides annulatus,* reared in tanks. *Aquaculture,* 284, 277–280.

Farrell, A. P. and Richards, J. G. 2009. Chapter 11 Defining Hypoxia: An Integrative Synthesis of the Responses of Fish to Hypoxia, In Jeffrey, G., Richards, A. P. F. and Colin, J. B. (Eds.), *Fish Physiology*. Academic Press pp 487-503

Fermin, A. C. and Seronay G. A. 1997. Effects of different illumination levels on Zooplankton abundance, feeding periodicity, growth and survival of the Asian sea bass, *Lates calcarifer* (Bloch), fry in illuminated floating nursery cages. *Aquaculture* 157, 227-237.

Fernandes, M. N., Barrionuevo, W. R. and Rantin, F. T. 1995. Effects of thermal stress on respiratory responses to hypoxia of a South American Prochilodontid fish, *Prochilodus scrofa*. *Journal of Fish Biology*, 46, 123-133

Fernandes, M. N. and Rantin, F. T. 1989. Respiratory responses of *Oreochromis niloticus* (Pisces, Cichlidae) to environmental hypoxia under different thermal conditions. *Journal of Fish Biology*, 35, 509-519

Food and Agriculture Organization of the United Nations (FAO) 2006-2013. Cultured Aquatic Species Information Programme. *Lates calcarifer*. Cultured Aquatic Species Information Programme. Text by Rimmer, M. A. In: FAO Fisheries and Aquaculture Department, Rome. Updated 3 June 2006. (Cited 23 July 2013). http://www.fao.org/fishery/culturedspecies/Lates_calcarifer/en.

Fletcher, W. J., Chesson, J., Fisher, M., Sainsbury, K. J. and Hundloe, T. J. 2004. National ESD reporting framework: the 'how to' guide for aquaculture. FRDC, Canberra, Australia. pp 88

Francois, O. and Durand, E. 2010. Spatially explicit Bayesian clustering models in population genetics. *Molecular Ecology Resources,* 10, 773-784.

Fry, F.E.J. 1971. The Effect of Environmental Factors on the Physiology of Fish. In: *Fish Physiology*. Academic Press, New York.

Fry, F. E. J. and Hart, J. S. 1948. The Relation of Temperature to Oxygen Consumption in the Goldfish. *The Biological Bulletin*, 94, 66-77

Frye, R. 1983. Climatic change and fisheries management. *Natural Resources Journal,* 23, 76-76.

Fu, S-J., Brauner, C. J., Cao, Z-D., Richards, J. G., Peng, J-L., Dhillon, R. and Wang, Y-X. 2011. The effect of acclimation to hypoxia and sustained exercise on subsequent hypoxia tolerance and swimming performance in goldfish (*Carassius auratus*). *Journal of Experimental Biology*, 214, 2080-2088

Gannicott, A. M. and Tinsely, R. C. 1998. Larval survival characteristics and behaviour of the gill monogenean *Discocotyle sagittata. Parasitology,* 117, 491–498.

Gardiner, N. M., Munday, P. L. and Nilsson, G. E. 2010. Counter-Gradient Variation in Respiratory Performance of Coral Reef Fishes at Elevated Temperatures. *PLoS ONE*, 5, e13299Gibson, D. I., Bray, R. A., and Harris, E. A. (Eds.) 2005. Host-Parasite Database of the

Natural History Museum, London. http://www.nhm.ac.uk/research-curation/research/projects/host-parasites/index.html

Glencross, B. 2008. A factorial growth and feed utilization model for barramundi, *Lates calcarifer* based on Australian production conditions. *Aquaculture Nutrition,* 14, 360-373.

Gillanders, B. M. and Kingsford M. J. 2002. Impact of changes in flow of freshwater on estuarine and open coastal habitats and the associated organisms. *Oceanography and Marine Biology: An Annual Review* 40, 233-309.

Glencross, B. D. 2008. A factorial growth and feed utilization model for barramundi, *Lates calcarifer* based on Australian production conditions. *Aquaculture Nutrition* 14, 360-373.

Glencross, B.D. and Bermudes, M. 2012. Adapting bioenergetic factorial modelling to understand the implications of heat stress on barramundi (*Lates calcarifer*) growth, feed utilisation and optimal protein and energy requirements - potential strategies for dealing with climate change? *Aquaculture Nutrition*, 18, 411-422.

Goldstein, D. B., Ruiz Linares, A., Cavalli-Sforza, L. L. and Feldman, M. W. 1995. An evaluation of genetic distances for use with microsatellite loci. *Genetics,* 139, 463-71.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). updated from Goudet 1995. Available from http://www.unil.ch/izea/softwares/fstat.html. . 2.9.3.2 ed.

Goudet, J., Raymon, M., De Meeus, T. and Rousset, F. 1996. Testing differentiation in diploid populations. *Genetics,* 144, 1933-1940.

Graham, C. T. and Harrod, C. 2009. Implications of climate change for the fishes of the British Isles. *Journal of Fish Biology*, 74, 1143-1205

Grau, A., Riera, F. and Carbonell, E. 1999. Some protozoan and metazoan parasites of the amberjack from the Balearic Sea (western Mediterranean). *Aquaculture International,* 7, 307–317.

Guo, F. C. and Woo, P. T. K. 2009. Selected parasitosis in cultured and wild fish. *Veterinary Parasitology*, 163,207–216.

Guy, J. A., Cross, E., Cowden, K. and Carroll, A. 2010. 'Land-based Mulloway (*Argyrosomus japonicus*) farming: A diversification opportunity for Northern NSW prawn farmers', paper presented to Australian Prawn Farmers Association & Australian Barramundi Farmers Association Conference 2010, Broadbeach, Gold Coast, Qld., 4-5 August.

Hamilton, S. 2010. Biogeochemical implications of climate change for tropical rivers and floodplains. *Hydrobiologia* 657, 19-35.

Hardy-Smith, P., Ellis, D., Humphrey, J., Evans, M., Evans, D., Rough, K., Valdenegro, V. and Nowak, B. 2012. *In vitro* and *in vivo* efficacy of anthelmintic compounds against blood fluke (*Cardicola forsteri*). *Aquaculture,* 334, 39–44.

Hayward, C. J. Bott. N. J., Itoh, N., Iwashita, M., Okihiro, M. and Nowak, B. F. 2007 Three species of parasites emerging on the gills of mulloway, *Argyrosomus japonicus* (Temminck and Schlegel, 1843), cultured in Australia. *Aquaculture,* 265, 27–40.

Hayward, C. J., Aiken, H. M. and Nowak, B. F. 2008. An epizootic of *Caligus chiastos* on farmed southern bluefin tuna *Thunnus maccoyii* off South Australia. *Diseases of Aquatic Organisms,* 79, 57–63.

Hayward, C. J., Ellis, D., Footec, D., Wilkinson, R. J., Crosbie, P. B. B., Bott, N. J. and Nowak, B. F. 2010. Concurrent epizootic hyperinfections of sea lice (predominantly *Caligus chiastos*) and blood flukes (*Cardicola forsteri*) in ranched Southern Bluefin tuna. *Veterinary Parasitology,* 173, 107–115.

Hemmer-Hansen, J., Nielsen, E. E., Frydenberg, J. and Loeschcke, V. 2007. Adaptive divergence in a high gene flow environment: Hsc70 variation in the European flounder (Platichthys flesus L.). *Heredity,* 99, 592-600.

Henriksson, P., Mandic, M. and Richards Jeffrey, G. 2008. The Osmorespiratory Compromise in Sculpins: Impaired Gas Exchange Is Associated with Freshwater Tolerance. *Physiological and Biochemical Zoology*, 81, 310-319

Henry, M. A., Alexis, M. N., Fountoulaki, E. Nengas, I. and Rigos, G. 2009. Effects of a natural parasitical infection (*Lernanthropus kroyeri*) on the immune system of European sea bass, Dicentrarchus labrax L. *Parasite Immunology,* 31, 729–740.

Herbert, B. W., Shaharom-Harrison F. M. and Overstreet, R. M. 1994. Description of a new blood.fluke, *Cruoricola lates* n. g., n. sp. (Digenea: Sanguinicolidae), from sea-bass Lates calcarifer (Bloch, 1790) (Centropomidae) *Systematic Parasitology,* 29, 51–60.

Herbert, B. W. and Shaharom F. M. 1995. A new blood fluke, *Parasanguinicola vastispina* gen. nov., sp. nov. (Trematoda: Sanguinicolidae), from sea bass *Lates calcarifer* (Centropomidae) cultured in Malaysia. *Parasitology Research,* 81, 349–354.

Hewitt, G. C. 1971. Two species of *Caligus* (Copepoda, Caligidae) from Australian Waters, with a description of some developmental stages. *Pacific Science,* 25, 145–164.

Hesp, S. A., Hobbs, R. P., and Potter, I. C. 2002. Infection of the gonads of *Glaucosoma hebraicum* by the nematode *Philometra lateolabracis*: occurrence and host response. *Journal of Fish Biology,* 60, 663–673.

Hirayama, T., Kawano, F. and Hirazawa, N. 2009. Effect of *Neobenedenia girellae* (Monogenea) infection on host amberjack *Seriola dumerili* (Carangidae). *Aquaculture,* 288, 159–165.

Hirazawa, N., Mitsuboshi, T., Hirata, T. and Shirasu, K. 2004. Susceptibility of spotted halibut *Verasper variegatus* (Pleuronectidae) to infection by the monogenean *Neobenedenia girellae* (Capsalidae) and oral therapy trials using praziquantel. *Aquaculture,* 238, 83–95.

Hirazawa, N., Takano, R., Hagiwara, H., Noguchi, M. and Narita, M. 2010. The influence of different water temperatures on *Neobenedenia girellae* (Monogenea) infection, parasite growth, egg production and emerging second generation on amberjack *Seriola dumerili*

(Carangidae) and the histopathological effect of this parasite on fish skin. *Aquaculture,* 299, 2–7.

Ho, J. S. and Lin, C. L. 2003. Solution to the taxonomic confusion surrounding *Caligus epinepheli* Yamaguti, a caligid copepod (Siphonostomatoida) parasitic on marine fishes. *Zoological Studies,* 42, 268–283. (record on *L. calcarifer*)

Ho, J., and Kim, I. 2004. Lernanthropid copepods (Siphonostomatoida) parasitic on fishes of the Gulf of Thailand. *Systematic Parasitology,* 58, 17–21.

Ho, J. and Lin, C. 2004. *Sea lice of Taiwan*. Sueichan Press, Taiwan. pp388

Hoshina, T. 1968. On the monogenetic trematode, *Benedenia seriolae*, parasitic on yellow-tail, *Seriola quinqueradiata. Bulletin de l'Office International des Epizooties,* 69, 1179–1191.

Huang, H. T., Hsu, J. P., Hung, H. H. and Chang, W. F. 1990. Streptococcal infection in maricultured sea bass *Lates-calcarifer*. *Journal of the Chinese Society of Veterinary Science*, 16, 171–180.

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A.M., Jess, M. and Williams, S. E. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B*, 367, 1665-1679

Huey, R. B. and R. D. Stevenson. 1979. Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. American Zoologist 19:357-366.

Hughes, L. 2003. Climate change and Australia: trends, projections and impacts. Austral Ecology 28:423-443.

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandoe, P., and Turnbull, J. F. 2006. Current issues in fish welfare. *Journal of Fish Biology,* 68, 332–372.

Hutson, K. S., Mooney, A. J., Ernst, I. and Whittington, I. D., 2007a. Metazoan parasite assemblages of wild yellowtail kingfish (*Seriola lalandi*) from eastern and southern Australia. *Parasitology International,* 56, 95–105.

Hutson, K. S., Ernst, I. and Whittington, I. D. 2007b. Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in South Australian sea-cage aquaculture. *Aquaculture,* 271, 85-99.

Hutson K. S., Mata L., Paul N. and de Nys R. 2012. Seaweed extracts as a natural control against the monogenean ectoparasites *Neobenedenia* sp. infecting farmed barramundi (*Lates calcarifer*). *International Journal for Parasitology,* 42,1135–1141.

Imholt, C., C. Soulsby, I. A. Malcolm, M. Hrachowitz, C. N. Gibbins, S. Langan, and Tetzlaff D. 2013. Influence of scale on thermal characteristics in a large montane river basin. *River Research and Applications* 29, 403-419.

IPCC, 2007a. Climate change 2007: synthesis report. In: Allali, A., Bojariu, R., Diaz, S., Elgizouli, I., Griggs, D., Hawkins, D., et al, editors. Cambridge, UK, New York, US: Intergovernmental Panel of Climate Change; 2007.

IPCC, 2007b. The physical science basis. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K., et al, editors. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. UK and New York, US, Cambridge.

Jahn, T. L., Kuhn, L. R., 1932. The life history of *Epibdella melleni*, MacCallum, 1927, a monogenetic trematode parasitic in marine fishes. *Biological Bulletin,* 62, 89–111.

Jakobsson, M. and Rosenberg, N. A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics,* 23, 1801-1806.

James, C., J. Vanderwal, S. Capon, L. Hodgson, N. Waltham, D. Ward, B. Anderson, and R. Pearson. 2013. Identifying Climate Refuges for Freshwater Biodiversity Across Australia: identification and characterisation of freshwater refuges in the face of climate change. 1925039560, National Climate Change Adaptation Research Facility.

Jensen, J. L., Bohonak, A. J. and Kelley, S. T. 2005. Isolation by distance, web service. *BMC Genetics,* 6, 13. v.3.23 http://ibdws.sdsu.edu/.

Jobling, M. 1994. Fish bioenergetics. Chapman & Hall.

Johansen, R., Ranheim, T., Hansen, M. K., Taksdal, T. and Totland, G. K. 2002. Pathological changes in the juvenile Atlantic halibut *Hippoglossus hippoglossus* persistently infected with nodavirus. *Diseases of Aquatic Organisms,* 50, 161–169.

Johansen, J.L. and Jones, G.P. 2011. Increasing ocean temperature reduces the metabolic performance and swimming ability of coral reef damselfishes. *Global Change Biology*, 17, 2971-2979.

Johnsen, B. O. and Jensen, A. J. 1991. The *Gyrodactylus* story in Norway. *Aquaculture,* 98, 289–302.

Johnson S.C., Albright L.J. 1991. Development, growth and survival of Lepeophtheirus salmonis (Copepoda: Calgidae) under laboratory conditions. *Journal of the Marine Biological Association of the UK,* 71:425–36.

Johnson, S. C., Treasurer, J. W., Bravo, S., Nagasawa, K. and Kabata, Z. 2004. A review of the impact of parasitic copepods on marine aquaculture. *Zoological Studies,* 43, 229–243.

Johnson, M. F., R. L. Wilby, and Toone J. A. 2013. Inferring air–water temperature relationships from river and catchment properties. *Hydrological Processes*, Early online.

Johnston, T. H. and Mawson, P. M. 1943. Some ascarid nematodes from Australian marine fish. *Transactions of the Royal Society of South Australia*, 67, 20–35.

Johnston, T. H. and Mawson, P. M. 1951. Report on some parasitic nematodes from the Australian Museum. *Records of the Australian Museum,* 22, 289–297.

Justic, D., Rabalais, N. N. and Turner, R. E. 2001. Implications of global climate change for coastal and estuarine hypoxia; hypothesis observations and models for the northern Gulf of Mexico, Athens, Georgia

Kaneko, J. J., Yamada, R., Brock, J. A. and Nakamura, R. M. 1988. Infection of tilapia, *Oreochromis mossambicus* (Trewavas), by a marine monogenean, *Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963 in Kaneohe Bay, Hawaii, USA, and its treatment. *Journal of Fish Diseases,* 11,295–300.

Kapetsky, J.M. 2000. Present applications and future needs of meteorological and climatological data in inland fisheries and aquaculture. International Workshop on Agrometerology in the 21st Century - Needs and Perspective, ACCRA, Ghana.

Katersky, R.S. and Carter, C.G. 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. *Aquaculture*, 250, 775-780.

Katersky, R. S. and Carter C. G. 2007a. High growth efficiency occurs over a wide temperature range for juvenile barramundi *Lates calcarifer* fed a balanced diet. *Aquaculture* 272, 444-450.

Katersky, R. S. and Carter C. G. 2007b. A preliminary study on growth and protein synthesis of juvenile barramundi, *Lates calcarifer* at different temperatures. *Aquaculture* 267, 157-164.

Keenan, C. P. 1994. Recent evolution of population structure in Australian barramundi, *Lates calcarifer* (Block): an example of isolation by distance in one dimension`. *Australian Journal of Marine and Freshwater Research,* 45, 1123-1148.

Keenan, C. P. 2000. Should we allow human-induced migration of the Indo-West Pacific fish, barramundi *Lates calcarifer* (Bloch) within Australia? *Aquaculture Research,* 31, 121-131.

Kearn, G. C. 1986. The eggs of monogeneans. *Advances in Parasitology,* 25, 175–273.

Kimmerer, W. J. 2002. Effects of freshwater flow on abundance of estuarine organisms: Physical effects or trophic linkages? *Marine Ecology Progress Series* 243, 39-55.

Kimura, M. 1968. Evolutionary rate at the molecular level. *Nature,* 217, 624-626.

King, T. A. and Cable, J. 2007. Experimental infections of the monogenean *Gyrodactylus turnbulli* indicate that it is not a strict specialist. *International Journal for Parasitology,* 37, 663–672

Koenker, K. 2013. quantreg: Quantile Regression. R package - version 5.01. http://CRAN.R-project.org/package=quantreg

Kua, B. C., Azmi, M. A. and Hamid, N. K. A. 2010. Life cycle of the marine leech (*Zeylanicobdella arugamensis*) isolated from sea bass (*Lates calcarifer*) under laboratory conditions. *Aquaculture,* 302, 153–157.

Kua, B. C., Noraziah, M. R., and Nik, R. A. 2012. Infestation of gill copepod *Lernanthropus latis* (Copepoda: Lernanthropidae) and its effect on cage-cultured Asian sea bass *Lates calcarifer*. *Tropical Biomedicine,* 29, 443–450.

Kuo, C. and Humphrey, J. D. 2008. Monitoring the Health of Prawns, Barramundi and Mud Crabs on Aquaculture Farms in the Northern Territory. Fishnote, Northern Territory Government 13pp.

Lackenby, J. A., Chambers, C. B., Ernst, I., Whittington, I. D., 2007. Effect of water temperature on reproductive development of *Benedenia seriolae* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia. *Diseases of Aquatic Organisms,* 74, 235–242.

Lafferty, K. D. and A. M. Kuris. 1999. How environmental stress affects the impacts of parasites. Limnology and Oceanography 44:925-931

Lafferty, K. D. 2009. The ecology of climate change and infectious diseases. *Ecology,* 90, 888–900.

Larsen, P. F., Nielsen, E. E., Williams, T. D. and Loeschcke, V. 2008. Intraspecific variation in expression of candidate genes for osmoregulation, heme biosynthesis and stress resistance suggests local adaptation in European flounder (*Platichthys flesus*). *Heredity,* 101, 247-59.

Last, P.R., Lyne, V.D., Williams, A., Davies, C.R., Butler A.J. and Yearsley, G.K. 2010. A hierarchal framework for classifying seabed biodiversity with application to planning and managing Australia's marine biological resources. *Biological Conservation,* 143, 1675-1686.

Leclercq, N., Gattuso, J.P., and Jaubert, J. 1999. Measurement of oxygen metabolism in open-top aquatic mesocosms: application to a coral reef community. *Marine Ecology-Progress Series* 177, 299-304.

Lekang, O.I. 2007. Aquaculture Engineering. Blackwell Publishing, UK

Leong, T. S. 1997. Control of parasites in cultured marine finfishes in Southeast Asia – an overview. *International Journal for Parasitology,* 27, 1177–1184.

Leong T.S. and Colorni A. 2002. Infection diseases of warm water fish in marine and brackish waters. In: Diseases and disorders of finfish in cage culture (ed. by P.T.K. Woo, D.W. Bruno, L.H.S Lim), pp. 193-230. CABI Publishing, United Kingdom

Leong, T. S., Tan, Z. and Enright, W.J . 2006. Important parasitic diseases in cultured marine fish in the Asia-Pacific region. *AQUA Culture AsiaPacific Magazine,* January/February 2006, 14–16.

Leong, T. S. and Wong, Y. S. 1986. Parasite fauna of sea bass, *Lates calcarifer* Bloch, from Thailand and from floating cage culture in Penang, Malaysia. In: Maclean, J. L. Dizon, L. B. and HosiUos, L. V. (Eds.) *The first Asian fisheries forum. Manila: Asian Fisheries Society*, pp 251-254.

Leong, T. S. and Wong, S. Y. 1990a. Parasites of health and diseased juvenile grouper (*Epinephelus malabaricus* (Bloch and Schneider)) and Seabass (*Lates calcarifer* (Bloch)) in floating cages in Penang, Malaysia. *Asian Fisheries Science,* 3, 319–327.

Leong T. S. and Wong S. Y. 1990b. Parasites of seabass, *Lates calcarifer* (Bloch) cultured in ponds and floating net cages in Thailand. In: Hirano R. and Hanyu I. (eds) *The Second Asian Fisheries Forum. Manila, Asian Fisheries Society,* pp 705–707.

Leong, T. S. and Wong, S. Y. 1992a. Parasites of marine finfishes cultured in ponds and cages in Indonesia. Proceedings of the Symposium on Tropical Fish Health Management, 14-16 May 1991, Bogor, Indonesia Biotrop Special Publication, 48, 119–124.

Leong, T. S. and Wong, S. Y. 1992a. Parasites of marine finfishes cultured in ponds and cages in Indonesia. Symposium on Tropical Fish Health Management, *Biotrop Special Publication*, 48, 119–124.

Leong, T. S. and Wong, S. Y. 1992b. The parasite fauna of cultured Seabass, *Lates calcarifer* Bloch from Kelantan and Penang, Malaysia. *Journal of Bioscience,* 3, 27–29.

Lester, R. J. G. 1994. Fish parasite problems in Australia. In: Ozcel, M. A. and Alkan, M. Z. (Eds.) *Abstracts, 8th International Congress of Parasitology*. Turkish Society of Parasitology,Izmir, Turkey. pp 53.. 10-14th October

Lewin-Koh, N. J., R. Bivand, E. Pebesma, E. Archer, A. Baddeley, H. Bibiko, S. Dray, D. Forrest, M. Friendly, and P. Giraudoux. 2011. maptools: Tools for reading and handling spatial objects. R package version 0.8-10, URL http://CRAN. R-project. org/package= maptools.

Lim, L. H. S. 1998. Diversity of monogeneans in Southeast Asia. *International Journal for Parasitology,* 28, 1495-1515.

Lin, C. L. and J.S. Ho. 1993. Life history of Caligus epidemicus Hewitt parasitic on the tilapia (Oreochromis mossambica) cultured in brackish water, in G. A. Boxshall and D. Defaye (Eds), Pathogens Of Wild And Farmed Fish: Sea Lice (London: Ellis Horwood), pp. 5-15.

Lin, C. L. and Ho, J. S. 1993. Life history of *Caligus epidemicus* Hewitt parasitic on the tilapia (*Oreochromis mossambica*) cultured in brackish water. In G. A. Boxshall and D. Defaye (Eds.), *Pathogens Of Wild And Farmed Fish: Sea Lice*, London, Ellis Horwood, pp 5–15.

Ling, C. X., J. Huang, and H. Zhang. 2003. AUC: a better measure than accuracy in comparing learning algorithms. Pages 329-341 Advances in Artificial Intelligence. Springer.

Loneragan, N. R. and Bunn S. E. 1999. River flows and estuarine ecosystems: Implications for coastal fisheries from a review and a case study of the Logan River, southeast Queensland. *Austral Ecology* 24, 431-440.

Lough, J.M., Gupta, A.S. and Hobday, A.J. 2012. Temperature in: E.S. Poloczanska, A.J.H.a.A.J.R. (Ed.), A Marine Climate Change Impacts and Adaptation Report Card for Australia 2012.

McCarthy, I. D., E. Moksness, D. A. Pavlov, and Houlihan D. F. 1999. Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*). *Canadian Journal of Fisheries and Aquatic Sciences* 56, 231-241.

Mackey, A. P. and Berrie A. D. 1991. The prediction of water temperatures in chalk streams from air temperatures. *Hydrobiologia* 210:183-189.

Magnuson, J.J. and DeStasio, B.T. 1997. Thermal niche of fishes and global warming. Pp. 377-408 in: C.M. Wood and McDonald D.G. (eds.), Global Warming -- Implications of Freshwater and Marine Fish. SEB Seminar Series. Cambridge University Press, Cambridge, U.K.

Mamun, S., Focken, U. and Becker, K. 2013. A respirometer system to measure critical and recovery oxygen tensions of fish under simulated diurnal fluctuations in dissolved oxygen. *Aquaculture International*, 21, 31-44

Manera M, Dezfuli, B.S. Lernanthropus kroyeri infections in farmed sea bass *Dicentrarchus labrax*: pathological features. *Diseases of Aquatic Organisms,* 57,177–80.

Marcogliese, D. J. 2001. Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology,* 79, 1331-1352.

Marcogliese, D. J. 2008. The impact of climate change on the parasites and infectious diseases of aquatic animals. *Revue scientifique et technique (International Office of Epizootics),* 27, 467–484.

Marra, J. 2005. When will we tame the oceans? *Nature,* 436,175–176

Marshall, C. R. E. 2005. Evolutionary genetics of barramundi (*Lates calcarifer*) in the Australian region. Western Australia: Murdoch University.

Meisner, J. D., J. L. Goodier, H. A. Regier, B. J. Shuter, and Christie W. J. 1987. An Assessment of the Effects of Climate Warming on Great Lakes Basin Fishes. *Journal of Great Lakes Research* 13, 340-352.

Merella, P., Cherchi, S., Garippa, G., Fioravanti, M. L., Gustinelli, A. and Salati, F. 2009. Outbreak of *Sciaenacotyle panceri* (Monogenea) on cage-reared meagre *Argyrosomus regius* (Osteichthyes) from the western Mediterranean Sea. *Diseases of Aquatic Organisms,* 86, 169–173.

Meyer, F. P. 1991. Aquaculture disease and health management. *Journal of Animal Science,* 69, 4201–4208.

Meynecke, J.-O., S. Y. Lee, N. C. Duke, and Warnken J. 2006. Effect of rainfall as a component of climate change on estuarine fish production in Queensland, Australia. *Estuarine, Coastal and Shelf Science* 69, 491-504.

Meynecke, J. O., Y. L. Shing, N. C. Duke, and Warnken J. 2007. Relationships between estuarine habitats and coastal fisheries in Queensland, Australia. *Bulletin of Marine Science* 80, 773-793.

Meynecke, J. O., Lee, S. Y. and Duke, N. C. 2008. Linking spatial metrics and fish catch reveals the importance of coastal wetland connectivity to inshore fisheries in Queensland, Australia. *Biological Conservation,* 141, 981-996.

Meynecke, J.-O. and Lee S. Y. 2011. Climate-coastal fisheries relationships and their spatial variation in Queensland, Australia. *Fisheries Research* 110, 365-376.

Militz, T. A., Southgate, P. C., Carton, A. G. and Hutson, K.cS. (in press, 2013). Efficacy of garlic (*Allium sativum*) extract applied as a therapeutic immersion treatment for *Neobenedenia* sp. management in aquaculture. *Journal of Fish Diseases*.

Mohseni, O., T. Erickson, and Stefan H. 2002. Upper Bounds for Stream Temperatures in the Contiguous United States. *Journal of Environmental Engineering* 128, 4-11.

Mohseni, O., H. G. Stefan, and Erickson T. R. 1998. A nonlinear regression model for weekly stream temperatures. *Water Resources Research* 34, 2685-2692.

Moore, R. and Reynold L. 1982. Migration patterns of barramundi, (*Lates calcarifer*) (Bloch), in Papua New Guinea. *Australian Journal of Marine and Freshwater Research* 33, 671-682.

Moravec, F., Orecchia, P. and Paggi, L. 1988. Three interesting nematodes from the fish *Parupeneus indicus* (Mullidae, Perciformes) of the Indian Ocean, including a new species, *Ascarophis parupenei* sp.n. (Habronematoidea). *Folia Parasitologica,* 35, 47–57.

Morrongiello, J. R., Beatty, S. J., Bennett, J. C., Crook, D. A., Ikedife, D. N. E. N., Kennard, M. J., Kerezsy, A., Lintermans, M., McNeil, D. G. and Pusey, B. J. 2011. Climate change and its implications for Australia's freshwater fish. *Marine and Freshwater Research*, 62, 1082-1098

Müeller, K. W., Watanabe, W. O. and Head, W. D. 1992. Effect of salinity on hatching in *Neobenedenia melleni*, a monogenean ectoparasite of seawater-cultured tilapia. *Journal of the World Aquaculture Society,* 23(3), 199–204.

Muhd-Faizul, H. A. H., Kuab, B. C. and Leaw, Y. Y. 2012. Caligidae infestation in Asian seabass, *Lates calcarifer*, Bloch 1790 cultured at different salinity in Malaysia. *Veterinary Parasitology,* 184, 68–72.

Munday, B. L., Kwang, J. and Moody, N. 2002. Betanodavirus infections of teleost fish: a review. *Journal of Fish Diseases,* 25, 127–142.

Murray, A. G. and Peeler, E. J., 2005. A framework for understanding the potential for emerging diseases in aquaculture. *Preventative Veterinary Medicine* 67, 223–235.

Narum, S. R., Campbell, N. R., Kozfkay, C. C. and Meyer, K. A. 2010. Adaptation of redband trout in desert and montane environments. *Molecular Ecology*, 19, 4622-37.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* 89, 583-590.

Newton, J.R., Smith-Keune, C. and Jerry D.R. 2010. Thermal tolerance varies in tropical and sub-tropical populations of barramundi (*Lates calcarifer*) consistent with local adaptation. *Aquaculture,* 308, S128-S132.

Newton, J. R. 2013. Investigating the genetics of thermal tolerance and adaptation to temperature amongst populations of Australian barramundi (*Lates calcarifer*). Ph.D. Thesis, James Cook University, Queensland.

Newton, J. R., Zenger, K. R. and Jerry, D. R. 2013. Next-generation transcriptome profiling reveals insights into genetic factors contributing to growth differences and temperature adaptation in Australian populations of barramundi (*Lates calcarifer). Marine Genomics,* 11C, 45-52

Nilsson, G. E., Hobbs, J-P., Munday, P. L. and Östlund-Nilsson, S. 2004. Coward or braveheart: extreme habitat fidelity through hypoxia tolerance in a coral-dwelling goby. *Journal of Experimental Biology*, 207 33-39

Nilsson, G. E., Östlund-Nilsson, S. and Munday, P. L. 2010. Effects of elevated temperature on coral reef fishes: Loss of hypoxia tolerance and inability to acclimate. C*omparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 156, 389-393

Northern Territory Government. 2012. Fishery Status Reports 2011., Northern Territory

Nosil, P., Funk, D. J. and Ortiz-Barrientos, D. 2009. Divergent selection and heterogeneous genomic divergence. *Molecular Ecology,* 18, 375-402.

Nowak, B. F. 2004. Assessment of health risks to southern bluefin tuna under current culture conditions. *Bulletin of the European Association for Fish Pathology,* 24, 45–51.

Nowak, B. F. 2007. Parasitic diseases in marine cage culture – An example of experimental evolution of parasites? *International Journal for Parasitology,* 37, 581–588.

Oberdorff, T., J.-F. Guégan, and B. Hugueny. 1995. Global Scale Patterns of Fish Species Richness in Rivers. Ecography 18:345-352.

Ogawa, K. 1996. Marine parasitology with special reference to Japanese fisheries and mariculture. *Veterinary Parasitology,* 64, 95–105.

Ogawa, K. and Fukudome, M. 1994. Mass mortality caused by blood fluke (*Paradeontacylix*) among amberjack (*Seriola dumerili*) imported to Japan. *Fish Pathology,* 29, 265–269.

Ohta, T. and Kimura, M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research,* 22, 201-204.

Ogawa, K. 1988. Development of Bivaginatai Monogenea: Microcotylidae. Nippon Suisan Gakkaishi-Bull. *Bulletin of the Japanese Society for the Science of Fish,* 54(1), 61–64.

Ogawa, K., Bondad-Reantaso, M. G., Fukudome, M. and Wakabayashi, H. 1995. *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963 (Monogenea: Capsalidae) from cultured marine fishes of Japan. *Journal of Parasitology,* 81, 223–227.

Ogawa, K. and Yokoyama, H. 1998. Parasitic diseases of cultured marine fish in Japan. *Fish Pathology*, 33, 303–309.

Ogawa, K., Miyamoto, J., Wang, H. C., Lo, C. F. and Kou, G. H. 2006. *Neobenedenia girellae* (Monogenea) infection of cultured cobia *Rachycentron canadum* in Taiwan. *Fish Pathology,* 41, 51–56.

Ohno, Y., Kawano, F. and Hirazawa, N. 2009. The effect of oral antibiotic treatment and freshwater bath treatment on susceptibility to *Neobenedenia girellae* (Monogenea) infection of amberjack (*Seriola dumerili*) and yellowtail (*S. quinqueradiata*) hosts. *Aquaculture,* 292,248–251.

Oliver, G. 1977. Pathogenic Effect of Fixation of *Diplectanum aequans* (Wagener, 1857) Diesing, 1858 (Monogenea, Monopisthocotylea, Diplectanidae) on the Gills of *Dicentrarchus labrax* (Linnaeus, 1758) (Pisees, Serranidae). In French. *Zeitschrift Fur Parasitenkunde-Parasitology Research*, 53, 7–11.

Ott, M. E., Heisler, N and Ultsch, G. R. 1980. A re-evaluation of the relationship between temperature and the critical oxygen tension in freshwater fishes. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* 67, 337-340

Palm, H. W. 1995. Untersuchengen zur Systematik von Russelbandwurmen (Cestoda: Trypanorhyncha) aus atlantischen Fischen. Thesis (In German), Berichte aus dem Institut für Meereskunde an der Christian-Albrechts Universität , Kiel, 275, 1–238.

Palm, H. W. and Beveridge, I. 2002. Tentaculariid cestodes of the order Trypanorhyncha (Platyhelminthes) from the Australian region.' *Records of the South Australian Museum,* 35, 49–78.

Palm, H., Poynton, S. L. and Rutledge, P. 1998. Surface ultrastructure of plerocercoids of *Bombycirhynchus sphyraenaicum* (Pintner, 1930) (Cestoda: Trypanorhyncha). *Parasitology Research,* 84, 195–204.

Paperna, I. and Overstreet, R. M. 1981. Parasites and diseases of mullets (Muglidae). In: Aquaculture of Grey Mullets, Oren, O. H. (Eds.), pp 411–493. Cambridge University Press, Cambridge.

Parmesan, C. and Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37-42.

Pearson, R. G., Crossland, M. R., Butler, B. and Manwaring, S. 2003. Effects of cane-field drainage on the ecology of tropical waterways,. James Cook University, Townsville, Queensland, Australia

Peakall, R. and Smouse, P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes,* 6, 288-295.

Pech, D., Leopoldina Aguirre-Macedo, M., Lewis, J. W. and Vidal-Martínez, V. M. 2010. Rainfall induces time-lagged changes in the proportion of tropical aquatic hosts infected with metazoan parasites. *International Journal for Parasitology,* 40, 937–944.

Perera, R., Fiske, R. and Johnson, S. 1998. Histopathology of hybrid tilapias infected with a biotype of *Streptococcus iniae*. *Journal of Aquatic Animal Health,* 10, 294–299.

Perry, A. L., P. J. Low, J. R. Ellis, and Reynolds J. D. 2005. Climate Change and Distribution Shifts in Marine Fishes. *Science* 308, 1912-1915.

Phillips, S. J., R. P. Anderson, and Schapire R. E. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190, 231-259.

Phillips, S. J. and Dudík M. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* 31, 161-175.

Pillai, N. K. 1985. The fauna of India. Copepod parasites of marine fishes. Calcutta: Zoological Society of India.

Pinsky, M. L., B. Worm, M. J. Fogarty, J. L. Sarmiento, and Levin S. A. 2013. Marine Taxa Track Local Climate Velocities. *Science* 341, 1239-1242.

Pirozzi, I. 2009. A factorial approach to defining the dietary protein and energy requirements of mulloway, *Argyrosomus japonicus*: optimizing feed formulations and feeding strategies. PhD thesis, James Cook University http://eprints.jcu.edu.au/11697/

Pirozzi, I., Booth, M.A. 2009. The routine metabolic rate of mulloway (Argyrosomus japonicus: Sciaenidae) and yellowtail kingfish (Seriola lalandi: Carangidae) acclimated to six different temperatures. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* 152, 586-592.

Pollock, M. S., Clarke, L. M. J. and Dubé, M. G. 2007. The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environmental Reviews*, 15, 1-14

Poulin, R., Conley, D. C. and Curtis, M. A. 1989. Effects of temperature fluctuations and photoperiod on hatching in the parasitic copepod *Salmincola edwardsii*. *Canadian Journal of Zoology,* 68,1330–1332.

Poulin, R. 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology,* 132,143–151.

Power, M. E. 1992. Top-Down and Bottom-Up Forces in Food Webs: Do Plants Have Primacy. *Ecology* 73, 733-746.

Priede, I.G. 1985. *Metabolic scope in fishes*. London, Croom-Helm.

Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetics,* 155, 945-959.

Prosser, C. L. and Brown, F. A. 1961. *Comparative Animal Physiology*, Saunders, Philadelphia, PA, USA

Pusey, B., Arthington, A. and Read, M. 1998. Freshwater fishes of the Burdekin River, Australia: biogeography, history and spatial variation in community structure. *Environmental Biology of Fishes*, 53, 303-318

Queensland Department of Employment, E. D. a. I. 2011a. Annual status report 2010 - East Coast Inshore Fin Fishery. DEEDI.

Queensland Department of Employment, E. D. a. I. 2011b. Annual status report 2010 - Gulf of Carpentaria Inshore Fin Fishery. DEEDI.

Rajkumar, M., Perumal1, P. and Trilles, J. P. 2005. *Cymothoa indica* (Crustacea, Isopoda, Cymothoidae) parasitizes the cultured larvae of the Asian seabass *Lates calcarifer* under laboratory conditions. *Diseases of Aquatic Organisms,* 66, 87–90.

Raymond, M. and Rousset, F. 1995. An exact test for population differentiation. *Evolution,* 49, 1280-1283.

Rayner, T. S., B. Pusey, and Pearson R. G. 2008. Seasonal flooding, instream habitat structure and fish assemblages in the Mulgrave River, north-east Queensland: towards a new conceptual framework for understanding fish-habitat dynamics in small tropical rivers. *Marine and Freshwater Research* 59, 97-116.

Robins, J., D. Mayer, J. Staunton-Smith, I. Halliday, B. Sawynok, and Sellin M. 2006. Variable growth rates of the tropical estuarine fish barramundi *Lates calcarifer* (Bloch) under different freshwater flow conditions. *Journal of Fish Biology* 69, 379-391.

Robins, J. B., I. A. Halliday, J. Staunton-Smith, D. G. Mayer, and Sellin M. J. 2005. Freshwater-flow requirements of estuarine fisheries in tropical Australia: a review of the state of knowledge and application of a suggested approach. *Marine and Freshwater Research 56*, 343-360.

Robinson, E.S. 1965. Cestoda (Tetraphyllidea and Trypanoryncha) from marine fishes of New South Wales. Records of the Australian Museum 26, 341–348.

Rodgers, L. J. and Bloomfield, J. P. 1993. Comparison of growth, condition and mortality between stocks of barramundi Lates calcarifer: 1. Cairns and Burrum River strains, In Barlow CG, Rimmer MA eds, Larval and Juvenile Culture of Barramundi *Lates calcarifer*, Fishing Industry Research and Development Council, Canberra pp 9.1 - 9.19

Rodgers, L. J. and Burke, J. B. 1988. Aetiology of 'red spot' disease (vibriosis) with special reference to the ectoparasitic digenean *Prototransversotrema steeri* (Angel) and the sea mullet, *Mugil cephalus* (Linnaeus). *Journal of Fish Biology*, 32, 655–663.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig, and Pounds J. A. 2003. Fingerprints of global warming on wild animals and plants. *Nature* 421, 57-60.

Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes,* 4, 137-138.

Roubal, F. R. and Diggles, B. K. 1993. The rate of development of *Polylabroides multispinosus* (Monogenea: Microcotylidae) parasitic on the gills of *Acanthopagrus australis* (Pisces: Sparidae). *International Journal for Parasitology,* 23(7), 871–875.

Rousset, F. 2008. genepop'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources,* 8, 103-106.

Routley, M. H., Nilsson, G. E., Renshaw, G. M. C. 2002. Exposure to hypoxia primes the respiratory and metabolic responses of the epaulette shark to progressive hypoxia. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology*, 131, 313-321

Rückert, S., Palm, H. W. and Klimpel, S. 2008. Parasite fauna of seabass (*Lates calcarifer*) under mariculture conditions in Lampung Bay, Indonesia. *Journal of Applied Ichthyology,* 24, 321–327.

Russell, D.J., Garrett, R.N. 1983. Use by juvenile barramundi, Lates calcarifer, and other fishers of temporary supralittoral habitats in a tropical estuary in northern Australia. *Australian Journal of Marine and Freshwater Research* 34, 805 - 811.

Russell, D. and R. Garrett. 1985. Early life history of barramundi, (Lates calcarifer) (Bloch), in north-eastern Queensland. *Australian Journal of Marine and Freshwater Research* 36:191-201.

Russell, D. J. and Garrett, R. N. 1988. Movements of juvenile barramundi, *Lates-calcarifer* (Bloch), In Northeastern Queensland. *Australian Journal of Marine and Freshwater Research,* 39, 117-123.

Salen-Picard, C., A. M. Darnaude, D. Arlhac, and Harmelin-Vivien M. L. 2002. Fluctuations of macrobenthic populations: A link between climate-driven river run-off and sole fishery yields in the Gulf of Lions. *Oecologia* 133, 380-388.

Salini, J. and Shaklee, J. B. 1988. Genetic structure of barramundi (*Lates calcarifer*) stocks from northern Australia. *Australian Journal of Marine and Freshwater Research,* 39, 317-329.

Sambrook, J. and Russell, D. W. 2001. *Molecular cloning: a laboratory manual,* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press.

Sanil, N. K., Asokan, P. K and Lijo John, Vijayan, K. K. 2011. Pathological manifestations of the acanthocephalan parasite, *Tenuiproboscis* sp. in the mangrove red snapper (*Lutjanus argentimaculatus*) (Forsskål, 1775), a candidate species for aquaculture from Southern India. *Aquaculture,* 310, 259–266.

Saoud, M. F. A. et al. 2002. Helminth parasites of fishes from the Arabian Gulf: 10. Trematodes of the genus *Stephanostomum* Looss, 1899 (Digenea: Acanthocolpidae Lühe, 1901), with description of *Stephanostomum qatarense* n. sp., and redescription of *Stephanostomum triacanthi* Madhavi, *Rivista di Parassitologia,* 19, 87–103. Not sighted, In Gibson, D. I., Bray, R. A., and Harris, E. A. (Compilers) 2005. Host-Parasite Database of the Natural History Museum, London.

Sawynok, B. and Platten, J. 2009. Growth, movement and survival of stocked fish in impoundments and waterways of Queensland 1987-2008. Available from http://info-fish.net/suntag/stocktag-reports/: Infish Services.

Schofield, P. J. and Chapman, L. J. 2000. Hypoxia tolerance of introduced Nile perch: implications for survival of indigenous fishes in the Lake Victoria basin. *African Zoology*, 35, 35-42

Schram, T. A., Iversen, L., Heuch, P. A. and Sterud, E. 2005. *Argulus* sp. (Crustacea: Branchiura) on cod, *Gadus morhua* from Finnmark, northern Norway. *Journal of the Marine Biological Association U.K.*, 85, 81–86.

Schurmann, H., Steffensen, J. F. 1997. Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *Journal of Fish Biology*, 50, 1166-1180

Seng, L. T. 1997. Control of parasites in cultured marine finfishes in Southeast Asia - An overview. *International Journal for Parasitology,* 27, 1177–1184.

Seng, L. T., Tan, Z. and Enright, W. J. 2006. Important parasitic diseases in cultured marine fish in the Asia-Pacific region. *AQUA Culture AsiaPacific Magazine,* 2, 14–16.

Shaklee, J. B., Salini, J. and Garrett, R. N. 1993. Electrophoretic Characterization of Multiple Genetic Stocks of Barramundi Perch in Queensland, Australia. *Transactions of the American Fisheries Society,* 122, 685-701.

Shaklee, J. B. and Salini, J. P. 1985. Genetic-variation and population subdivision in Australian barramundi, *Lates-calcarifer* (bloch). *Australian Journal of Marine and Freshwater Research,* 36, 203-218.

Sharp, N., Diggles, B. K., Poortenaar, C. W. and Willis, T. J. 2004. Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi lalandi*. New Zealand. *Aquaculture,* 236, 67–83.

Shumway, S. E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. *Journal of the World Aquaculture Society* 21, 65-104.

Sievers, G., Lobos, C., Inostroza, R. and Ernst, S. 1996. The effect of the isopod parasite Ceratothoa gaudichaudii on the body weight of farmed Salmo salar in southern Chile. *Aquaculture,* 143, 1–6.

Slatkin, M. and Voelm, L. 1991. FST in a hierarchical island model. *Genetics,* 127, 627-9.

Small, L., Humphrey, J. and Shilton, C. 2002. Report on investigation into gill ectoparasites in sea-caged barramundi at Port Hurd. Northern Territory Government, Internal Report pp 15

Smith, C., Benzie, J. A. H. and Wilson, K. J. 2003. Isolation and characterization of eight microsatellite loci from silver-lipped pearl oyster Pinctada maxima. *Molecular Ecology Notes,* 3, 125-127.

Southwell, T. 1915. Notes from the Bengal Fisheries Laboratory, Indian Museum. No. 2. On some Indian parasites of fish, with a note on carcinoma in trout. *Records of the Indian Museum, Calcutta*, 11, 311-330, 26-28.

Srivastava, H. D. 1938 A new parasite of the family Acanthocolpidae Luhe, 1909, from an Indian host. *Indian Journal of Veterinary Science and Animal Husbandry,* 8, 247–248. Not sighted. In: Bray, R. A. and Cribb, T. H. 2003. Species of *Stephanostomum* Looss, 1899 (Digenea: Acanthocolpidae) from fishes of Australian and South Pacific waters, including five new species. *Systematic Parasitology,* 55, 159–197.

Stead, D. G., 1914. (Notes and exhibits.) Proceedings Linnean Society New South Wales 39, 20.

Stromberg, B. E. and Averbeck, G. A. 1999. The role of parasite epidemiology in the management of grazing cattle. *International Journal for Parasitology,* 29, 33–39.

Stuart, I. G. and Berghuis, A. P. 2002. Upstream passage of fish through a vertical-slot fishway in an Australian subtropical river. *Fisheries Management and Ecology*, 9, 111-122

Staunton-Smith, J., J. B. Robins, D. G. Mayer, M. J. Sellin, and Halliday I. A. 2004. Does the quantity and timing of fresh water flowing into a dry tropical estuary affect year-class strength of barramundi (Lates calcarifer)? *Marine and Freshwater Research* 55, 787-797.

Stefan, H. G. and Preud'homme E. B. 1993. Stream temperature estimation from air temperature. *JAWRA Journal of the American Water Resources Association* 29, 27-45.

Svendsen, Y. S. and Haug, T. 1991. Effect of formalin, benzocaine, and hypo- and hypersaline exposures against adults and eggs of *Entobdella hippoglossi* (Müller), an ectoparasite on Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture,* 94, 279–289.

Swets, J. A. 1988. Measuring the accuracy of diagnostic systems. *Science* 240, 1285-1293.

Tanimoto, M., J. B. Robins, M. F. O'Neill, I. A. Halliday, and Campbell A. B. 2012. Quantifying the effects of climate change and water abstraction on a population of barramundi (*Lates calcarifer*), a diadromous estuarine finfish. *Marine and Freshwater Research* 63, 715-726.

Tewksbury, J. J., Huey, R. B. and Deutsch, C. A. 2008. Putting the Heat on Tropical Animals. *Science*, 320, 1296-1297

Tingbao, Y., Kritsky, D. C., Yuan, S., Jianying, Z., Suhua1, S. and Agrawal, N. 2006. Diplectanids infesting the gills of the barramundi *Lates calcarifer* (Bloch) (Perciformes: Centropomidae), with the proposal of *Laticola* n. g. (Monogenoidea: Diplectanidae). *Systematic Parasitology,* 63, 127–141.

Tokşen, E., Buchmann, K. and Bresciani, J. 2007. Occurrence of *Benedenia sciaenae* van Beneden, 1856 (Monogenea: Capsalidae) in cultured meager (*Argyrosomus regius* Asso, 1801) (Teleost: Sciaenidae) from western Turkey. *Bulletin of the European Association of Fish Pathology,* 27, 250–253.

Townsend, S. A., Boland, K. T. and Wrigley, T. J. 1992. Factors contributing to a fish kill in the Australian wet/dry tropics. *Water Research*, 26, 1039-1044

Townsend, S. A. and Edwards, C. A. 2003. A fish kill event, hypoxia and other limnological impacts associated with early wet season flow into a lake on the Mary River floodplain, tropical northern Australia. *Lakes & Reservoirs: Research and Management*, 8, 169-179.

Tripathi, Y. R. 1957. Studies on the parasites of Indian fishes II. Monogenea, Family: Dactylogyridae. *Indian Journal of Helminthology,* 7, 5–24.

Tripathi, Y. R. 1962. Parasitic copepods from Indian fishes. III. Family Anthosomatidae and Dichelesthiidae. *First All-India Congress on Zoology, 1959*, 191–217.

Troadec, J. P. 2000. Adaptation Opportunities to Climate Variability and Change in the Exploitation and Utilisation of Marine Living Resources. *Environmental Monitoring and Assessment* 61, 101-112.

Tubbs, L. A., Poortenaar, C. W., Sewell, M. A. and Diggles, B. K. 2005. Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*. *International Journal for Parasitology,* 35, 315–327.

Unmack, P. J. 2001. Biogeography of Australian freshwater fishes. *Journal of Biogeography,* 28, 1053-1089.

VanDerWal, J., L. Falconi, S. Januchowski, L. Shoo, and C. Storlie. 2011. SDMTools: Species distribution modelling tools: Tools for processing data associated with species distribution modelling exercises. R package version 1.

VanDerWal, J., L.P. Shoo, C.N. Johnson, and Williams S.E. 2009. Abundance and the environmental niche: environmental suitability estimated from niche models predicts the upper limit of local abundance. *The American Naturalist* 174, 282-291.

Van Oosterhout, C., Hutchinson, W. F., Willis, D. P. M. and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* 4, 535-538.

Van Putten, M. and Rassam, G. 2001. Fisheries, climate change, and working together. *Fisheries,* 26, 4.

Velasquez, C.C. 1962. Some Hemuirid trematodes from Philippine fishes. *The Journal of Parasitology,* 485, 39-544.

Venmathi Maran, B. A., Leong, T. S., Ohtsuka, S. and Nagasawa, K. 2009. Records of *Caligus* (Crustacea: Copepoda: Caligidae) from Marine Fish Cultured in Floating Cages in Malaysia with a Redescription of the Male of *Caligus longipedis* Bassett-Smith, 1898. *Zoological Studies,* 48, 797–807.

Vinoth, R., Ajith Kumar, T. T., Ravichandran, S., Gopi, M. and Rameshkumar, G. 2010. Infestation of copepod parasites in food fishes of Vellar Estuary, Southeast Coast of India. *Acta Parasitologica Globalis,* 1, 1–5.

Waller, P. J. 1997. Nematode Parasite Control of Livestock in the Tropics/Subtropics: the Need for Novel Approaches. *International Journal of Parasitology,* 21(10),1193–1201.

Walters, C. J. and J. S. Collie. 1988. Is research on environmental factors useful to fisheries management? *Canadian Journal of Fisheries and Aquatic Sciences* 45, 1848-1854.

Wang, C. M., Lo, L. C., Zhu, Z. Y. and Yue, G. H. 2006. A genome scan for quantitative trait loci affecting growth-related traits in an F1 family of Asian seabass (*Lates calcerifer*). *BMC Genomics,* doi:10.1186/1471-2164-7-274, 13.

Wang, C. M., Zhu, Z. Y., Lo, L. C., Feng, F., Lin, G., Yang, W. T., Li, J. and Yue, G. H. 2007. A microsatellite linkage map of Barramundi, *Lates calcarifer*. *Genetics,* 175, 907-915.

Watanabe, W.O., 1991. Saltwater culture on tilapia in the Caribbean. *World Aquaculture,* 22, 49–54.

Webster, I. T., Rea, N., Padovan, A. V., Dostine, P., Townsend, S. A. and Cook, S. 2005. An analysis of primary production in the Daly River, a relatively unimpacted tropical river in northern Australia. *Marine and Freshwater Research,* 56, 303-316

Weir, B. S. and Cockerham, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution,* 38, 1358-1370.

Weiss, R. F. 1970. The solubility of nitrogen, oxygen and argon in water and seawater. *Deep Sea Research and Oceanographic Abstracts*, 17, 721-735

Whitfield, A. K. 2005. Fishes and freshwater in southern African estuaries - A review. Aquatic Living Resources 18:275-289.

Whittington, I. D. 2001. A revision of *Benedenia* Diesing, 1858 including a redescription of *B. sciaenae* (van Beneden, 1856) Odhner, 1905 and recognition of *Menziesia* Gibson, 1976 (Monogenea: Capsalidae). *Journal of Natural History,* 35, 663–777.

Whittington, I. D. 2004. The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note about species complexes. *Folia Parasitologica,* 51, 109–122.

Whittington I.D. 2005. Monogenea Monopisthocotylea (ectoparasitic flukes). In: Marine Parasitology. Rohde K. (Ed.), pp. 63–72. CSIRO Publishing, Melbourne, Australia.

Whittington, I. D. 2012. *Benedenia seriolae* and *Neobenedenia* species. In: Woo, P. T. K. and Buchmann, K. (Ed.), *Fish Parasites: Pathobiology and Protection*, CABI publishing, Oxford.

Whittington, I. D., Chisholm, L. A. and Rohde, K. 2000. The larvae of Monogenea (Platyhelminthes). *Advances in Parasitology,* 44,139–232.

Whittington I.D. and Chisholm L.A. 2008. Diseases caused by Monogenea. In: Fish Diseases Volume 2. Eiras J.C., Segner H., Wahlii T. & Kapoor B.G. (Eds.), pp. 683–816 (Chapter 13). Science Publishers, Inc., New Hampshire, USA.

Williams, A. 1989. Some monogenean parasites of the genera *Calceostoma* van Beneden, 1852 and Diplectanum Diesing, 1858 Irom *Argyrosomus hololepidotus* (Lacepède, 1802) (Sciaenidae: Teleostei) in Western Australia. *Systematic Parasitology,* 14, 187–201.

Williams, A. and Jones, H. 1994. Parasitic worms of fish. Taylor and Francis, London and Bristol, 593pp.

Wu, R. S. S. 1990. Environmental tolerance of some marine fish: applications in mariculture management, U.S. Environmental and Protection Agency, Guangzhou, PRC.

Yamaguti S. 1961: Studies on the helminth fauna of Japan. Part 57. Nematodes of fishes, III. *Journal of Helminthology*, R.T. Leiper Suppl., 217–228.

Yue, G. H., Li, Y., Chao, T. M., Chou, R. and Orban, L. 2002. Novel microsatellites from Asian sea bass (*Lates calcarifer*) and their application to broodstock analysis. *Marine Biotechnology,* 4, 503-511.

Yue, G. H., Yang, L. and Orban, L. 2001. Characterization of microsatellites in the *IGF-2* and *GH* genes of asian seabass (*Lates calcarifer*). *Marine Biotechnology,* 3, 1-3.

Yoshinaga, T., Segawa, I., Kamaishi, T. and Sorimachi, M. 2000. Effects of temperature, salinity and chlorine treatment on egg hatching of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder. *Fish Pathology,* 35(2), 85–88.

Young, P. 1970. The species of Monogenoidea recorded from Australian fishes and notes on their zoo geography. Anales del Instituto de Biología, Universidad Nacional Autónoma de México. *Serie zoología (0368-8720)*, 41 (1), 163.

Zhu, Z. Y., Lin, G., Lo, L. C., Xu, Y. X., Feng, F., Chou, R. & Yue, G. H. 2006. Genetic analysis of Asian seabass stocks using novel polymorphic microsatellites. *Aquaculture,* 256, 167-173.

APPENDICES

Appendix A1: Summary of P1 suite microsatellite markers and associated primers derived from (Zhu et al., 2006). PCR conditions including final primer concentrations and fluorescent labels, are those utilized in the present study and these conditions and the observed allele size range may differ from Zhu et al., (2006). All reverse primer sequences (R) were PIG tailed (Brownstein et al., 1996) as indicated by the underlined sequence. * indicates locus possesses 1bpr alleles.

Locus	Motif	[Final primer] (μM)	Allele size range (bp)	Primer Sequence (5′–3′)
Lca08	$(GA)_n$	0.2	*259 - 266	**F:VIC-**GACGGTTGGATTTAAGGATTTT **R:**GTTTCTTTGCTTTTCATTAGTGTTTCCCACAC
Lca20	$(CA)_n$	0.8	124 - 160	**F:VIC**-TTGCCCACCCAAAGACC **R:**GTTTCTTTACAGGCTTAACAGTGTGC
Lca21	$(CA)_n$	0.8	187 - 198	**F:NED**-GTGCCACCTGCCTGACC **R:**GTTTCTTTGCCATGACTGATTGCTGAGA
Lca58	$(GT)_n$	0.24	405 - 463	**F:FAM**-AAACAGGCAGCCAGATAGACAGAG **R:**GTTTCTTAAGAGGTGGTGGGACTAATTTGAGA
Lca64	$(AC)_n$	0.2	283 - 313	**F: FAM-**AGGCATATGCACCTCACAAGAGTG **R:**GTTTCTTCCCACGGTTTATTTATCTGTCATTATC
Lca69	$(GT)_n$	1.28	359 - 367	**F:VIC-**GCCTTTCTGTTTTCTGATTTATCTTCAT **R:**GTTTCTTAACACCCCGAAATACTGCTACTACAG
Lca70	$(CAG)_n$	0.64	288 - 320	**F:NED**-AGCCTTCTCGACCCAGTCACAAG **R:**GTTTCTTCAAGCCCTGGGGTGTAAGTGTTG
Lca74	$(CA)_n$	1.28	170 - 216	**F:FAM**-CATCATTTACACTCTGTTTGCCTCAT **R:**GTTTCTTGACAGACAGGTGTTTTAGCCTATTTG
Lca98	$(TG)_n$	1.2	198 - 227	**F:PET**-CAAAGGGGCCACTGCACATAAT **R:**GTTTCTTCTCCAGCTCACCCAGGTTCACT

Appendix A2: Summary of G suite microsatellite markers and associated primers indicating source of original primer sequences as locus superscripts; 1 (Yue et al., 2001), 2 Yue et al. 2002), 3 (Zhu et al., 2006) 4 (Wang et al., 2006). PCR conditions, including final primer concentrations and fluorescent labels, are those utilized in the present study and these conditions and the observed allele size range may differ from the original primer publications. All reverse primer sequences were PIG tailed (Brownstein et al., 1996) as indicated by the underlined sequence. * indicates locus possesses 1bpr alleles.

Locus	Motif	[Final primer] (μM)	Allele size range (bp)	Primer Sequence (5′–3′)
Lca03[1]	$(CA)_n$	0.1	219 - 251	**F:NED**-TCAAATCAGTTTGTGACACG **R:**GTTTCTTTCTTGGCTCTGGATCAGTG
Lca16[2]	$(CA)_n$	0.2	* 259 - 287	**F:FAM**-ACAAGGGCTGCGCTCAGGTG **R:**GTTTCTTTGCTCTGCCAGGGTTGTTGTCCT
Lca40[2]	$(GT)_n$	0.2	126 - 146	**F:NED**-GAGGAAGCATCAGCTGTAATCA **R:**GTTTCTTTCAGGACGCAAACACTGAAAT
Lca57[3]	$(GT)_n$	0.2	205 - 230	**F**:**VIC**-GTTCCACCGTACACCACAGTTG **R:**GTTTCTTATGGCAAAGTTCTATTCTTCAAATGA
Lca154[4]	$(TG)_n$	0.1	149 - 162	**F:PET**-AAGCGTCTCTGCAGTAAAAAGATA **R:**GTTTCTTAAAACAGGGCTATAGATCCAGAAT
Lca178[4]	$(GA)_n$	0.2	421 - 459	**F:FAM**-TCCCAGGCTGTGGATGTGTCTAA **R:**GTTTCTTTCGCATATGAGGGGGAAACATTAT
Lca287[4]	$(TC)_n$	0.2	174 - 223	**F:FAM**-TAATGTTTGGGTATCCGTGTCC **R:**GTTTCTTTGACCGAATGAGCTGTTGATAAT
Lca371[4]	$(CA)_n$	0.2	378 - 390	**F:VIC**-GGGCCGGTGATCAGAGACG **R:**GTTTCTTGGCAGATCCACATGGACGAGTG

Appendix A3: Summary of the assigned subpopulation (Subpop #), freshwater fish bioregion (FW BIOREGION) and freshwater fish subprovince (FW SubProv) from Unmack (2001), as well as the assigned Level 1 and Level 2 drainage basins from National Catchment Boundaries v1.1.4 (*http://www.ga.gov.au/topographic-mapping/national-surface-water-information.html*) for each of the 48 wild barramundi collections used in the current genetic audit (Component A). Collection CODES are as per Table 1.

collection #	Collection CODE	Subpop #	FW BIOREGION	FW SubProv	Level 1 Basin	Level 2 Basin
1	PIL	1	Pilbara Province	Pilbara	2	245
2	BME	2	Paleo Province	West Plateau	12	10
3	STG	3	Kimberley Province	West Kimberley	12	210
4	ADM	4	Kimberley Province	West Kimberley	12	210
5	SWI	5	Kimberley Province	West Kimberley	12	210
6	DRY	5	Kimberley Province	East Kimberley	12	222
7	SMB	5	Kimberley Province	East Kimberley	12	222
8	KGR	5	Kimberley Province	East Kimberley	12	222
9	BER	5	Depauperate Nth Subprovince (West)	Victoria-Ord Rivers	12	222
10	HEL	5	Depauperate Nth Subprovince (West)	Victoria-Ord Rivers	12	243
11	NNC	5	Depauperate Nth Subprovince (West)	Victoria-Ord Rivers	12	243
12	ORD	5	Depauperate Nth Subprovince (West)	Victoria-Ord Rivers	12	243
13	BPG	5	Depauperate Nth Subprovince (West)	Victoria-Ord Rivers	12	19
14	MOYK	5	Speciose Nth Subprovince (West)	Daly River	12	45
15	DLYK90	6	Speciose Nth Subprovince (West)	Daly River	12	45
16	DLY08	6	Speciose Nth Subprovince (West)	Daly River	12	45
17	BTIK	7	Speciose Nth Subprovince (West)	Daly River	12	66
18	DHBK	7	Speciose Nth Subprovince (West)	Daly River	12	59
19	SHOK	7	Speciose Nth Subprovince (West)	Daly River	12	59
20	MRR	8	Speciose Nth Subprovince (West)	Arnhemland	12	80
21	ALG	9	Speciose Nth Subprovince (West)	Arnhemland	12	104
22	LVP	10	Speciose Nth Subprovince (West)	Arnhemland	12	119
23	ANBK	11	Speciose Nth Subprovince (West)	Arnhemland	12	134
24	ROP	12	Depauperate Nth Subprovince (East)	West Gulf of Carpentaria	1	148
25	MAC	13	Depauperate Nth Subprovince (East)	West Gulf of Carpentaria	1	220
26	LICK90	14	Depauperate Nth Subprovince (East)	Nicholson River	1	35
27	ALB11	14	Depauperate Nth Subprovince (East)	Nicholson River	1	35
28	GIL	14	Depauperate Nth Subprovince (East)	South Gulf of Carpentaria	1	65
29	MITK	14	Speciose Nth Subprovince (East)	East Gulf of Carpentaria	1	85
30	HOLK	14	Speciose Nth Subprovince (East)	East Gulf of Carpentaria	1	108
31	ARCK93	14	Speciose Nth Subprovince (East)	Archer River	1	113
32	ARC11	14	Speciose Nth Subprovince (East)	Archer River	1	113
33	JAR	15	Speciose Nth Subprovince (East)	Cape York	1	138
34	JCK	16	Speciose Nth Subprovince (East)	Cape York	5	2
35	ESC	16	Speciose Nth Subprovince (East)	Cape York	5	2
36	PCB	17	North-Eastern Subprovince	South-East Cape York	5	205
37	BIZ	17	North-Eastern Subprovince	South-East Cape York	5	194
38	JOR	18	North-Eastern Subprovince	North-East Queensland	5	31
39	HCC	18	North-Eastern Subprovince	North-East Queensland	5	55
40	CLE	18	North-Eastern Subprovince	North-East Queensland	5	76
41	BOWK88	18	Eastern	Burdekin River	5	83
42	BOW08	18	Eastern	Burdekin River	5	83
43	BURK89	18	Eastern	Burdekin River	5	101
44	BUR08	18	Eastern	Burdekin River	5	101
45	BRSK	19	Eastern	Fitzroy River	5	136
46	FTZK88	20	Eastern	Fitzroy River	5	156
47	FTZ13	20	Eastern	Fitzroy River	5	156
48	MARK	21	Eastern	South-East Queensland	5	173

Appendix A4: Summary of hierarchical AMOVA analyses of 16 microsatellite loci for 48 collections of barramundi across northern Australia. Collections and associated subpopulations, freshwater (FW) bioregions, FW subprovinces, Level 1 and 2 basins are as described in Appendix A3.

Source of variation	d.f.	Sum of Squares	Variance Component	*p*-value	Percent of variation
A: among/within collections					
Among collections	47	1094	0.36	**0.000**	7.9
Within collections	2498	10552	4.22	**0.000**	92.2
Total	2545	11646	4.58		
B: among/within subpopulations (significant pairwise Fst >0.010)					
Among subpopulations	20	967	0.39	**0.000**	8.4
Among collections within subpopulations	27	127	0.01	**0.029**	0.2
Within collections	2498	10552	4.22	**0.000**	91.4
Total	2545	11646	4.62		
C: among/within FW Bioregions					
Among Bioregions	8	733	0.30	**0.000**	6.5
Among collections within Bioregions	39	361	0.10	**0.000**	2.1
Within collections	2498	10552	4.22	**0.000**	91.4
Total	2545	11646	4.62		
D: among/within FW subprovinces					
Among subprovinces	17	883	0.32	**0.000**	7.0
Among collections within subprovinces	30	211	0.05	**0.000**	1.2
Within collections	2498	10552	4.22	**0.000**	91.8
Total	2545	11646	4.60		
E: among/within Level 1 basins					
Among Level 1 Basins	3	572	0.32	**0.000**	6.9
Among collections within Level 1 Basins	44	522	0.15	**0.000**	3.1
Within collections	2498	10552	4.22	**0.000**	90.0
Total	2545	11646	4.69		
F: among/within Level 2 basins					
Among Level 2 Basins	31	1011	0.35	**0.000**	7.6
Among collections within Level 2 Basins	16	83	0.02	**0.001**	0.4
Within collections	2498	10552	4.22	**0.000**	92.0
Total	2545	11646			

Appendix 5: Current and future temperature (°C), rainfall and evaporative (mL) mean of each genetically distinct subpopulation. *Future climatic variables are derived from the 50th percentile of GCMs representing RCP 8.5 in 2085.

Subpopulation ID	Min. monthly mean	Min. monthly mean (2085*)	Difference	Max. monthly mean	Max. monthly mean (2085)	Difference	Min. monthly minimum	Min. monthly minimum (2085)	Difference	Max. monthly maximum	Max. monthly maximum (2085)	Difference	Precipitation (July – Sept)	Precipitation (July – Sept 2085)	Difference	Precipitation (Jan – Mar)	Precipitation (Jan – Mar 2085)	Difference	Mean monthly potential Evap.	Potential Evap. (2085)	Difference
1	20.6	24.6	4.0	32.4	36.7	4.3	14.2	17.7	3.5	38.3	42.9	4.6	22	12	-10	168	160	-8	150	169	19
2	21.8	25.9	4.1	30.6	34.9	4.2	14.1	17.9	3.9	35.3	39.7	4.4	6	8	2	524	505	-19	128	143	15
3	22.2	26.1	4.0	30.1	33.8	3.7	13.6	17.4	3.8	36.2	40.1	3.9	8	14	5	892	919	27	155	176	21
4	23.2	27.2	4.0	30.8	33.8	3.0	14.8	19.8	4.9	36.7	39.4	2.7	8	10	1	860	867	7	136	164	28
5	23.9	27.8	3.8	31.2	35.0	3.8	16.7	20.2	3.5	36.7	40.7	4.0	7	9	2	725	776	51	145	161	16
6	23.8	27.6	3.9	29.8	33.5	3.7	16.1	20.4	4.2	35.3	39.3	3.9	18	16	-2	1014	1093	79	153	169	16
7	24.7	28.3	3.5	29.6	32.6	3.0	18.5	22.3	3.8	34.2	37.2	3.0	21	21	0	1083	1167	84	144	155	11
8	23.6	27.4	3.9	30.1	33.5	3.4	15.8	19.9	4.1	36.4	39.9	3.5	17	13	-3	903	978	75	134	147	12
9	24.8	28.7	3.9	30.3	33.6	3.3	18.8	23.0	4.3	35.8	39.0	3.2	9	8	-2	939	982	43	151	165	14
10	23.9	27.7	3.8	29.6	33.1	3.6	17.7	21.7	4.0	34.1	37.4	3.3	5	5	0	805	823	19	138	151	13
11	24.2	26.1	1.8	29.3	35.2	5.9	19.2	18.9	-0.3	33.5	40.9	7.4	10	5	-6	815	662	-154	132	158	26
12	22.2	27.8	5.6	31.1	32.8	1.7	14.9	23.1	8.2	37.3	36.7	-0.6	6	9	3	615	824	209	143	144	1
13	22.2	26.2	3.9	30.9	34.7	3.8	16.9	20.9	4.0	35.8	39.6	3.8	5	5	0	645	732	87	131	144	13
14	23.0	26.4	3.4	30.1	33.8	3.7	16.4	19.9	3.5	35.8	39.6	3.8	4	2	-1	940	1080	139	142	157	15
15	24.9	27.8	2.9	28.9	32.2	3.3	21.3	24.3	3.0	32.5	35.9	3.3	12	15	2	1232	1281	48	119	128	9
16	24.5	27.4	2.9	28.6	31.8	3.2	20.8	23.8	3.0	32.2	35.4	3.2	26	29	3	1232	1251	19	116	125	9
17	23.0	26.6	3.6	29.2	32.5	3.2	17.1	21.1	4.0	34.5	37.6	3.1	7	10	3	841	935	94	136	147	11
18	19.7	23.0	3.3	27.8	31.1	3.3	14.9	18.3	3.4	31.8	35.2	3.4	100	98	-3	804	932	128	122	138	11
19	17.6	21.3	3.7	27.6	30.8	3.2	11.7	15.2	3.5	32.1	35.5	3.5	75	70	-5	490	575	85	121	132	11
20	17.1	20.7	3.6	27.1	30.3	3.2	11.8	15.4	3.6	31.1	34.4	3.3	87	84	-4	346	364	18	114	123	10
21	16.3	19.5	3.2	26.1	28.8	2.8	10.7	13.8	3.1	30.4	33.1	2.7	141	131	-9	429	491	62	111	120	9
Mean	22.3	25.9	3.7	29.6	33.1	3.5	16.0	19.8	3.8	34.6	38.1	3.5	28	27	-1	776	828	52	134	148	14
Min.	16.3	19.5	1.8	26.1	28.8	1.7	10.7	13.8	-0.3	30.4	33.1	-0.6	4	2	-10	168	160	-154	111	120	1
Max.	24.9	28.7	5.6	32.4	36.7	5.9	21.3	24.3	8.2	38.3	42.9	7.4	141	131	5	1232	1281	209	155	176	28
SD	2.6	2.6	0.7	1.5	1.8	0.8	2.8	2.9	1.4	2.2	2.5	1.4	38	36	4	277	291	71	13	17	6

Appendix 6: Staff Lists and contribution

Professor Dean Jerry - Genetics

Dr Carolyn Smith-Keune - Genetics

Dr Igor Pirozzi - Physiology

Dr Guy Carton - Physiology

Dr Jeremy vanderWaal – Climate Modelling

Dr Kate Hutson - Parasites

Lauren Hodgson – Climate Modelling

Appendix 7: Publications

Gamble, S., Carton, A.G., Pirozzi, I. (under review). The routine metabolic rate of barramundi, *Lates calcarifer*, measured in both closed and open-top static respirometers. Marine & Freshwater Behaviour & Physiology

Collins, G.M., Clark, T.D., Rummer, J.L. and Carton, A.G. (in press). Hypoxia tolerance is conserved across genetically distinct sub-populations of an iconic, tropical Australian teleost (Lates calcarifer). Marine and Freshwater Behaviour and Physiology

Militz, TA, Southgate, PC, Carton, AG, Hutson, KS (2013) Dietary supplementation of garlic (*Allium sativum*) to prevent monogenean infection in aquaculture. Aquaculture, 408-409, 95-99.

Militz, TA, Southgate, PC, Carton, AG, Hutson, KS (2013)Efficacy of garlic (*Allium sativum*) extract applied as a therapeutic immersion treatment for *Neobenedenia* sp. management in aquaculture. Journal of Fish Diseases DOI:OI: 10.1111/jfd.12129.

Conference presentations
Militz, TA (2013) Efficacy of garlic extract for the prevention of a marine ectoparasite. 2013 Fisheries Research and Development Corporation (FRDC) Conference. 8 - 12 July 2013. Cairns, Queensland.

Electronic Appendix E1 – E5: Raw genotyping data and summary genetic files

https://research.jcu.edu.au/researchdata/default/detail/0d7bb8111a792482b5ef063211e75423/