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Barramundi origins

Determining the contribution of stocking to the barramundi catch on Queensland's east coast

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Abbreviations

AGM	Annual General Meeting
AIMS	Australian Institute of Marine Science
AMOVA	analysis of molecular variance
ANOVA	analysis of variance
CBA	cost-benefit analysis
DAF	Queensland Department of Agriculture and Fisheries
DNA	deoxyribonucleic acid
DES	Queensland Department of Environment and Science
FIS	Inbreeding coefficient of an individual (I) relative to the subpopulation (S)
FRDC	Fisheries Research and Development Corporation
FST	Inbreeding coefficient of subpopulation (S) relative to the total population (T)
ISO	International Organisation for Standardization
JCU	James Cook University
LA-ICP-MS	Laser Ablation Inductively Coupled Plasma Mass Spectrometry
LDA	linear discriminant analysis
LOD	Log-Order Difference
NATA	National Association of Testing Authorities, Australia
NIR	near infrared
NIRS	near infrared spectroscopy
NIST	National Institute of Standards and Technology
NRM	natural resource management group
OOB	out-of-the-bag cross-validation estimation method
PC-QDA	principal component quadratic discriminant analysis
PCR	polymerase chain reaction
QBFP	Queensland Boating and Fisheries Patrol
QDA	quadratic discriminant analysis
RAC	Research Advisory Committee
RF	random forest classification algorithm
RSD	relative standard deviation
SG	Savitsky-Golay smoothing
UWA	University of Western Australia

Executive Summary

Researchers from Queensland's Department of Agriculture and Fisheries, James Cook University, and the University of Western Australia tested a range of otolith-based and genetic methods to identify hatchery-born from wild-born Barramundi. The project took place in the Dry Tropics region, where extensive historical and ongoing impoundment stocking (release of hatchery-born Barramundi into freshwater bodies) may be contributing to the downstream wild-capture marine and estuarine fishery. Fish samples were collected from the commercial and recreational wild-capture marine and estuarine fishery in 2019 and 2020, following the major Townsville floods in February 2019. The team identified a cost-effective means of using trace elements in fish otoliths to reliably distinguish hatchery-origin from wild-origin fish, measure the contribution of stocked fish to the wild population, and assess the sustainability of the wild-capture fishery.

Background

Extensive stocking of Barramundi fingerlings has occurred in Queensland since the 1980s, primarily to create and boost local recreational fisheries. Over time, tens of millions of fingerlings have been released into dams and weirs, predominantly on the east coast. Many of those fish have since had the opportunity to escape from their impoundments during moderate or exceptional wet seasons. The contribution of these fish to the total Barramundi population in Queensland has confounded stock assessments.

Aims and Objectives

This project aimed to identify the most accurate and cost-effective method to distinguish if a legal-size Barramundi captured in the wild-catch marine and estuarine fishery was born in the wild, or was born in a hatchery setting. Natural lifelong chemical traces in otoliths (calcified structures typically used to estimate fish age) were used to differentiate fish that had experienced wild or hatchery conditions in early life. These chemical traces were measured directly using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) to quantify otolith microchemical composition, and indirectly using hyperspectral imaging in the near infrared (NIR) spectral region. Otolith-based methods have the potential to be retrospectively applied to historical otolith collections as a means of quantifying stocked fish contribution to the fishery over the past 15 years for which archival otoliths are available. Provenance classification using a genetic method was also used. The central research goal was addressed through four objectives:

(1) Develop a near infrared spectroscopy (NIRS) model that can distinguish between wild-origin and hatchery-origin Barramundi.
(2) Develop an otolith chemistry model that can distinguish between wild-origin and hatchery-origin Barramundi.
(3) Compare the results from the models developed in (1) and (2) against a genetic parentage analysis approach and assess agreement between the three different methods of distinguishing wild- from hatchery-origin fish in wild caught Barramundi.
(4) Evaluate and complete a cost-benefit analysis of the approaches developed.

Methodology

More than 200 known-origin juvenile Barramundi were used to calibrate otolith NIRS and microchemical provenance classification models. Fish samples were collected directly from participating hatcheries and stocking groups, and by a network of volunteer recreational and commercial fishers in 2019 and 2020. Over 600 "unknown-origin" samples were collected during routine monitoring of the Dry Tropics Barramundi fishery in 2019. Provenance-determination methods were assessed based on their relative accuracy and inter-method agreement rates.

A cost-benefit analysis was subsequently undertaken for each provenance-determination method to meet three potential management needs: (1) Developing a provenance determination model for Barramundi in a new region; (2) Implementing provenance determination for Barramundi as a routine monitoring tool in the Dry Tropics, where a suitable model has now been established; and (3) Applying a provenance determination model to a subset of the historic Barramundi otolith collection.

Results

Provenance determination using whole and sectioned otolith NIRS was not successful in this instance. It may be that the provenance-related differences in otolith microchemical composition, or their proxies, fall below the detectability limit of the NIRS hyperspectral sensor used in this study. Further attempts to assess the potential of the NIRS technique for provenance determination should use a much larger collection of known-origin samples than were available in the current study.

Provenance determination using otolith core microchemistry was highly accurate (>98% accuracy) and was driven by consistent, biophysical differences in the water chemistry of farm versus wild habitats (e.g. low manganese availability in filtered, well oxygenated farm water).

Provenance confirmation using microsatellite parentage analysis was 83% accurate on known wild-origin samples; accuracy on known hatchery-origin samples could not be assessed as they were used to define the classification threshold. The high level of misclassification of known wild-origin samples (17%) indicated a high probability of genetic introgression, in which genetic material from stocked individuals is found in subsequent generations of wild-born individuals, indicating breeding of stocked fish with the wild population has occurred.

Extrapolation of the otolith microchemistry provenance classification method to the 2019 commercial catch in the Dry Tropics region estimated that 3% of the Barramundi landed were hatchery-born and 96% were wild-born, indicating that the fishery is primarily capturing wild-born individuals. Extrapolation of the genetic classification method to the 2019 commercial catch in the Dry Tropics region estimated that 21% of the Barramundi landed displayed predominantly hatchery ancestry and 79% had predominantly wild ancestry.

Otolith microchemical profiles allowed for opportunistic reconstruction of Barramundi movement history, and indicated that extended residency (≥1 year) in freshwater habitats during early/juvenile life was very common. Extrapolation of a simple juvenile freshwater residency model to the 2019 commercial catch in the Dry Tropics region estimated that 33% of the Barramundi landed had spent at least one full year during their juvenile period in a freshwater nursery habitat (39% by weight). Individuals that displayed evidence of juvenile freshwater residency were more strongly represented in the larger and heavier size classes as adults. Spatiotemporal patterns in the representation of juvenile freshwater residency indicate that productivity of the wild-capture marine and estuarine Barramundi fishery in some areas may be limited by barriers to juvenile fish movement into suitable freshwater nursery habits.

Implications for relevant stakeholders

(1) The wild-capture marine and estuarine Barramundi fishery in the Dry Tropics region is primarily composed of wild-born fish. This suggests that biomass estimates in the recent stock assessment reflect a predominantly wild-born stock that is not being significantly supplemented by fish stocking.
(2) Stocked fish represent 3% of the Barramundi fishery, but hatchery ancestry was detected in 21% of the catch, indicating that stocked fish successfully breed with wild fish and contribute genetic material to subsequent generations. The strong representation of hatchery ancestry among the wild-born population (17%) highlights the importance of fish stocking regulations to support local genetic diversity and evolutionary traits.
(3) Juvenile access to suitable freshwater habitats is important in sustaining the Barramundi fishery (33% by number, 39% by weight) and must be maintained. In addition, it may be possible to enhance

productivity of this fishery by increasing juvenile fish access to suitable freshwater habitats (e.g. installation of fishways and habitat remediation).

(4) Otolith microchemistry was the most accurate and reliable method for provenance detection in Barramundi, with high potential for use in other regions. Collection of complete otolith microchemical profiles for provenance determination also allows collection of fish movement history data (e.g. detecting juvenile freshwater residency).

Recommendations

(1) We recommend the use of complete cross-sectional otolith microchemical profiles for routine monitoring of fish provenance and juvenile habitat use in the Barramundi fishery. This will clarify whether the contributions of stocked fish (3%) and juvenile freshwater residency (33%) identified in the current study are consistent through time. Such monitoring would provide early indications of changes in population dynamics (e.g. increased proportion of stocked fish indicating failure of wild recruitment; reduced proportion of juvenile freshwater residents indicating reduced juvenile habitat availability) and fishable biomass. Targeted application of otolith microchemistry could occur in other regions where stakeholders have expressed concern regarding the contribution of stocked fish to the fishery and/or limitations on juvenile habitat availability.

(2) Although Barramundi stocking has minimal contribution to the wild-capture marine and estuarine fishery, it is critical to establishing (e.g. Ross Dam) and maintaining (e.g. Burdekin Dam) significant recreational impoundment fisheries that otherwise would not exist. As a result, Barramundi stocking may indirectly support wild-capture fisheries by shifting recreational fishing effort away from estuaries and marine environments. Quantifying spatial and temporal changes in recreational fishing effort would be a valuable means of assessing a potential indirect impact of fish stocking on downstream wild-capture fisheries.

(3) Consideration should be given to the genetic composition of stocked fish, particularly when stocking in impoundments from which significant numbers of individuals can escape and eventually interbreed with the wild population. In order to limit inbreeding accumulation, we recommend stocked fish originate from at least 50 broodstock over a period of 5 years. In order to conserve local adaptive traits, we recommend that only wild-collected broodstock should be used. We advise that broodstock that have been selected for aquaculture traits should not be used for stocking into impoundments from which significant numbers of individuals can escape and eventually interbreed with the wild population.

(4) Juvenile freshwater residency is a major driver of the Barramundi fishery, but is highly variable through time (i.e. between year classes) and does not seem to correlate to wet season severity. The mechanisms driving annual variation in juvenile Barramundi freshwater residency merit further investigation, as they appear to be much stronger drivers of recruitment to the fishery than Barramundi stocking in this region.

(5) We recommend management policies, as well as incentives for on-ground organisations and landholders, to increase availability and accessibility of suitable freshwater habitats for juvenile fish, which will contribute to the sustainability of the Barramundi fishery and can potentially be used to increase fishery biomass in this region.

(6) We recommend implementation of a pilot study on Barramundi provenance determination using Single Nucleotide Polymorphisms (SNPs) from both tissue samples and archival otoliths. This will confirm whether SNPs are a suitable tool for high-resolution parentage analysis that can be rapidly deployed as a monitoring tool following events such as dam overtopping, large farm escape events, etc. Use of a high-resolution genetic parentage tool such as SNPs should provide much greater confidence than microsatellites can for identifying wild vs hatchery-born individuals in instances where genetic introgression may be occurring.

Keywords

Barramundi, *Lates calcarifer*, provenance, natal origin, fish stocking, otolith microchemistry, NIRS, microsatellites, parentage analysis, cost-benefit analysis, nursery habitat

Introduction

Background

Fish stocking occurs in aquatic systems around the world for conservation purposes, to create or enhance recreational fisheries, and to enhance wild-catch commercial fisheries (Warren-Myers et al. 2018). Identifying and quantifying the contribution of stocking efforts to the wild population is crucial to informing these management objectives. However, routinely monitoring the effects of stocking requires an accurate, cost-effective, and replicable means of distinguishing hatchery-origin from wild-origin fish.

In Queensland, hatchery-born Barramundi (*Lates calcarifer*) fingerlings are released into impoundments (i.e. dams and weirs) and waterways primarily to create and boost local recreational freshwater fisheries (MacKinnon and Cooper 1987; Rutledge et al. 1990). Large wet season freshwater flows enable downstream movement of these stocked fish into the wild-capture marine and estuarine commercial fishery, increasing the biomass of Barramundi available to the fishery and confounding underlying changes in natural population dynamics (Streipert et al. 2019). Due to the challenges of accurately and cost-effectively distinguishing hatchery-origin from wild-origin Barramundi at a fishery-relevant scale, the contribution of stocked Barramundi to the wild-capture marine and estuarine commercial fishery on Queensland's east coast is unclear. Early work in the Johnstone River catchment in Far North Queensland used external wire tags to estimate that fish stocked from 1992 onwards contributed between 10 and 15% of the 580-650 mm cohort of Barramundi (Russell and Rimmer 1997). This implies that fish stocking has the potential to make a significant numerical contribution to the wild-capture marine and estuarine fishery, which is a significant source of uncertainty in Barramundi stock assessments and management of Barramundi stocks in line with Queensland's Sustainable Fisheries Strategy.

Several methods can be used to reliably distinguish between hatchery-origin and wild-origin fish. However, to be useful for stock assessment a method must be (1) accurate (i.e. correct within a specific tolerance range for error), (2) cost-effective when applied to a large number of samples per year, and (3) replicable (i.e. able to be undertaken consistently over multiple years to provide a time series of data).

The use of genetic methods for provenance determination typically provides a high degree of confidence. In particular, parentage analysis, in which alleles of unknown-origin individuals are matched against a database of potential parents, appears to be highly successful in some systems (e.g. Barramundi, Russell et al. 2013, Noble et al. 2014; coho salmon, Beacham et al. 2017). However, this approach requires an accessible and complete database of the hatchery broodstock used over the course of the stocking period. In addition, genetic provenance determination assumes some level of genetic difference between hatchery-born and wild-born offspring, which may not be the case if hatchery broodstock or their recent ancestors were sourced from the local wild population, or if there has been high levels of F1 generation introgression between hatchery-origin and wild Barramundi. In such situations, the relative frequency of alleles and simulation of hypothetical progeny genotypes can be used to estimate fish provenance (e.g. Russell et al. 2013). Genetic methods typically require fresh or properly preserved tissue samples from which to extract undamaged DNA (Rodriguez-Ezpeleta et al. 2013). In the absence of preserved tissue samples, forensic DNA techniques have successfully been used on historic otolith collections to estimate population size and connectivity (Poulsen et al. 2006; Toomey et al. 2016), as well as to retrospectively determine provenance (e.g. Robbins et al 2008). Despite recent advances in efficiency (Campbell et al. 2015), genetic approaches can be expensive when applied en masse to wild fishery surveys, and can produce low quantity and poor quality DNA when extracted from otoliths (Toomey et al. 2016).

Alternatively, hatchery-origin fish can be physically, chemically, isotopically, or thermally tagged prior to release to facilitate identification at capture (Warren-Myers et al. 2018). Applying physical external tags requires individually handling large numbers of fingerlings (Gillanders 2009), with the risk that the tags may be shed/discarded later in life (Boucek and Adams 2011). External tagging is generally most successful with large juvenile and adult fish (Gillanders 2009), rather than the fingerling stage at which Barramundi are typically stocked (i.e. ~100 mm). Permanent chemical, isotopic, and thermal tagging of hard structures (e.g. otoliths, fin rays) has been successfully implemented in a number of species (Gillanders 2009). Batch marking of stocked fish remains underutilised in Australian fisheries despite extensive research and promising cost-benefit analyses around the low cost of marking, the low cost of detecting the marking, the longevity of marks, and the low mortality rates of fingerlings/larvae during the marking process (Warren-Myers et al. 2018). This is the case for stocked Barramundi in Queensland, which may be sourced from hatcheries which breed and rear fish for a range of purposes (e.g. for human consumption).

An increasingly common alternative is to identify and leverage natural lifelong chemical marking of hatchery-origin fish, which occurs through differential mineral and isotopic deposition in otoliths resulting from differences in ambient water chemistry and in diet between hatchery and wild environments experienced in early life (Pracheil et al. 2014; Hüssy et al. 2020). Otoliths are chemically stable and grow throughout the life of a fish, and therefore provide a permanent record of the mineral environment in which the fish has lived (Campana and Neilson 1985). The core of the otolith is formed shortly after larval fish hatching, and reflects the microchemical availability of trace elements such as strontium, barium, and magnesium in the fish's natal environment. The edge of the otolith is formed shortly before fish capture, and reflects the microchemical availability of trace elements in the environment in the days and weeks leading up to capture. A transect across a sectioned otolith surface captures the entire microchemical life history of an individual, including natal origin, movement history, and capture location. If successful, otolith-based provenance-determination can be applied to archival otolith collections, such as those maintained by DAF, to quantify the contribution of hatchery-origin fish to the fishery historically and into the future.

Otolith elemental fingerprinting using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS, hereafter referred to as "laser ablation") of thinly-sectioned otoliths has become a standard method for establishing fish provenance (Gillanders 2009; Pracheil et al. 2014; Warren-Myers et al. 2018). Laser ablation provides a direct measure of otolith elemental composition and isotopic ratios. It can be highly successful if there are measurable and consistent differences in water chemistry among natal locations. However, otolith microchemistry is difficult to scale up to the large numbers typically required to inform fisheries management, primarily due to the high cost per sample. The high cost of microchemical analysis is a result of the level of technical skill needed to prepare an otolith for ablation without contamination from equipment and other samples, as well as the cost of operating the specialised ablation instruments.

Near Infrared Spectroscopy (NIRS) and hyperspectral imaging are non-invasive, non-destructive means of using optical light to determine chemical composition of various materials, and may provide a more cost-effective method for resolving otolith provenance. NIRS technology has been used for decades as a diagnostic tool in a wide range of disciplines, primarily because it offers a rapid, repeatable and cost-effective method of predicting properties of interest. NIRS is a vibrational spectroscopy technique, and is based on the interaction of electromagnetic energy with covalent bonds in organic molecules. The bonds associated with different functional groups (C-H, N-H, -OH) absorb near infrared (NIR) energy at unique frequencies, resulting in some molecules changing their vibration from one energy level to another. NIRS techniques harness and translate these vibrations (or unique spectral signatures) using simple, rapid analytical procedures. NIR light penetrates into otolith material, thereby reflecting bond energies on the otolith surface, and bond energies inside the otolith itself up to a maximum depth of potentially 5-6 mm (Passerotti et al. 2020).

NIRS relies on developing a calibration equation (statistical model or algorithm, usually of multi-variate regression form) that relates the property of interest in the material being assessed to the NIR spectra measured by a spectrophotometer or imaging camera (charged couple device). In the current project, the chemical composition of the otolith core provides the spectral information ("reference data") that is related to the environment in which the fish was hatched. If hatchery-origin fish are sufficiently different in otolith chemistry (e.g. trace element composition or suitable proxies) from wild-origin fish, there should be a spectral signature from the otolith core area that is unique to fish reared in a hatchery environment. NIRS has the potential to provide the means to cost-effectively scale up discrimination of fish provenance using natural differences in otolith microchemistry, and has been applied to measure fish age based on otolith chemistry differences (Wedding et al. 2014, Helser et al. 2019, Wright et al. 2021). However, it remains untested for fish provenance determination.

Need

The current project was developed to address the Queensland Research Advisory Committee's (RAC) November 2017 priority: "Determine the proportion of Queensland East Coast (marine and estuarine) wild Barramundi catch that is of hatchery origin." Stocking of Barramundi fingerlings in impoundments and waterways has been identified as a significant source of uncertainty in quantitative stock assessment of the Queensland Barramundi fishery (Streipert et al. 2019). This uncertainty is amplified following above-average wet seasons, during which large freshwater flows connect stocked waterways to estuaries and present the opportunity for stocked fish to move into the wild-capture marine and estuarine commercial fishery (e.g. overtopping of Awoonga dam, Wesche et al. 2013).

Stocking of Barramundi in Queensland is significant, with over 14 million fingerlings stocked in impoundments and waterways statewide between the earliest recorded stocking events in 1987 and 2017 (DAF, *unpublished data*). In the Dry Tropics region that is the focus of the current study, over 3.7 million Barramundi fingerlings have been stocked in dams, weirs, and floodplain lagoons from 1988 to 2020 (S. Leahy, *unpublished data*) (Figure 1). Retrospective calculations estimate that as many as 3 million of the 3.7 million Barramundi fingerlings stocked in the Dry Tropics region could have survived and moved into the wild-catch fishery by 2021, while a further 300,000 fingerlings may yet have the opportunity to move out of their stocking location (S. Leahy, *unpublished data*). Tag returns from hatchery-origin fish captured as adults in marine systems by the wild-harvest commercial net fishery, charter, and recreational sectors provides evidence that some stocked Barramundi can later be caught in the wild-capture marine and estuarine fishery (Rimmer and Russell 1998; Sawynok and Platten 2009).

Recent assessment of the Queensland Barramundi fishery has highlighted the importance of quantifying the magnitude of the contribution of stocked fish to Queensland's East Coast Barramundi fishery in order to reduce uncertainty in the quantitative stock assessment (Streipert et al. 2019). Ideally, estimates of the contribution of stocked fish to the wild-capture fishery should balance accuracy, cost-effectiveness, and be carried out in multiple years to provide a time series of data from which Fisheries Queensland can make assessments regarding the sustainability of the fishery. To this end, the current project aims to assess the efficacy of different otolith-based methods to distinguish hatchery-origin from wild-origin Barramundi. These are: whole otolith spectroscopy, sectioned otolith spectroscopy, and sectioned otolith microchemistry. The most cost-effective method can subsequently be applied to Fisheries Queensland's otolith collection to quantify the contribution of stocked fish to historic and future catches, and thereby support more robust stock status reporting and quantitative stock assessment for east coast Barramundi.

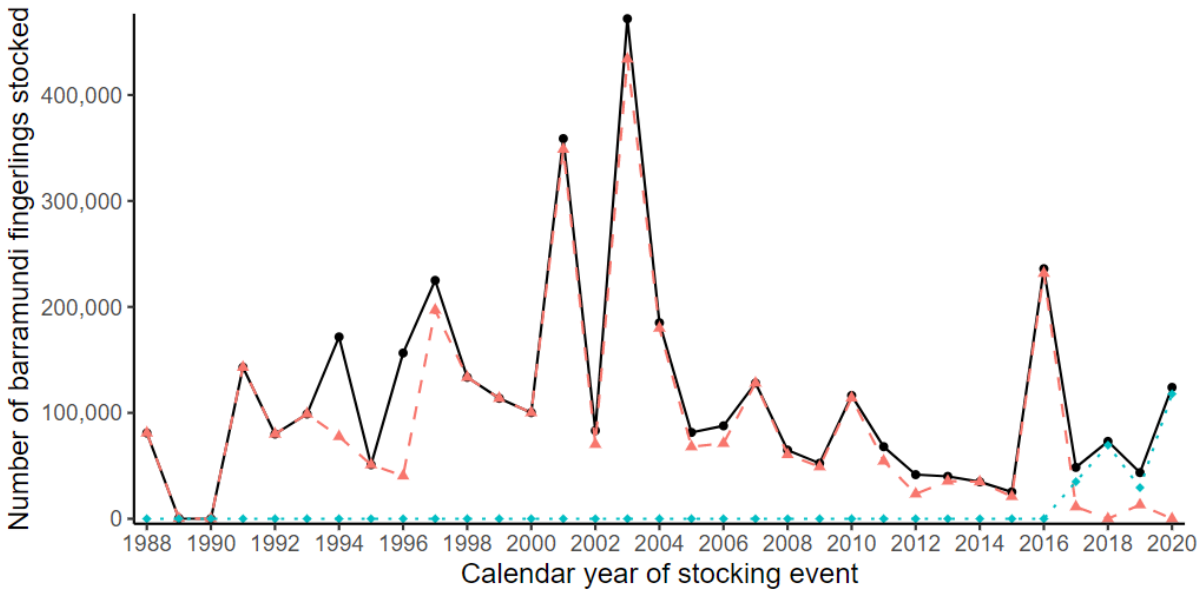

Figure 1. *Dry Tropics Barramundi stocking totals, by year of stocking event. Black solid line: total number of fingerlings released that calendar year. Red dashed line: total number of Barramundi stocked that year that had the opportunity to escape from their stocking location two or more years later. Blue dotted line: total number of Barramundi stocked that year that have not yet had the opportunity to move into the wild-capture marine and estuarine fishery (as of 2021).*

Objectives

(1) To develop a near infrared spectroscopy (NIRS) model that can distinguish between wild-origin and hatchery-origin Barramundi.

(2) To develop an otolith chemistry model that can distinguish between wild-origin and hatchery-origin Barramundi.

(3) Original objective (3): To compare the results from the models developed in (1) and (2) against an established method (genetics) to distinguish between wild and hatchery origin fish in wild-caught Barramundi.

Revised objective (3): To compare the results from the models developed in (1) and (2) against a genetic parentage analysis approach and assess agreement between the three different methods of distinguishing wild from hatchery-origin fish in wild-caught Barramundi. Objective (3) was revised due to the possibility of introgression of hatchery-origin genotypes into wild populations, such that the genetic approach detected fish with hatchery ancestry, rather than solely fish that had been born in hatcheries.

(4) To evaluate and complete a cost-benefit analysis of the approaches developed.

Methods

Study region

Dry Tropics Barramundi (19˚S to 20˚S) are a sub-stock of Queensland's North East Coast Barramundi stock (15°S to 20°S) (Streipert et al. 2019). The Dry Tropics region is defined by extremely seasonal rainfall, with dry winters and wet summers resulting in typically ephemeral surface water flows in unregulated watercourses (Davis et al. 2014). Dams and weirs on the Burdekin River, Haughton River, and Ross River were built for water storage and flood mitigation (Davis et al. 2014; Townsville City Council 2021), and have been the main impoundments receiving stocked Barramundi fingerlings (Figure 2). Remnant wetlands and artificial freshwater holding areas maintained by surface water flows for extensive agricultural irrigation on the lower Burdekin floodplain (Davis and Moore 2016) have also been the target of fish stocking activities.

A number of community groups have been involved in Barramundi stocking across a range of impoundments in this region (Table 1, Figure 2). The current research project focused on impoundments that are regularly and currently stocked (i.e. not Bowen River weir or Alligator Creek), and impoundments from which successful escape events are possible (i.e. not Eungella dam due to the height of the dam wall).

Table 1. *Stocked Barramundi impoundments, contributing stocking groups, total fingerlings stocked, and stocking period. Impoundments that were not included in the current study (due to absence of recent stocking history, or impossibility of successful escape events) are indicated in grey.*

Catchment	Impoundment	Stocking group	Fingerlings stocked	Stocking period
Burdekin	Burdekin Dam & floodplain	Burdekin Fish Restocking Association	>2,330,000	1988 to present
	Burdekin upper reaches	Charters Towers & Dalrymple Fish Stocking Group	>270,000	1999 to present
	Bowen River weir	Bowen River Fish Stocking Association	>280,000	1999 to 2011
	Eungella Dam	Mackay Area Fish Stocking Association	>400,000	1994 to present
Haughton	Haughton River weirs	Cungulla Fishing Club in conjunction with Burdekin Fish Restocking Association	>27,000	1997 to present
Alligator	Alligator Creek	Initially Burdekin Fish Restocking Association, then Townsville Barramundi Restocking Group	>47,000	2000 to 2004
Ross	Ross River Dam and weirs	Townsville Barramundi Restocking Group	>350,000	1992 to present

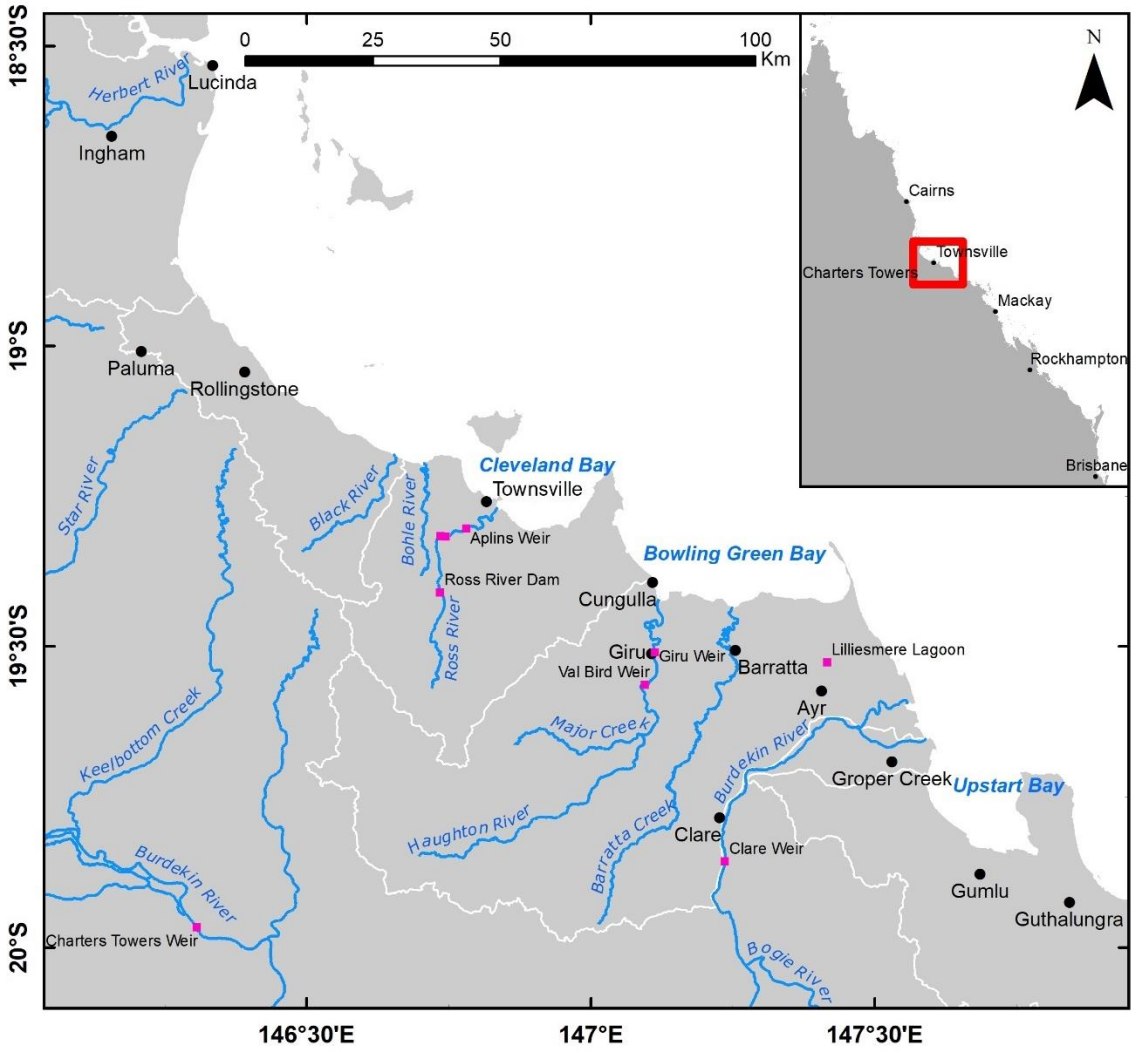

Figure 2. *Map of the study region. Major stocking areas are indicated with pink squares. Not illustrated: the Burdekin Dam, a major stocked impoundment south of the map display; numerous stocked lagoons in the Burdekin floodplain, east and south of Lilliesmere Lagoon. Inset: map of east coast of Queensland for context.*

Fish sampling

Developing classification models to determine Barramundi provenance requires the collection of known-origin samples from which calibration models are developed. A total of 130 known hatchery-origin fish were sourced from the four different Barramundi aquaculture facilities that have supplied Barramundi fingerlings to stocking groups in the Dry Tropics region within the previous decade (2009-2019). Approximately 80% of the Barramundi stocked in the Burdekin, Haughton, and Ross River systems between 2009 and 2019 were spawned at one of three different hatcheries (referred to as Hatcheries 1, 2, and 3) and reared at one of four different "grow-out" facilities (referred to as Farms A, B, C, and D). Understanding this chain of movement is important for understanding the potential source of water that stocked Barramundi may have experienced before their release in waterways. Contributing Barramundi aquaculture facilities, whether hatcheries or grow-out facilities, are hereafter referred to as "farms".

Hatchery-origin fish were collected between February 2019 and April 2020 directly from each of the four contributing farms at the typical stocking size of approximately 100 mm, or from stocking groups immediately prior to stocking events. These fish were euthanised in line with Animal Ethics permit SA 2018-12-671. For two of the farms, fingerlings were sourced from two different cohorts (one reared in

the dry season, one reared in the wet season) in order to capture inter-cohort variability in otolith chemistry that may arise from wet season induced changes in farm water chemistry.

A total of 263 known wild-origin Barramundi were collected from freshwater creeks and lagoons, estuaries, and inshore marine habitats along the east coast of Queensland between 18.7°S and 20°S (Ingham to Guthalungra, Figure 2) between March 2019 and October 2020. Collection was carried out by volunteer recreational fishers (General Fisheries Permit 200672) and targeted scientific collection (General Fisheries Permit 186281) in line with Animal Ethics permit SA-2018-12-671. Young-of-the-year Barramundi (i.e. younger than one year old) have a limited movement range (Russell and Garrett 1988), therefore juvenile Barramundi between 100-300 mm collected from known unstocked creek systems were assumed to be wild-born. Individuals >400 mm and known stocked areas/waterways were avoided to minimise the chance of escaped stocked fish from previous years being mistaken for wild-origin fish. However, due to difficulties capturing the target size class in the wild, fish up to 470 mm were retained in areas where few or no smaller fish were collected (Figure 3).

All known-origin fish were processed fresh or defrosted. Fish total length and head length were recorded to the nearest millimetre. A genetic sample was taken from the caudal fin or exposed muscle tissue, placed in a labelled 1.5 mL vial containing molecular grade ethanol (100% EtOH), and stored at -4˚C until processed for DNA extraction. Both sagittal otoliths were extracted, cleaned of adhering tissue or fluid, and stored dry for subsequent otolith-based analyses.

Unknown-origin fish were sampled during routine Fisheries Queensland Fishery Monitoring activity during the 2019 Barramundi season (1 February 2019 to 1 November 2019). A subset of commercial catch from the Dry Tropics region (Lucinda to Cape Upstart) was measured, and where possible tissue samples were taken and otoliths extracted. The collection of unknown-origin fish was supplemented with samples from the recreational sector in areas for which commercial samples were not available (e.g. the Haughton River estuary and stocked impoundments). Collection from the recreational fishing sector took place during the 2019 and 2020 Barramundi seasons. Matching tissue samples and otoliths were collected from a total of 721 commercially-caught unknown-origin Barramundi and 22 recreationally-caught unknown-origin Barramundi. Tissue samples, but no otoliths, were taken from a further 102 commercially-caught unknown-origin Barramundi.

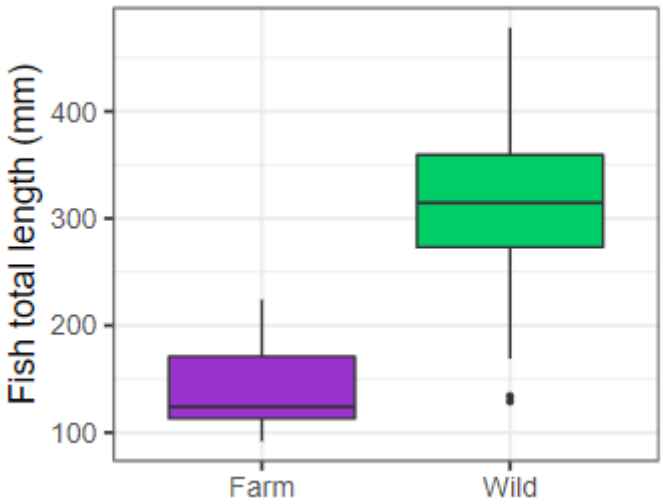

Figure 3. *Range of fish total lengths (in mm) used for provenance analyses, by origin.*

Water sampling

Water samples were collected and analysed for trace element composition at each of the farms where hatchery-origin fish were hatched and/or reared, as well as from locations at which wild-origin Barramundi had been captured. This served to identify whether there were differences in trace element concentrations between farm and wild habitats which were likely to affect the relative concentrations of trace elements deposited in the otoliths of fish reared in those environments. In addition, water sampling at key farms and wild habitats were temporally replicated to capture variability in the trace element profiles of these environments within and between seasons (three dry seasons and two wet seasons). At each site, a water sample was collected in a triple-rinsed 5 L bucket, of which 1 L of unfiltered water was stored in a triple-rinsed bottle, and 250 ml of water was passed through a sterile 0.45 µm Sartorius Minisart® hermetically sealed single use filter and stored in a nitric acid treated bottle triple-rinsed with filtrate, and subsequently buffered with 1.25 ml of 1% v/v nitric acid. Water samples were stored in a cool dark container for transport, and the following analyses were performed by the NATA (ISO 17025) accredited Queensland Government's Department of Environment and Science's Chemistry Centre Laboratory in Brisbane: conductivity (analogous to salinity), concentration of major cations (calcium, magnesium, sodium, potassium, phosphorus, silica), concentration of major anions (sulphate), and concentration of dissolved metals (aluminium, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lanthanum, lithium, magnesium, manganese, molybdenum, nickel, lead, selenium, silver, strontium, thallium, tin, titanium, uranium, vanadium, zinc). A subset of water samples were also analysed for $^{87}Sr/^{86}Sr$ (strontium) isotopic ratio and strontium concentrations by the Isotope Geoscience Group of the University of Melbourne.

Otolith preparation

For a subset of 818 fish (N = 49 known hatchery-origin, 163 known wild-origin, and 606 unknown-origin), the left sagittal otolith was blocked in clear casting resin. The right otolith was blocked if the left otolith was damaged or unavailable. All plasticware used was soaked in 10% nitric acid for 24 hours and rinsed with Milli-Q water (Millipore Corporation). Blocked otoliths were sectioned transversely through the core (i.e. primordium) using a low speed saw lubricated with Milli-Q water to produce two 300 µm thick sections exposing the otolith core. The section that best captured the otolith core ("primary section") was prepared for spectroscopy and microchemical analysis. The primary section was rinsed in analytical grade ethanol to remove surface contaminants potentially introduced by the saw, and hand polished with 15 µm aluminium oxide lapping film moistened with Milli-Q water. Primary sections were stored in acid-washed plastic vials, and mounted on microscope slides using clear casting resin; air dried slides were stored in individual plastic bags. For adult fish, secondary sections were prepared for age determination by mounting on microscope slides using clear casting resin and a glass coverslip.

A pilot sample of 8 hatchery-origin, 18 wild-origin, and 4 unknown-origin otoliths (total N = 30 otoliths) were prepared as above for trial NIRS and microchemical analysis to identify ideal spectral settings, placement of ablation track, and target trace elements and isotopes. NIRS and microchemical data collection and analysis of the pilot sample of 30 sectioned otoliths are described in 'Appendix 4. Pilot study'. The remaining 788 sectioned otoliths were subsequently prepared for NIRS and microchemical data collection as above.

Where available, the matching whole otolith for each fish was stored clean and dry for whole-otolith NIRS data collection and analysis.

Otolith NIRS

NIR spectra were collected from 818 slide-mounted otolith sections (N = 49 known hatchery origin, N = 163 known wild origin, N = 606 unknown origin). NIR spectra were also collected from a subset of the corresponding whole otoliths (N = 49 known hatchery origin, N = 163 known wild origin, N = 19 unknown origin) (Figure 4).

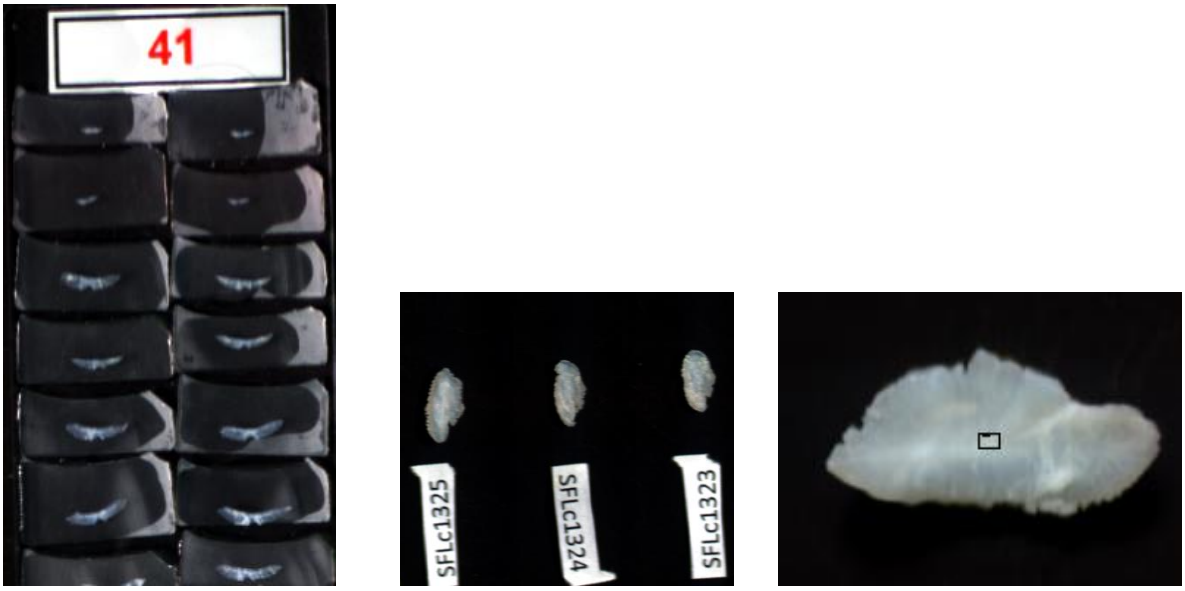

Figure 4. *True colour hyperspectral image of sectioned otoliths mounted on a glass slide (left), whole otoliths (centre) and zoomed area of interest on a whole otolith (right).*

NIR spectra collection

A Resonon Pika XC hyperspectral camera (Resonon Inc., USA) covering the spectral region of 400-1000 nm range was used to capture the spectral characteristics of both the sectioned and whole otoliths.

Spectra were collected in diffuse reflectance mode, with incident NIR energy provided by a single 100 watt halogen light source mounted at 45° to the camera (Figure 5), on a benchtop stage with a motorised sample platform. Frame rate and integration times were adjusted to avoid light saturation, while maximising lighting to enhance spectral information. A lens with a focal length of 23 mm was utilised at a pathlength of 200 mm, providing a pixel size of 0.051 mm. Spectral data were collected using the Spectronon Pro program Version 2.122 (Resonon Inc., USA) utilised by the Resonon camera.

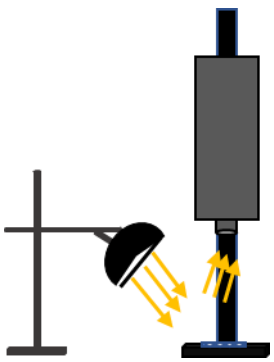

Figure 5. *Setup of the Resonon hyperspectral camera and lighting platform.*

Data analysis

The best location on the otolith to identify provenance-related differences is the otolith core, avoiding both the sulcus and edge effects as these contain materials from the end of the fish's life (Appendix Figure 1). Spectral data pixels from the otolith core were manually selected and spectrally averaged in the Spectronon Pro program Version 2.122 (Resonon Inc., USA) and then exported into R (version 4.0.5) for data analysis using the statistical package MASS (Ripley et al. 2021).

Wavelength selection was informed by the pilot analysis (Appendix 4. Pilot study) and constrained to the NIR wavelength region of 783 to 959 nm. A 35-point second derivative Savitsky-Golay (SG) smoothing transformation was applied to enhance spectral features. A predictive model for qualitative classification was developed using principal components quadratic discriminant analysis (PC-QDA). PC-QDA is a classification technique where the number of groups and the samples that belong to each group are pre-defined (Otto 1999; Naes et al. 2002). This technique produces a number of discriminant functions that maximise the separation between the groups, yet minimises the variance within groups. Data dimensionality was reduced using principal component analysis (PCA) prior to running the QDA.

The predictive model for NIRS of sectioned otoliths was constructed from N = 41 hatchery-origin and N = 145 wild-origin samples (total N = 186 known-origin samples). The predictive model for NIRS of whole otoliths was constructed from N = 49 hatchery-origin and N = 163 wild-origin samples (total N = 212 known-origin samples). Accuracy of each model was assessed using 100/0, 80/20, and 60/40 calibration/validation random subsets.

Unknown-origin samples underwent the same wavelength selection and SG smoothing procedure described above. Their NIRS signatures were subsequently dimensionally-reduced using the Principal Component eigenvalues developed from the known-origin samples and fitted to the QDA classification model.

Otolith microchemistry

Data collection

All otolith sections (total N = 814; made up of N = 49 known hatchery-origin, N = 163 known wild-origin, and N = 606 unknown origin samples) were analysed for trace element composition at the University of Western Australia (Perth, WA) using an Analyte G2 laser ablation system coupled with an ElementXR Sector-field ICP-MS. Prepared otolith slides were ultrasonicated prior to ablation to remove any surface contaminants.

Ablation occurred in a helium-flushed chamber mixed with argon and nitrogen. For each otolith, the ablation surface was cleaned four times using pre-ablation tracks with a spot size of 50-150 µm and a pulse rate of 12 Hz, after which the concentration of 14 trace elements (aluminium, barium, boron, copper, iron, magnesium, manganese, nickel, phosphorus, rubidium, sulphur, strontium, yttrium, zinc) and calcium were quantified. Background measurements were collected for 60 seconds before and after each otolith ablation track, and standards were analysed every 30-50 minutes throughout each session to correct for any short-term instrument drift.

Trace element composition was collected along a distal-to-proximal polyline ablation transect ("vertical" transect, Appendix Figure 2), providing trace element composition information across the complete lifetime of each fish. A rectangular laser spot (hereafter referred to as "slit") was used for all samples to increase chronological precision of the trace element data. For large, adult otoliths, trace element composition was collected using a 30 µm by 110 µm rectangular laser slit moving at a speed of 5 $\mu m \cdot s^{-1}$. For small, typically wild-origin otoliths, ablation was carried out using a 45 µm by 75 µm rectangular laser slit moving at a speed of 5 $\mu m \cdot s^{-1}$. For extremely small, typically hatchery-origin

otoliths, ablation was carried out using a 45 µm by 75 µm rectangular laser slit moving at a speed of 2 $\mu m \cdot s^{-1}$. The difference in laser slit dimensions accommodated the large difference in otolith dimensions between the small, known-origin samples and the large, unknown-origin samples, but resulted in a similar amount of material ablated per pulse (~3,300 μm^2 for large otoliths, ~3,375 μm^2 for small otoliths). The reduced ablation speed for the smallest samples was necessary to collect sufficient otolith material.

Trace element data were calibrated against a National Institute of Standards and Technology (NIST) 614 standard for internal precision, a NIST 612 secondary standard for external precision, and an in-house University of Western Australia carbonate standard. Mean relative standard deviation (RSD) for each trace element with each standard are reported in Appendix Table 1.

Data reduction was carried out in Iolite (Paton et al. 2011). All trace element concentrations are expressed as a ratio to calcium in $mmol \cdot mol^{-1}$ to account for variable deposition rates of the calcium carbonate otolith structure, but are referred to by the trace element name for convenience in this report.

Data analysis

Ablation data were converted from "seconds of ablation" to "distance ablated" to account for differing ablation speeds between differently-sized otoliths. Ablation data for each otolith and each trace element were then inspected for errors. Spikes in any trace element concentrations more than four standard deviations from the mean for each element and each otolith were considered extreme outliers and were removed from the dataset. Spikes in iron were used to identify and remove data resulting from ablation of resin rather than otolith material; iron concentration was not used in provenance classification analyses.

The uptake rate of each trace element into otolith material was inspected using matching water samples and otolith edge ("terminus") information. For all known-origin fish, otolith terminal area was identified as the final 10 µm of each ablation track, and trace element concentrations for each fish were averaged across this period. Where spatially and temporally matched water samples were available, otolith terminus trace element concentrations were correlated with water trace element concentrations.

Identification of otolith provenance was carried out using otolith "core" areas, which was manually identified for each sample as the central 400 µm of each ablation transect around which major trace element concentrations demonstrated the "compressed symmetry" typical of vertical ablation tracks (Figure 6). In addition, a local peak in magnesium concentration (Hüssy et al. 2020) and a marine water "plateau" in strontium concentration (indicative of the saline natal phase in barramundi, McCulloch et al. 2005) were used as confirmation of the otolith core location. The 400 µm core width captured the environmental signature at the time of hatching (marine water for all Barramundi) as well as immediately after hatching. Concentration of each trace element was then averaged across the core of each otolith.

Classification of the known-origin Barramundi was carried out using a random forest approach, which is a machine learning classification method. Random forest approaches are increasingly used to classify otolith microchemistry datasets as they do not assume normal distribution of predictor variables among sampling units the way linear discriminant analysis does. The random forest algorithm (Breiman 2001) was applied using the randomForest package (Liaw 2018) in R, with the default settings for number of variables randomly sampled as candidates for each split, 1,000 trees, and sampling of cases carried out without replacement. The number of trace elements in the predictive model was reduced to eight as a result of iterative removal of elements with low Gini impurity index values. Cross validation

is included in model development due to random forest's bootstrapping approach. However, accuracy of each model was re-assessed using 80/20 and 60/40 calibration/validation random subsets.

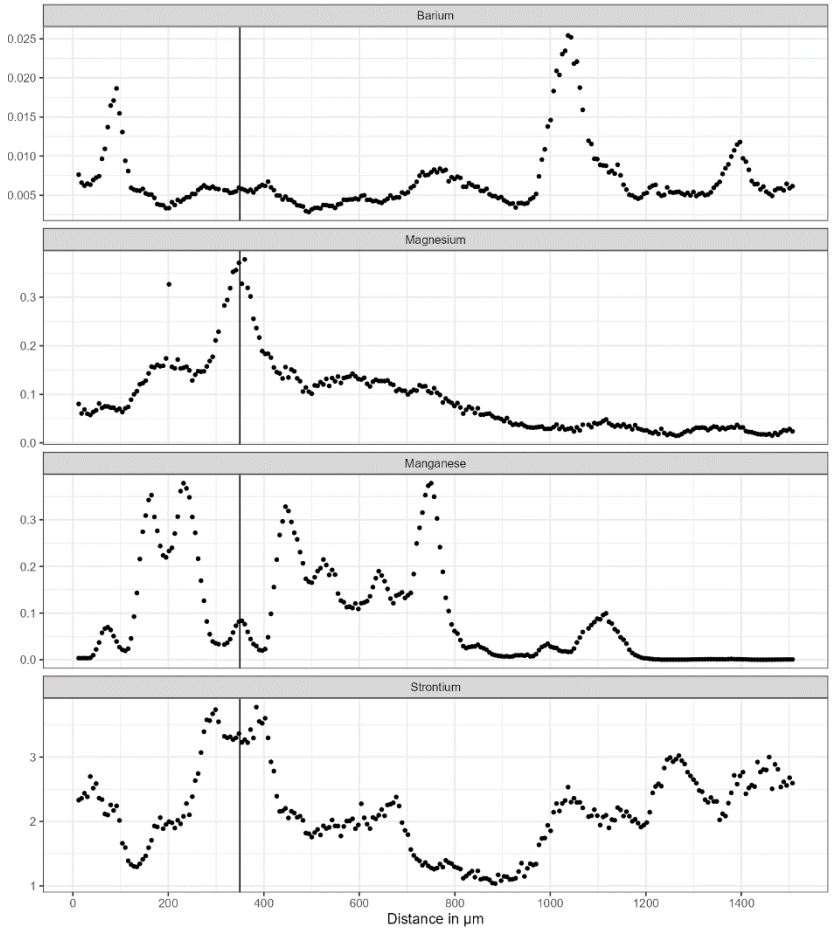

Figure 6. *Example ablation track (fish ID: SFLc0231, total length: 680 mm, age: 3+ years) illustrating the concentration of four major trace elements along an edge-to-edge ablation track. The vertical line indicates fish start-of-life, i.e. the centre of the otolith core; ablation data left of the core represents a "compressed mirror image" of the ablation data on the right side of the otolith core.*

Sample collection notes and preliminary classification analyses indicated that otoliths of two juvenile fish collected in a wild habitat immediately outside of a participating farm microchemically matched hatchery-origin fish. This could be a result of the individuals being escapees from the aquaculture facility, or due to these two individuals living in the outflows from the aquaculture facility. As a precaution, all samples collected from this wild habitat were excluded from classification model-building and were re-coded as "unknown-origin" fish (N = 4).

The predictive random forest model using otolith microchemistry was constructed from N = 49 known hatchery-origin samples and N = 163 known wild-origin samples, and was subsequently applied to 606 unknown origin samples.

In addition to provenance, the collection of cross-section ablation transects resulted in complete lifetime microchemical "profiles" for each fish, thus providing the opportunity to quantify other life history characteristics of Barramundi, in particular freshwater and saltwater residency periods (Elsdon et al. 2008). Strontium (Sr:Ca) and barium (Ba:Ca) patterns are well-established proxies for salinity in many species (Hüssy et al. 2020), including barramundi (Pender and Griffin 1996; McCulloch et al. 2005). Visual inspection of strontium and barium patterns in each ablation profile were used to coarsely identify if each adult fish had spent at least one full year (indicated by coincidence with at least one pair of translucent and opaque otolith bands) early in life in freshwater (Sr:Ca ≤ 1.5, "juvenile

freshwater resident"), or its whole life residing in saltwater (Sr:Ca ≥ 2, "marine resident"), allowing for brief seasonal periods of brackish or freshwater exposure. A third "uncertain" category was applied to fish that appeared to spend some amount of time in freshwater, but not a full year.

Parentage analysis

Tissue samples for N = 60 known hatchery origin, N = 237 known wild origin, and 746 unknown origin Barramundi were collected and prepared for parentage analysis.

DNA Extractions

DNA extraction was performed on a small (1 mm^2) subsample of the fin or muscle tissue. Each tissue sample was placed in a well of a 96-well microtitre plate containing 100 µl of Tween®-20 lysate buffer (670 Mm Tris-HCl, pH 8.0, 166 Mm ammonium sulphate, 0.2% v/v Tween®-20, 0.2% v/v IGEPAL®CA-630) and 5 µl of 20 mg/ml proteinase K and left to digest overnight at 55°C. Following digestion, the plates were centrifuged at 1000 *g* for 1 min, incubated at 95°C for 15 min to deactivate proteinase K, and stored at -20°C for 24 h. A 0.5 µl aliquot of the crude lysate supernatant was used as genomic DNA (gDNA) template for the subsequent PCR reactions.

PCR amplification of microsatellites

Two multiplex PCR suites of nine (Lca008, Lca020, Lca021, Lca058, Lca064, Lca069, Lca070, Lca074, Lca098) and seven (Lca003, Lca016, Lca040, Lca057, Lca154, Lca178, Lca371) microsatellite markers (Zhu et al. 2006) were used for the PCR reactions. Each marker had the forward primer labelled with a recommended fluorescent dye, leaving the reverse primer unlabelled. Primer mixes (x10) were prepared in advance (1-3 µM of each forward and reverse primer, adjusted to the specifications of the fluorescent intensities). Each 10 µl PCR reaction consisted of: 1 µl of 10x primer mix, 5 µl of MyTaq™HS Mix (Bioline), 3.5 µl of water and 0.5 µl of the lysate DNA. Amplification was performed on a C1000 Thermal Cycler (Bio-Rad) with all PCR reactions beginning with a 5 min denaturation step at 95°C, followed by 10 cycles at 95°C for 30 s, 57°C for 90 s and 72°C for 30 s, then 20 cycles of 95°C for 30 s, 55°C for 90 s and 72°C for 30 s, with a final 30 min extension step at 60°C. A 2 µl aliquot of the amplicons was loaded onto a 1.5% TAE agarose gel containing GelGreen® (Biotium Inc.) and electrophoresed for 25 min at 80 V to visualise successful amplification. Products were purified through filtration spin columns (700 *g* for 3 min) containing Sephadex ®G-50 Fine (GE Healthcare) and stored at 4°C.

Genotyping and allele analysis

Genotyping of the microsatellites was outsourced to the Australian Genome Research Facility (AGRF) using fragment separation analysis via an Applied Biosystems (ABI) 3730 DNA analysis system. A size standard, GeneScan 500 LIZ Ladder (Applied Biosystems) was included with each PCR product. Allele data files were analysed using Genemarker 2.4.0 software (Soft Genetics). Positive and negative controls were run simultaneously to certify allele scoring consistency and to confirm no apparent contamination had occurred during processing. Samples that had four or more failed alleles were deemed deficient and excluded from the sample data set.

Parentage analysis

DNA parentage analyses of sampled Barramundi were analysed against the commercial-in-confidence broodstock genetic database held by James Cook University, which covers putative broodstock genotyped within Hatchery 1 (N = 65), Hatchery 2 (N = 569), and Hatchery 3 (N = 904) between 2008 and 2020. Together, these three hatcheries produced approximately 80% of the Barramundi stocked in the study region in the past decade. CERVUS 3.0 (Marshall et al. 1998) was used to assign sampled fish against broodstock fish in this database. An initial simulation to calculate confidence of correct

assignment (Log Order Difference (LOD)) was conducted based on allele frequencies of broodstock within each hatchery and the parameters: sex unknown (as sometimes the sex of broodfish were not provided by hatcheries), 10,000 in silico simulated offspring, 0.2 proportion of possible parents sampled, 0.01 loci mistyped.

To establish a reliable LOD threshold for identifying if an unknown-origin sample likely originated from one of the three hatcheries, the broodstock database was subset into three datasets depending on the broodstock source (Hatchery 1, 2, or 3), and each hatchery-derived progeny sample tested against each hatchery source independently and the LOD score of the most likely parental match examined. By investigating the assignment LOD score of progeny from known sources, a Trio (Parent1-Parent2-Progeny) LOD score > 10 was identified as a suitable threshold for acceptance of parental assignments. Determination of LOD > 10 as the threshold that represented a parent-progeny trio match with high confidence was also validated by using LOD scores from a published study involving progeny from a large independent commercial spawn from one of the hatcheries, where all possible parental contributors were known (Domingos et al. 2021).

Allelic diversity and population genetic analysis

The number of private (i.e. unique) alleles in each population sample (PA), the number of alleles or allelic richness (AR), observed (Ho) and expected (He) heterozygosity were calculated for all broodstock, unknown-origin samples, and known-origin samples using the R package 'diveRsity' (Keenan et al. 2013). The inbreeding coefficient (FIS) and its 95% confidence intervals were calculated at the population level using 99,999 bootstraps within FSTAT Version 2.9.4 (Goudet 1995). To assess the levels of genetic variance present between wild and hatchery-origin populations a pairwise population FST analysis and an analysis of molecular variance (AMOVA) was conducted in Arlequin v 3.5.2.2 (Excoffier et al. 2005) with significance tested using the 95% confidence intervals over 10,000 bootstraps.

Extrapolating results to the wider stock

The contribution of stocked fish to the 2019 Dry Tropics Barramundi commercial catch was estimated using both length-frequency and age-frequency approaches. Microchemical provenance assignments with hatchery assignment probabilities < 40% were categorised as "wild origin"; assignments with hatchery assignment probabilities > 60% were categorised as "hatchery origin"; assignments with intermediate assignment probabilities (40-60%) were categorised as "uncertain" (Figure 7). The proportion of samples in each of these categories across the microchemical dataset of 580 commercially-captured fish was applied to Fisheries Queensland's Fishery Monitoring dataset of 850 commercial samples for which fish length data had been collected in a representative (i.e. not size biased) way. Length classes were also converted to weight to provide results in a format relevant to future harvest strategies for the East Coast Inshore Fin Fish Fishery.

Fish length was converted to age-frequency using an Age-Length Key (ALK) developed from the Fishery Monitoring age-at-length dataset for the 2019 Dry Tropics commercial catch. Fish age was assessed by a qualified reader following established protocols (Fisheries Queensland 2020) using the secondary otolith section produced during the preparation of thin-sectioned otoliths for NIRS and microchemistry. Fish age was subsequently converted to age class and year class (Fisheries Queensland 2020).

The length- and age-based extrapolation of project results to the 2019 Dry Tropics commercial catch was repeated using the genetic parentage results from 720 commercial samples, as well as using the juvenile residency patterns derived from the otolith microchemical profiles.

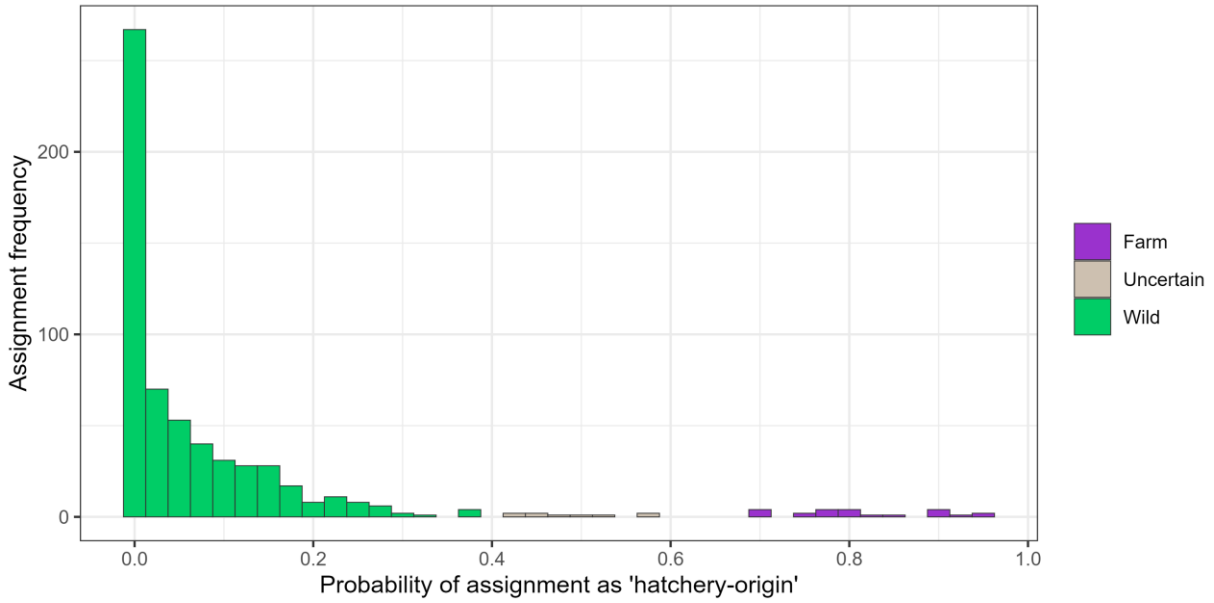

Figure 7. *Microchemical provenance assignment probability range and frequency histogram for 606 unknown-origin samples (580 from the commercial fishing sector and 26 from the recreational fishing sector). Colours indicate provenance assignment class.*

Cost-Benefit Analysis

Determining minimum sample size

A simulation was carried out in which the random forest provenance classification using otolith core microchemistry was applied to varying size subsets of each provenance type (hatchery origin and wild origin), with the goal of determining the minimum number of known-hatchery and known-wild training samples necessary to build a robust microchemical classification model. Random subsets were selected without replacement for sample sizes ranging from 10 to 49 hatchery-origin samples, and from 10 to 100 wild-origin samples. All configurations of minimum sample numbers for each origin type were tested (i.e. fully crossed design).

Similar minimum sample size calculations were not possible for the otolith NIRS models, as the spectral range of known-origin samples available in the current study was inadequate to capture the full range of spectral variability in the population. Spectral variability can arise from differences in the water chemistry of Barramundi natal environments, or spectral interference resulting from variation in otolith preparation (e.g. differences in otolith thickness, resin thickness, or imperfections in glass slides). In the absence of an objective measure of minimum sample size for the NIRS calibration dataset, for the purpose of producing a meaningful cost-benefit analysis we conservatively calculated minimum known-origin sample size as: approximately four times the number of wild-origin fish than was provided in the current study (4 * 163 ≈ 650 wild-origin samples), and an equal number of hatchery-origin samples (650 hatchery-origin samples).

Cost-benefit analysis

A cost-benefit analysis was prepared for three different types of application. Application scenarios assume collection, otolith extraction, and age estimation of unknown-origin samples (i.e. legal size adult fish) are carried out as per existing routine Fishery Monitoring activities in that region, and therefore are not included as project costs. Scenarios were:

(1) Development of a provenance determination model for Barramundi in a new region, with collection of known hatchery-origin samples from Barramundi farms known to contribute to local stocking activities, and collection of known wild-origin samples via:
    a. Local recreational fishing volunteers,
    b. Targeted electrofishing, or
    c. Targeted charter fishing.
(2) Implementation of provenance determination for Barramundi as a routine monitoring tool in a region for which a classification model has already been established (i.e. Dry Tropics), assuming collection of 400 unknown-origin adult fish per year as part of routine monitoring activities by Fisheries Queensland, and collection and processing of an additional 10% of the known-origin fish calibration dataset per year as part of classification model maintenance.
(3) Application of a provenance determination model to a representative subset of the Barramundi historic otolith collection for a region for which a classification model has already been established (i.e. Dry Tropics).

The cost of purchase of known hatchery-origin juveniles is estimated for fish of comparable size to wild-collected individuals (>200 mm), and is therefore much higher than the cost of procuring typical stocking-size hatchery-origin juveniles (~100 mm) in the current study. The larger target size for hatchery-origin samples in the cost-benefit analysis is because the otolith size differences in the current study confounded the whole otolith NIRS provenance model, and because the otoliths of very small fish were disproportionately challenging to handle and process.

Costs of collection of known wild-origin juveniles were estimated using three different methods (volunteers, electrofishing, charter fishing, Table 2). Catch rates (fish per day) are conservative estimates based on average catch rates recorded during sample collection for the present study across all habitat types and levels of site accessibility. A second, lower electrofishing catch rate (5 fish/day) is presented for ease of comparison with the charter option input costs, but was not used in the cost-benefit analysis as it did not reflect catch rates observed during the present study. For ease of interpretation, each collection method is assumed to be used to collect the full set of required known-origin fish. However, in the current study 88% of samples were collected by volunteer recreational fishers; the remainder were collected via targeted freshwater electrofishing and brackish and saltwater charter fishing. A mix of different sample collection methods may be required to adapt to local conditions and challenges, but such scenarios are not presented here.

Costs of collection for wild-origin fish are presented for both small (N = 60) and relatively large (N = 150) sample sizes (Table 2). This is to illustrate the relative insensitivity of the volunteer fisher option to increased sample sizes, and the greater increase in field costs associated with the more targeted electrofishing and charter options.

Each collaborating team (NIRS, microchemistry, genetics) supplied details on sample processing costs (Table 3). For genetic assignment methods, the cost-per-sample was provided for both the microsatellite approach used in this study, and a SNP approach more recently recommended in the literature, as well as a cost-per-sample to attempt extraction of DNA from preserved otoliths in the historic otolith collection (“forensic DNA extraction”).

For all methods, the cost of implementation of provenance determination as a routine monitoring tool was estimated under a “likely-case” scenario, in which otolith variability through time requires the addition of 10% more known-origin otoliths to the calibration dataset per year to capture interannual variation in the population. In addition, none of the costings presented budget for collection of unknown-origin fish, and instead assume that collection of unknown-origin adult fish is carried out as part of routine monitoring activities by Fisheries Queensland.

Table 2. *Estimated costings for known-origin sample collection, including replication for different wild-origin target numbers (N = 60 and N = 150). All travel expenses include: staff time costed at Queensland Government TO3.4 or equivalent pay rate, $300 per person per trip transport expenses, $120 per person per night accommodation, and $120 per people per day travel allowances. *Denotes items, catch rates, and costs applied to the subsequent cost-benefit analysis to standardise sample collection costs across each provenance-determination method.*

Hatchery-origin fish	Description	Cost / unit	Total cost
Purchase of fish*	~200 mm fish	$20 / fish*	
Staff travel to farms*	2 trips x 3 days each • Assumes fish collection from 2 different cohorts per farm	$2,122 / trip*	
N = 60 wild-origin fish			
Volunteer fisher option	• 6 trips x 5 days each for in-field engagement time • 24 days in-office engagement time per year (equivalent to 2 days per month) • Consumables (sample bags, waterproof permits, labels, and printing costs)	$3,335 / trip $367 / day $215	$29,036
Electrofishing option 1	Assumes catch rate of 6 fish / day • 2 trips x 5 days each • Electrofisher including staff hire rate: $1,700/day • Travel expenses for 2 staff for 5 days: $3,000 / trip	$11,500 / trip	$23,000
Electrofishing option 2	Assumes catch rate of 5 fish / day • 3 trips x 4 days each • Electrofisher including staff hire rate: $1,700/day • Travel expenses for 2 staff for 4 days: $2,520 / trip	$11,020 / trip	$33,060
Charter option	Assumes catch rate of 5 fish / day • 3 trips x 4 days each • Charter vessel: $1,000 / day • Travel expenses for 2 staff for 4 days: $2,520 / trip	$6,520 / trip	$19,560
N = 150 wild-origin fish			
Volunteer fisher option	• 6 trips x 5 days each for in-field engagement time • 24 days in-office engagement time per year (equivalent to 2 days per month) • Consumables (sample bags, waterproof permits, labels, and printing costs)	$3,335 / trip $367 / day	$29,036
Electrofishing option 1	Assumes catch rate of 6 fish / day • 5 trips x 5 days each • Electrofisher including staff hire rate: $1,700/day • Travel expenses for 2 staff for 5 days: $3,000 / trip	$11,500 / trip	$57,500
Electrofishing option 2	Assumes catch rate of 5 fish / day • 6 trips x 5 days each • Electrofisher including staff hire rate: $1,700/day • Travel expenses for 2 staff for 5 days: $3,000 / trip	$11,500 / trip	$69,000
Charter option*	Assumes catch rate of 5 fish / day • 6 trips x 5 days each • Charter vessel: $1,000 / day • Travel expenses for 2 staff for 5 days: $3,000 / trip	$8,000 / trip*	$48,000

Table 3. *Estimated processing costs and rates per sample for each provenance determination method (otolith NIRS, otolith microchemistry, and genetic parentage analysis).*

Whole otolith preparation	**Work rate**	**Staff level**
Otolith extraction	80 otoliths/day	TO3.4
Consumables (dissection tools, vials, transport)	$1/sample	N/a
Whole otolith NIRS		
Otolith handling time	1 otolith/minute	TO3.4
Pixel selection	20 samples/hour	TO3.4
Consumables, instrument depreciation, software licences	$6,000/year	N/a
Data analysis and model development	3 weeks	PO4.4
Data analysis, model maintenance, and ongoing application	1 week	PO4.4
Data analysis and application to historic collection	2 weeks	PO4.4
Sectioned otolith preparation for NIRS and/or microchemistry		
Extraction	80 otoliths/day	TO3.4
Blocking	80 otoliths/day	TO3.4
Sectioning	30 otoliths/day	TO3.4
Polishing	60 otoliths/day	TO3.4
Mounting	80 otoliths/day	TO3.4
Labelling + data entry	200 otoliths/day	TO3.4
Consumables (resin, catalyst, slides, depreciation on saw, replacement saw blade, analytical grade ethanol, vials, waterproof paper and printing, lapping film, “Milli Q” ultrapure water, slide boxes, cover slips for ageing, transport)	$12.27/sample	N/a
Sectioned otolith NIRS		
Otolith handling time (assume 5 otoliths per slide)	1 slide/minute	TO3.4
Pixel selection	20 samples/hour	TO3.4
Consumables, instrument depreciation, software licences	$6,000/year	N/a
Data analysis and model development	3 weeks	PO4.4
Data analysis, model maintenance, and ongoing application	1 week	PO4.4
Data analysis and application to historic collection	2 weeks	PO4.4
Sectioned otolith microchemistry		
Laser ablation	$60/otolith	External
Data analysis and model development for a new region	3 weeks	PO4.4
Data analysis, model maintenance, and ongoing application	1 week	PO4.4
Data analysis and application to historic collection	2 weeks	PO4.4
Genetic sample collection & processing		
Tissue sample collection	200 samples/day	TO3.4
Consumables (genetic vials, vial boxes, molecular grade ethanol, waterproof paper / labels and printing, cold transport)	$6.98/sample	N/a
JCU commercial charge rate for microsatellites	$40/sample	External
JCU commercial charge rate for SNPs	$35/sample	External
JCU commercial charge rate for forensic DNA extraction	$15/sample	External
Labelling + data entry	200 samples/day	TO3.4

Note 1: ideal batch size for genetic processing is multiples of 94 samples; ideal batch size for microchemical laser ablation is to maximise the number of samples mounted per slide. **Note 2:** external costs-per-sample for microsatellites, SNPs, and laser ablation can decrease with very large sample numbers.

Results

Otolith NIRS

Classification of known-origin samples using sectioned otolith NIRS

The classification model displayed an overall accuracy rate of 89% using the full calibration dataset (Table 4). Overall accuracy declined to 68% using an 80/20 calibration/validation subset, and the predictive performance improved to 77% using a 60/40 calibration/validation subset. The classification model's performance was consistently poor for known hatchery origin samples.

Table 4. *Predicted fish origin compared to known fish origin for NIRS of sectioned otoliths.*

	Predicted hatchery origin	Predicted wild origin	Percent correct
Full calibration, no validation set			
Overall accuracy			89%
Known hatchery origin	32	9	78%
Known wild origin	11	134	92%
80/20 calibration/validation set			
Overall accuracy			68%
Known hatchery origin	3	5	38%
Known wild origin	7	22	76%
60/40 calibration/validation set			
Overall accuracy			77%
Known hatchery origin	7	9	44%
Known wild origin	9	49	84%

Classification of known-origin samples using whole otolith NIRS

The classification model displayed an overall accuracy rate of 92% using the full calibration dataset (Table 5). Overall accuracy declined to 84% using an 80/20 calibration/validation subset, and decreased further to 79% using a 60/40 calibration/validation subset. The classification model's accuracy was consistently lower for hatchery-origin samples than for wild-origin samples.

Table 5. *Predicted fish origin compared to known fish origin for NIRS of whole otoliths.*

	Predicted hatchery origin	Predicted wild origin	Percent correct
Full calibration, no validation set			
Overall accuracy			92%
Known hatchery origin	40	9	82%
Known wild origin	9	154	94%
80/20 calibration/validation set			
Overall accuracy			84%
Known hatchery origin	5	5	50%
Known wild origin	2	31	94%
60/40 calibration/validation set			
Overall accuracy			79%
Known hatchery origin	13	7	65%
Known wild origin	11	54	83%

Classification of unknown-origin samples using sectioned otolith NIRS

Application of the PC-QDA calibrated using the full training dataset (N = 186) to the collection of 606 unknown-origin sectioned otoliths resulted in assignment of 13 samples as hatchery-origin and 593 samples as wild-origin. Relative assignment accuracy of these detections is addressed in the section on 'Inter-method agreement'.

Classification of unknown-origin samples using whole otolith NIRS

Application of the PC-QDA calibrated using the full training dataset (N = 212) to the subset collection of 19 unknown-origin whole otoliths resulted in assignment of 0 samples as hatchery-origin and all 19 samples as wild-origin. Relative assignment accuracy of these detections is addressed in the section on 'Inter-method agreement'.

Water chemistry

Overall trace element water chemistry varied significantly by salinity (i.e. freshwater vs brackish/marine water), by origin (i.e. farm or wild site), their interaction, and by season (Table 6). Magnesium, manganese, phosphorus and zinc concentrations differed significantly between farms and wild sites (significant origin effect), but independently of salinity (non-significant interaction, Table 6, Figure 8). Boron, barium, potassium, selenium, sodium, and sulphur concentrations differed significantly between farms and wild sites in patterns that varied by salinity (significant interaction, Table 6).

Consistency in water trace element signatures through time is critical for inferring consistency in otolith microchemistry for cohorts of fish where water samples are unavailable (e.g. historical otolith collections). Repeated water sampling at farms over multiple wet and dry seasons indicated that most trace element concentrations in each farm's water were extremely consistent through time (Figure 9a). Trace element differences between farms were typically attributable to water source (i.e. marine water higher in boron, potassium, magnesium, sodium, strontium and sulphur; bore water higher in barium).

Regular water sampling at wild Barramundi habitats identified that trace element water chemistry in these wild environments was generally more variable through time. Spikes in most elements coincided with wet season sampling (Figure 9b), and particularly large outliers occurred in samples collected immediately following a dust storm that briefly affected the study region during the 2019 wet season (Queensland Government 2020). Most elements demonstrated a distinct separation of marine, brackish, and freshwater sites that was consistent across the sampling period and the study region.

Table 6. *Results of analysis of variance identifying significant effects of salinity (freshwater or marine), origin (farm or wild), the interaction of salinity and origin, and seasonality on the concentration of each trace element in water samples, expressed as a ratio to calcium. Asterisks denote statistical significance: * for p < 0.05, ** for p < 0.01, *** for p < 0.001. Effects for which p > 0.06 are listed as "n.s." (non-significant).*

	Salinity effect	Origin effect	Salinity*origin interaction	Season
Aluminium	n.s.	n.s.	n.s.	n.s.
Boron	< 0.001 ***	0.004 **	0.012 **	n.s.
Barium	< 0.001 ***	< 0.001 ***	< 0.001 ***	n.s.
Copper	0.037 *	n.s.	n.s.	n.s.
Magnesium	< 0.001 ***	< 0.001 ***	n.s.	0.002 **
Manganese	0.024 *	0.003 **	n.s.	0.06
Nickel	n.s.	n.s.	n.s.	0.017 *
Phosphorus	n.s.	0.027 *	n.s.	n.s.
Potassium	< 0.001 ***	0.015 *	< 0.001 ***	n.s.
Selenium	0.025 *	n.s.	0.020 *	n.s.
Sodium	< 0.001 ***	n.s.	0.001 **	0.048 *
Strontium	< 0.001 ***	n.s.	n.s.	n.s.
Sulphur	< 0.001 ***	n.s.	0.008 **	n.s.
Zinc	0.039 *	0.036 *	n.s.	0.001 **
Overall	< 0.001 ***	< 0.001 ***	< 0.001 ***	< 0.001 ***

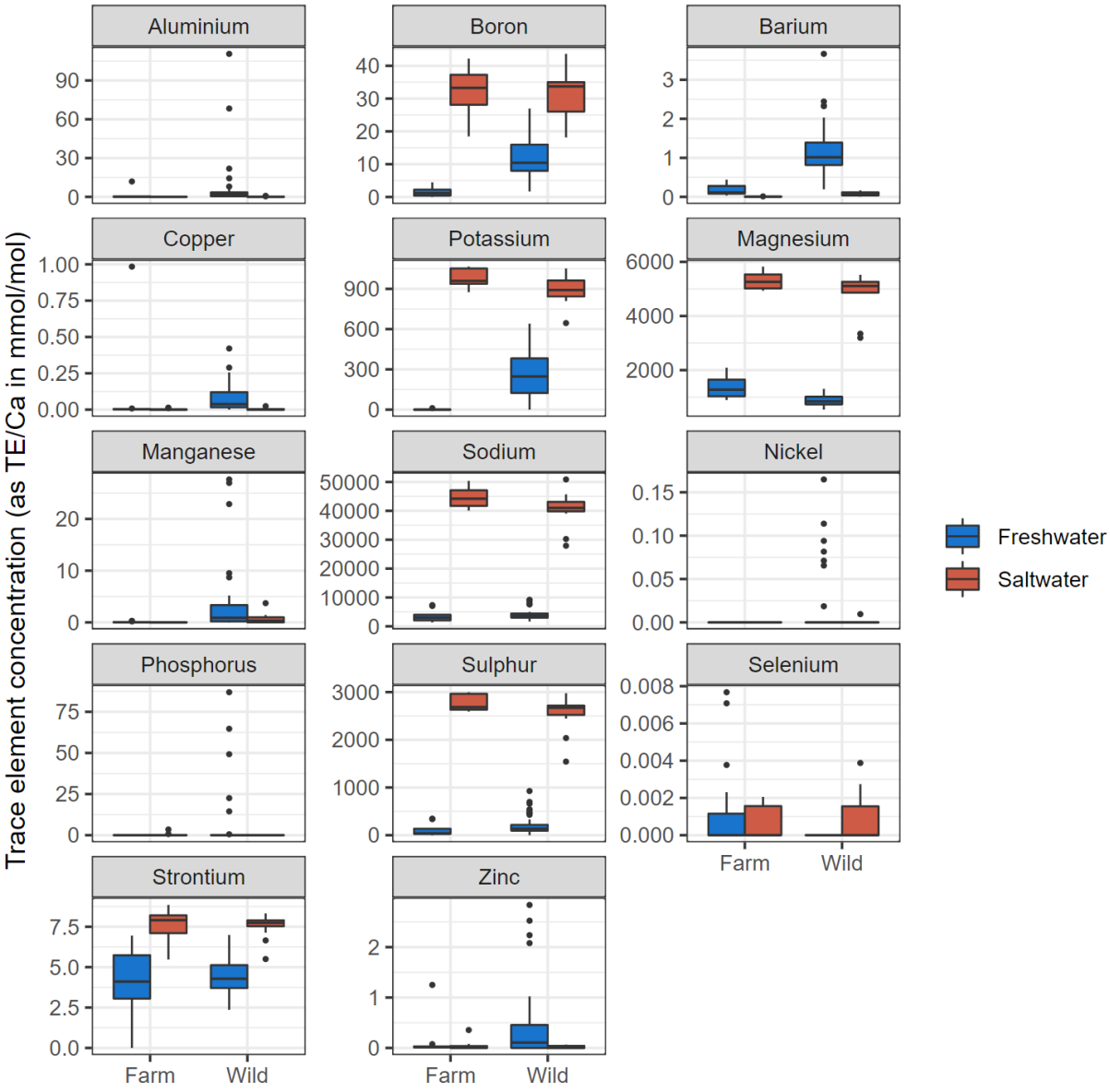

Figure 8. *Concentration of each trace element in water samples from freshwater and marine Barramundi farms and freshwater and marine wild Barramundi habitats, across all sampling years and seasons, expressed as a ratio to calcium. Significant effects of salinity and origin are presented in Table 6.*

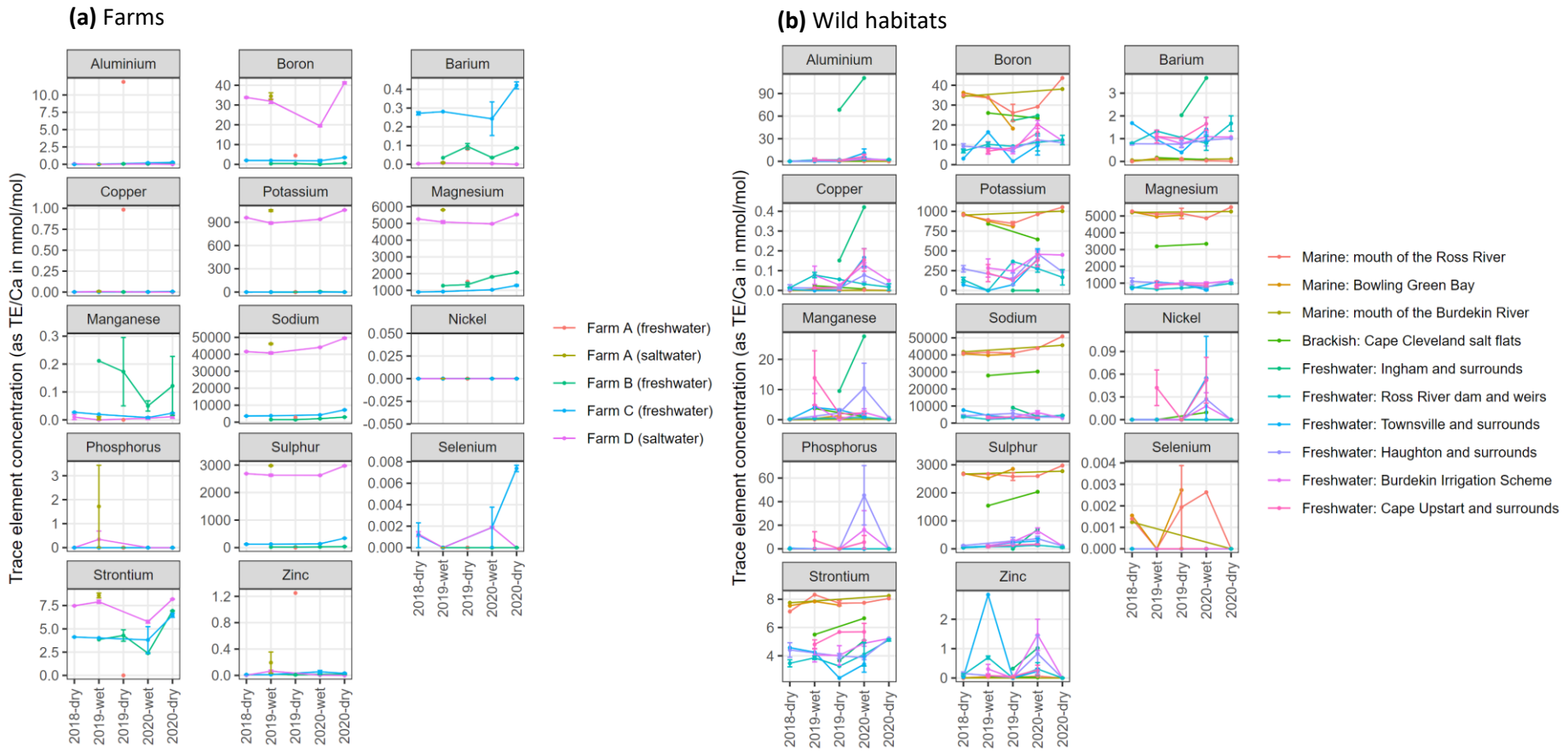

Figure 9. *Concentration of each trace element in water samples from (a) contributing Barramundi farms and (b) wild juvenile Barramundi habitats, over five seasons (2 years) of water sampling. Trace element concentrations are expressed as a ratio to calcium.*

Otolith microchemistry

Relationship between water and otolith microchemistry

Only barium, potassium, and strontium demonstrated a significant relationship between ambient water microchemistry and otolith terminus microchemistry at matching sites and seasons once the confounding effect of salinity was addressed (Table 7, Figure 10). A range of other confounding influences on trace element otolith deposition rates were not addressed in this study, including water temperature, diet, and water oxygenation levels (Hüssy et al. 2020).

In addition, the water-versus-otolith correlational approach assumes that juvenile Barramundi collected in wild habitats were resident in the sampled water body for a long enough period to allow for uptake of local trace elements and deposition at the terminal edges of their otoliths, a process which takes 3 to 14 days (Milton and Chenery 2001). This may not always be the case in estuarine and marine environments, where water masses change diurnally with tidal water movement and juvenile fish may move between tidal creeks (Russell and Garrett 1988).

Table 7. *Results of analysis of variance identifying significant effects of trace element concentration in water, salinity, and their interaction on the concentration of each trace element at the otolith terminus of known-origin fish. Asterisks denote statistical significance: * for p < 0.05, ** for p < 0.01, *** for p < 0.001. Effects for which p > 0.08 are listed as "n.s." (non-significant).*

	Water TE	Salinity	Water TE * salinity interaction
Aluminium	n.s.	n.s.	n.s.
Boron	0.07	n.s.	0.08
Barium	< 0.001 ***	n.s.	n.s.
Copper	n.s.	n.s.	0.02 *
Magnesium	n.s.	n.s.	n.s.
Manganese	n.s.	n.s.	n.s.
Phosphorus	n.s.	n.s.	n.s.
Potassium	0.020 *	n.s.	n.s.
Strontium	0.035 *	0.07	n.s.
Sulphur	n.s.	n.s.	n.s.
Zinc	n.s.	n.s.	n.s.
Overall	n.s.	n.s.	n.s.

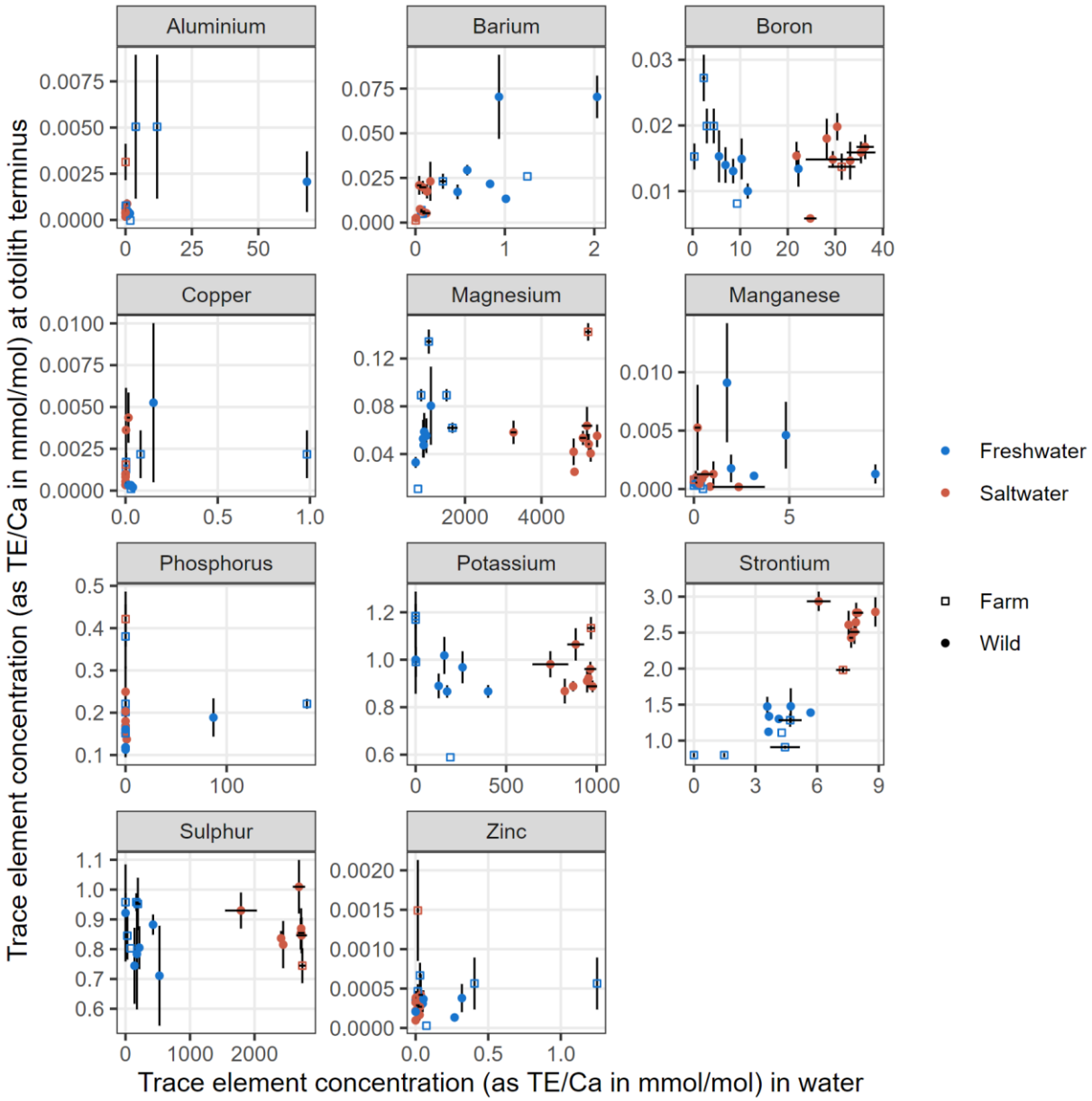

Figure 10. *Mean (±SE) concentration of trace elements (TE as a ratio to calcium (Ca)) in otolith terminus as a function of mean concentration of trace elements in spatially and temporally matched water samples. Otolith standard errors are calculated from the number of fish collected at a given site and time. Water standard errors are calculated from spatially similar (e.g. neighbouring watercourses) or temporally similar (e.g. multiple dry seasons) water samples.*

Classification of known-origin samples using sectioned otolith microchemistry

Mean otolith core microchemistry significantly differed between hatchery and wild-origin fish for all trace elements (Table 8, Figure 11). Microchemical differences between the otolith cores of hatchery- and wild-origin samples resulted in distinct and consistent separation of hatchery and wild-origin samples in multidimensional space (Figure 12). Wild-origin samples typically contained higher concentrations of barium, magnesium, zinc, and manganese, and lower concentrations of rubidium, than hatchery-origin fish. Strontium, sulphur, and yttrium appeared to be more sensitive to salinity than to provenance. Otolith core microchemistry "signatures" were consistent between cohorts for all sampling locations for which data was available from multiple cohorts (Figure 13). Generally, intra-

cohort variation was similar to or greater than inter-cohort variation in otolith microchemical composition.

Observed differences in the concentrations of trace elements between the otoliths of hatchery-origin and wild-origin samples have robust physical and physiological explanations. For example, the trace element manganese was almost completely absent from the otoliths of all hatchery-origin fish, across all farms and cohorts sampled (Figure 11, Figure 12, Figure 13). Manganese concentration was extremely variable in wild-origin fish, but was consistently orders of magnitude higher than in hatchery-origin fish. This is likely due to manganese being a sediment-bound element, which is therefore common in turbid waters such as freshwater lagoons and estuaries (Hüssy et al. 2020), and much rarer in clearwater systems, such as the filtered water in Barramundi hatcheries. In addition, manganese readily oxidises into bio-unavailable forms. This means that manganese is readily available and incorporated into Barramundi otoliths in oxygen-poor environments, but is of limited availability and incorporated at very low levels into otoliths in oxygen-rich environments (Limburg et al. 2015) such as well-aerated water typical of aquaculture facilities. Manganese concentration in Barramundi otolith cores should therefore be considered as a consistent and reliable indicator of provenance.

Table 8. *Results of analysis of variance identifying significant effects of origin (hatchery or wild) on trace element concentration in otolith cores of juvenile, known-origin Barramundi. Asterisks denote statistical significance: * for p < 0.05, ** for p < 0.01, *** for p < 0.001. Effects for which p > 0.10 are listed as "n.s." (non-significant).*

	Origin effect
Aluminium	< 0.05 *
Boron	< 0.001 ***
Barium	< 0.001 ***
Copper	< 0.001 ***
Magnesium	< 0.001 ***
Manganese	< 0.001 ***
Nickel	< 0.001***
Phosphorus	< 0.001 ***
Potassium	< 0.001 ***
Rubidium	< 0.001 ***
Strontium	< 0.001 ***
Sulphur	< 0.001 ***
Yttrium	< 0.001 ***
Zinc	< 0.001 ***
Overall	< 0.001***

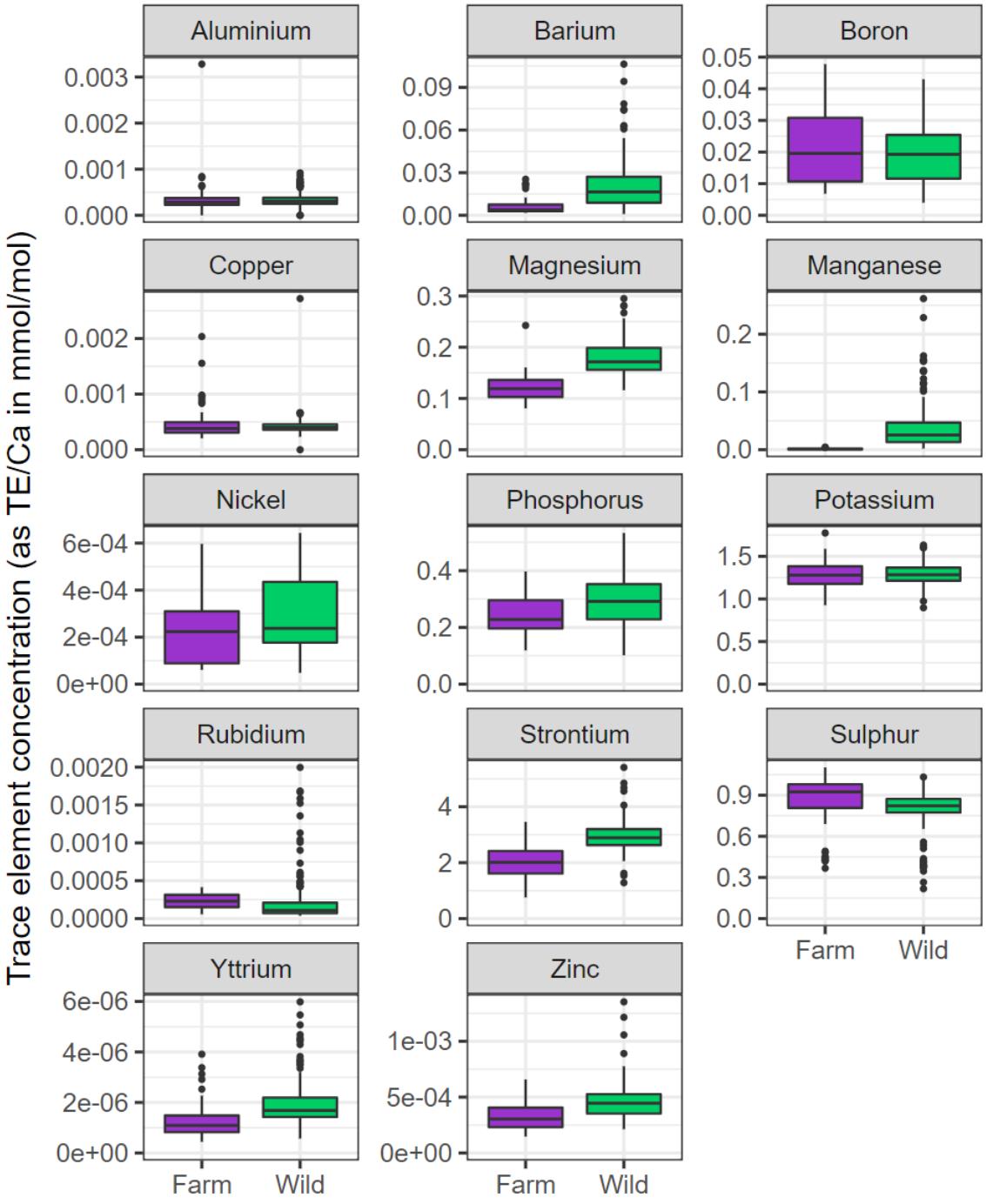

Figure 11. *Concentration of each trace element in the otolith cores of known-origin Barramundi, expressed as a ratio to Calcium.*

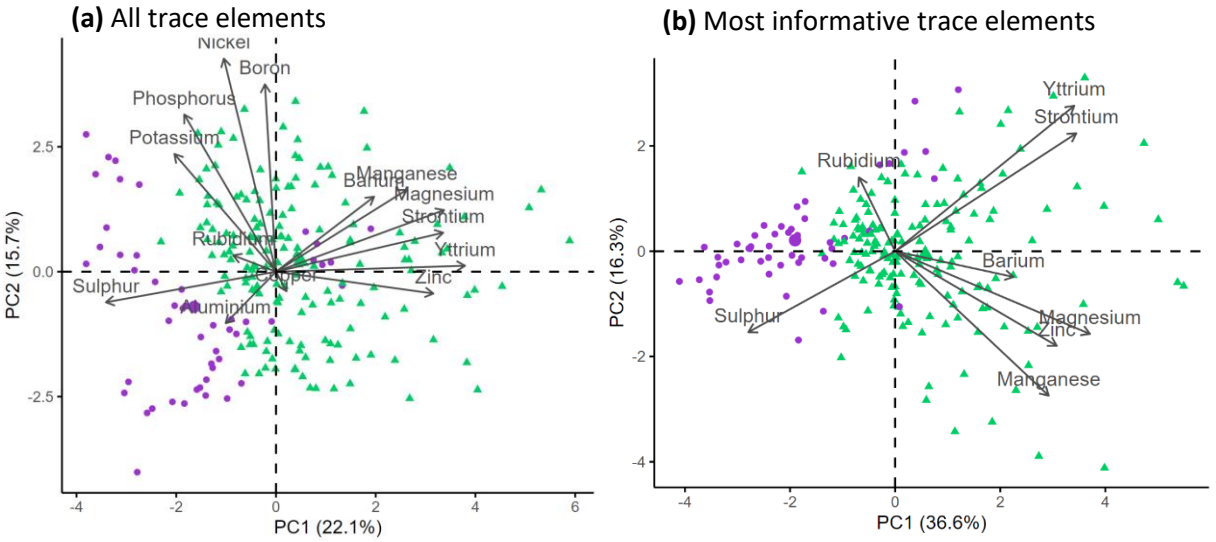

Figure 12. *First two principal components illustrating separation of hatchery-origin (purple) from wild-origin (green) samples using otolith core microchemistry, (a) using all available trace elements, (b) using the eight most informative trace elements identified by the random forest classification method.*

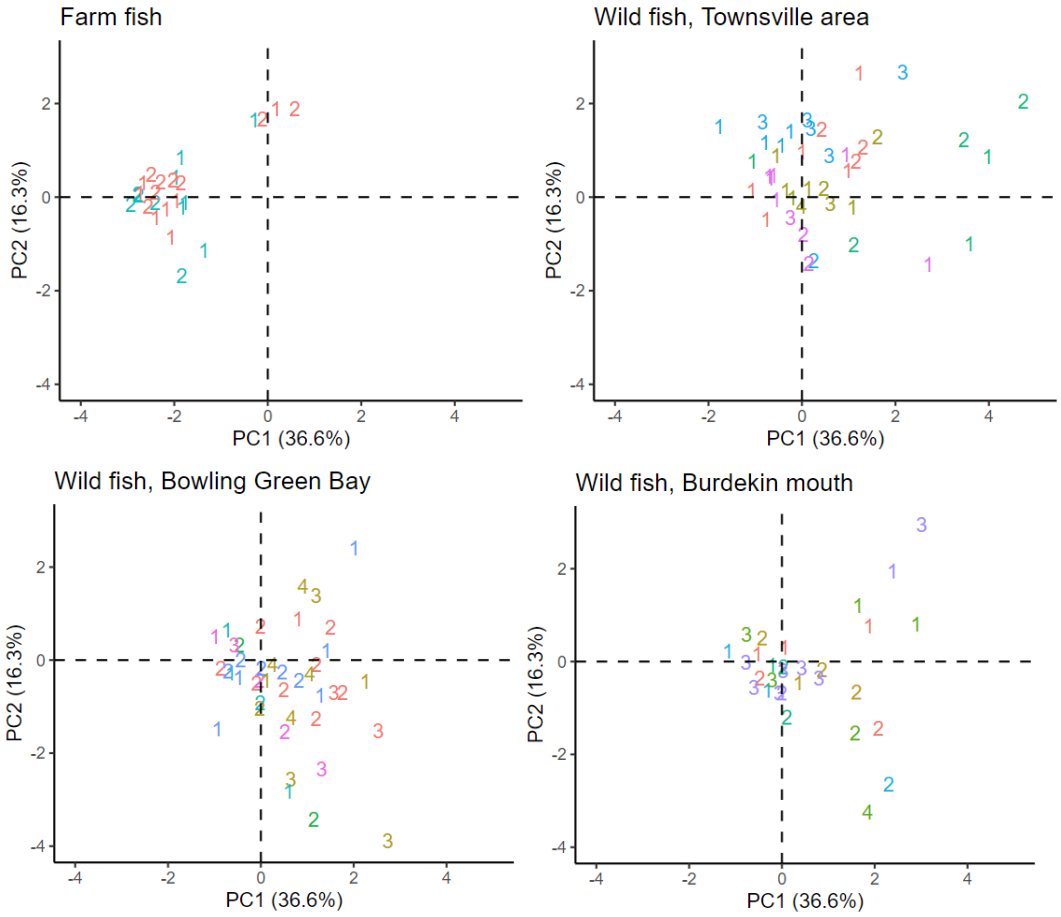

Figure 13. *PCA illustrating intra-cohort variability and inter-cohort consistency among known-origin fish sampled in the study. Colours indicate unique locations, numbers indicate individuals from sequential collection events at that location. Axes correspond to the PCA illustrated in Figure 12b.*

Assessment of the cross-validated predicted performance of the random forest model indicated that the model's error rate would be unchanged if the number of predictor variables was reduced from 14 to eight. The random forest model was therefore reapplied using only the eight most informative predictor variables (as identified by both the "mean decrease in accuracy" score and "mean decrease in the Gini impurity index"): manganese, magnesium, strontium, barium, sulphur, zinc, yttrium, rubidium (Figure 12). The random forest classification model based on these eight trace elements displayed an overall accuracy rate of 98% using the full calibration dataset (Table 9). Overall accuracy declined to 93% using an 80/20 calibration/validation subset, and improved to 99% using a 60/40 calibration/validation subset. Model performance was similar on the known-origin and wild-origin classes.

Table 9. *Predicted fish origin compared to known fish origin for microchemistry of sectioned otoliths.*

	Predicted hatchery origin	Predicted wild origin	Percent correct
Full calibration, no validation set			
Overall accuracy			98%
Known hatchery origin	47	2	96%
Known wild origin	2	161	99%
80/20 calibration/validation set			
Overall accuracy			93%
Known hatchery origin	9	1	90%
Known wild origin	1	32	97%
60/40 calibration/validation set			
Overall accuracy			99%
Known hatchery origin	20	0	100%
Known wild origin	1	64	98%

Classification of unknown-origin samples using sectioned otolith microchemistry

Application of the random forest algorithm, calibrated using the full dataset of known-origin samples (N = 212), to the collection of 606 unknown-origin sectioned otoliths assigned 26 samples as hatchery-origin and 580 samples as wild-origin. Relative assignment accuracy of these detections is addressed in the section on 'Inter-method agreement'.

The microchemical provenance model classified all 14 recreationally-caught unknown-origin samples provided from the Haughton River estuary in 2019 and 2020 as wild-origin with a high degree of confidence (84-100%), despite extensive stocking of the Haughton River weirs. Juvenile residency patterns identified using otolith microchemical profiles indicated that 43% of the legal-size Barramundi provided from the Haughton River estuary had resided in freshwater for at least one year as juveniles. Another 43% had not accessed freshwater, and exclusively inhabited marine water; the remaining 14% showed some use of freshwater early in life, but less than one full year.

The microchemical provenance model classified all three of the large (>1 m) Barramundi sampled from the Lilliesmere Lagoon fish kill in February 2019 (Townsville Bulletin 2019) as wild-origin with a high degree of confidence (78-90%). Lilliesmere Lagoon and adjoining freshwater bodies have been part of regular stocking activities on the Burdekin floodplain since 2000, and several large (>1 m) tagged stocked Barramundi were reported at the same fish kill event. All three individuals for which microchemical profiles are available were born in 2012, moved upstream into freshwater within their first few months of life, and remained there until the fish kill in 2019.

Parentage Analysis

Establishment of parentage analysis thresholds and parentage results

Analyses using maximum LOD scores of the progeny coming from each known hatchery (N = 60 in total) indicated that based on the genotypes in the broodstock database, the most likely parents for that hatchery returned a LOD score ≥10 (Figure 14). This LOD threshold of ≥10 was distinct for progeny from Hatcheries 2 and 3, but more variation in the maximum assigned LOD was observed in progeny from Hatchery 1. This is likely a result of genetic relatedness of broodstock among the different hatcheries, due to either historical sharing of broodstock, or historical purchasing of fingerlings and retaining them as breeding animals. As an independent assessment of the LOD score that represents a known parent-progeny trio, the LOD scores of progeny genotyped and assigned to parents based on a small sub-set of known hatchery broodstock supported an LOD score of ≥10 as a confident assignment (Figure 14, reproduced from Domingos et al. 2021). In Domingos et al. (2021), confident assignments of Barramundi progeny to a set of parents had LOD between 9 and 18 (Figure 14). Accordingly, a threshold value of LOD ≥10 was used to classify fish sampled from the wild to be either likely hatchery-origin or wild-origin.

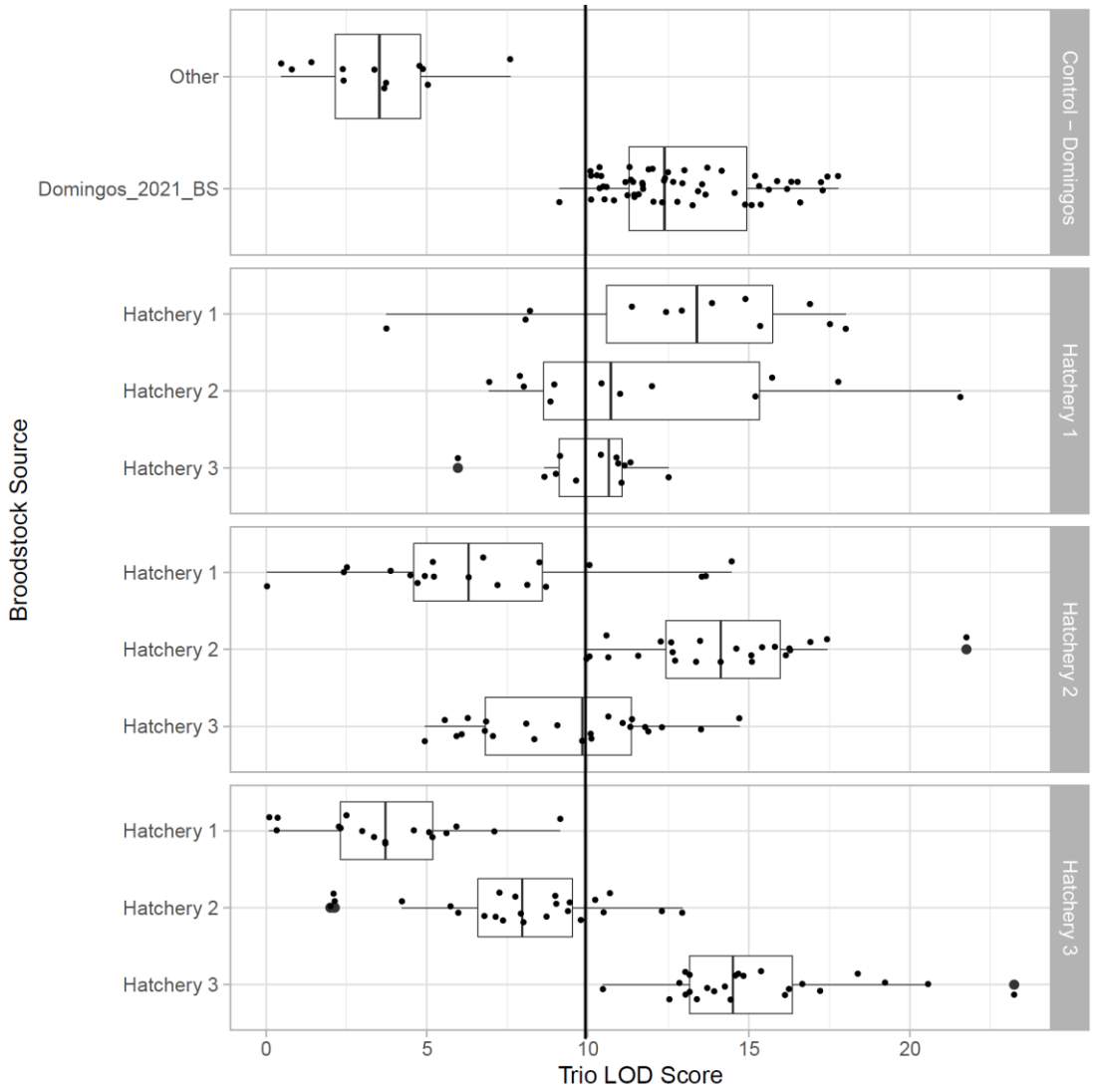

Figure 14. *A comparison of the LOD scores returned for the most likely trio assignments for farmed individuals known to have originated from certain broodstock sources. Individuals from Domingos et al. (2021) are listed under Control – Domingos.*

Classification of known-origin samples using parentage analysis

Applying the trio LOD threshold ≥10 for parentage assignment cut-offs, a total of 40 out of 237 known-wild juvenile fish (17%) were incorrectly classified as being hatchery-origin (Table 10). All known wild-origin samples were collected from locations, at sizes, and at times that provided extremely high confidence that these were wild-origin individuals. While some rare exceptions are possible, we consider it highly unlikely that this error occurred in 17% of the dataset. Instead, we consider this evidence that the microsatellite parentage analysis, using a "parent-progeny trio" approach and a membership threshold LOD score of 10, is over-detecting hatchery provenance in these samples.

Table 10. *Predicted fish origin compared to known fish origin for microsatellite parentage analysis. Note that known hatchery-origin samples were used to define provenance classification thresholds for all other samples, and therefore assessment of their own classification accuracy is not applicable.*

	Predicted hatchery origin	Predicted wild origin	Percent correct
Full calibration, no validation set			
Overall accuracy			N/a
Known hatchery origin	60	0	N/a
Known wild origin	40	197	83%

Classification of unknown-origin samples using parentage analysis

Applying the same trio LOD threshold ≥10 for parentage assignment cut-offs, a total of 152 of 746 unknown-origin fish (20%) were identified as being hatchery-origin.

Population diversity and genetic differentiation

All populations (both wild- and hatchery-origin) displayed moderate amounts of genetic diversity (Table 11) and were similar to those reported in wild Australian Barramundi populations (Loughnan et al. 2019). Population genetic metrics were comparable across populations, except in Hatchery 1 where a slight decrease in allelic richness (Ar) (from 3.77 to 2.66) and expected heterozygosity (He) (from 0.54 to 0.43) was observed. This likely reflects the smaller number of broodstock used in this hatchery compared to the other hatcheries involved in the study. As is typical of hatchery-produced Barramundi (Loughnan et al. 2013, Domingos et al. 2014), the known-hatchery progeny populations exhibited lower estimates of FIS (inbreeding coefficient (F) of an individual (I) relative to the subpopulation (S). FIS of the hatchery-born fish ranged from -0.252 to -0.119, indicating slight presence of inbreeding (or increased homozygosity) in the progeny cohorts, while FIS in the wild populations was close to zero (Table 11).

An AMOVA revealed that genetic variance was mostly partitioned within populations (97.75%; $p < 0.0001$); however; there was still significant differentiation among populations (2.25%; $p < 0.0001$). Pairwise FST (inbreeding coefficient (F) of subpopulations (S) relative to the total population (T)) comparisons between all population samples indicates that the Hatchery 1 progeny (known hatchery-origin) samples were significantly differentiated from other populations sampled (FST values from 0.059 to 0.086; Table 12).

Table 11. *Summary population genetic diversity statistics of the sampled populations (mean ± SE). The number of individuals genotyped (N), alleles (Na), and proportion of private alleles (Pa) are presented along with observed (Ho) and expected (He) heterozygosity, allelic richness (Ar) and inbreeding coefficient (FIS) with 95% confidence intervals.*

Source	Population	N	Na	Pa	Ho	He	Ar	FIS
Broodstock	Hatchery 1	63.88 (± 0.5)	5.31 (± 2.89)	0.13 (± 0.09)	0.54 (± 0.22)	0.54 (± 0.2)	3.77 (± 1.73)	0.023 (-0.043 to 0.07)
Broodstock	Hatchery 2	558.19 (± 11.18)	6.44 (± 3.16)	0.94 (± 0.19)	0.52 (± 0.22)	0.53 (± 0.22)	3.63 (± 1.71)	0.013 (-0.008 to 0.032)
Broodstock	Hatchery 3	893.25 (± 19.11)	5.38 (± 2.7)	0.06 (± 0.06)	0.52 (± 0.19)	0.51 (± 0.18)	3.49 (± 1.51)	-0.016 (-0.029 to -0.004)
Known hatchery-origin	Hatchery 1	11.88 (± 0.34)	2.69 (± 1.01)	0 (± 0)	0.55 (± 0.28)	0.43 (± 0.22)	2.66 (± 1)	-0.252 (-0.387 to -0.229)
Known hatchery-origin	Hatchery 2	22.5 (± 1.26)	3.5 (± 1.71)	0 (± 0)	0.58 (± 0.25)	0.51 (± 0.21)	3.19 (± 1.42)	-0.119 (-0.217 to -0.074)
Known hatchery-origin	Hatchery 3	23.38 (± 1.54)	3.5 (± 1.59)	0 (± 0)	0.56 (± 0.24)	0.49 (± 0.19)	3.22 (± 1.37)	-0.139 (-0.29 to -0.052)
Known wild-origin	Ayr	40.94 (± 2.02)	4.88 (± 3.14)	0 (± 0)	0.53 (± 0.2)	0.53 (± 0.2)	3.7 (± 1.96)	0.013 (-0.071 to 0.072)
Known wild-origin	Cleveland	53.13 (± 1.5)	4.81 (± 2.88)	0 (± 0)	0.55 (± 0.22)	0.55 (± 0.21)	3.68 (± 1.83)	0.003 (-0.07 to 0.051)
Known wild-origin	Giru	84.19 (± 2.66)	4.94 (± 2.38)	0 (± 0)	0.55 (± 0.2)	0.54 (± 0.19)	3.63 (± 1.56)	-0.015 (-0.066 to 0.026)
Known wild-origin	Ingham	24.38 (± 1.15)	4.38 (± 2.75)	0 (± 0)	0.59 (± 0.25)	0.53 (± 0.19)	3.61 (± 1.96)	-0.1 (-0.201 to -0.045)
Known wild-origin	Townsville	23.44 (± 1.15)	4 (± 2.13)	0 (± 0)	0.5 (± 0.25)	0.49 (± 0.21)	3.41 (± 1.58)	0.002 (-0.118 to 0.08)
Known wild-origin	Upstart	21.56 (± 1.5)	4.19 (± 2.17)	0 (± 0)	0.53 (± 0.19)	0.52 (± 0.19)	3.61 (± 1.67)	0.013 (-0.073 to 0.057)
Unknown-origin	Wild-capture fishery	733.94 (± 22.18)	5.94 (± 3.36)	0 (± 0)	0.54 (± 0.21)	0.54 (± 0.21)	3.66 (± 1.79)	0.007 (-0.01 to 0.022)

Table 12. *Pairwise FST values between the broodstock, known hatchery-origin juveniles, known wild-origin juveniles, and unknown-origin samples. Significant pairwise comparisons based on a false discovery rate (FDR) alpha value of 0.009 are indicated with an *.*

		Broodstock			Known hatchery-origin			Known wild-origin						Unknown-origin
		Hatchery 1	Hatchery 2	Hatchery 3	Hatchery 1	Hatchery 2	Hatchery 3	Ayr	Cleveland	Giru	Ingham	Townsville	Upstart	Wild-capture fishery
Broodstock	**Hatchery 1**	0												
Broodstock	**Hatchery 2**	0.012 *	0											
Broodstock	**Hatchery 3**	0.021 *	0.025 *	0										
Known hatchery-origin	**Hatchery 1**	0.046 *	0.065 *	0.052 *	0									
Known hatchery-origin	**Hatchery 2**	0.001	0.013	0.035 *	0.064 *	0								
Known hatchery-origin	**Hatchery 3**	0.024 *	0.035 *	0.002	0.062 *	0.044 *	0							
Known wild-origin	**Ayr**	0.019 *	0.028 *	0.027 *	0.072 *	0.024 *	0.021 *	0						
Known wild-origin	**Cleveland**	0.017 *	0.025 *	0.03 *	0.072 *	0.022 *	0.028 *	-0.003	0					
Known wild-origin	**Giru**	0.021 *	0.026 *	0.025 *	0.078 *	0.03 *	0.021 *	-0.002	0.002	0				
Known wild-origin	**Ingham**	0.013	0.017 *	0.022 *	0.081 *	0.025 *	0.028 *	0.003	0.001	-0.001	0			
Known wild-origin	**Townsville**	0.012	0.019	0.024 *	0.086 *	0.022	0.026 *	-0.001	0.006	-0.001	0.003	0		
Known wild-origin	**Upstart**	0.020	0.032 *	0.025 *	0.059 *	0.027	0.014	-0.002	0.008	-0.001	0.011	0.003	0	
Unknown-origin	**Wild-capture fishery**	0.017 *	0.025 *	0.024 *	0.067 *	0.024 *	0.023 *	-0.001	0.000	0.001	-0.001	0.004	0.004	0

Inter-method agreement

Four method comparison

Inter-method agreement using all overlapping samples from the four provenance-determination methods (NIRS of sectioned otoliths, NIRS of whole otoliths, otolith microchemistry, microsatellite parentage genetics, Table 13) was generally high for classification of known-origin samples (Figure 15a-d). However, inter-method agreement was poor when each method was applied to the unknown-origin samples. In particular, NIRS of whole otoliths did not detect any hatchery-origin samples, despite strong collection evidence that at least one sample (SFLc1466) had likely been stocked. (Figure 15e, Table 14).

Whole otolith NIRS demonstrated strong performance on known-origin samples without validation samples, deteriorating performance on known-origin samples with validation samples (Table 5), and poor performance at extrapolating to unknown-origin samples (Figure 15e). This suggests that the whole otolith NIRS classification algorithm may be detecting and extrapolating otolith spectral patterns unrelated to provenance. In particular, the algorithm may be relying on spectral differences relating to otolith thickness, which is directly related to fish size and age, rather than provenance. In this instance, the total lengths of known-hatchery and known-wild origin samples used to build the provenance-classification model were imbalanced, with hatchery-origin fish generally between 120 and 170 mm, and wild-origin fish generally between 270 and 350 mm (Figure 3). As a result, a classification algorithm that correlates otolith size with provenance would incorrectly extrapolate that all otoliths from unknown-origin fish (typically >580 mm, the minimum legal size for Barramundi) are wild-origin with a high degree of confidence, as was observed here (Table 14). A more suitable known-origin calibration set for NIRS of whole otoliths would have fish of the same age/size in the two provenance classes. This would ensure any separation of the classes is not due to external factors such as age or size.

Furthermore, the existing known-origin calibration samples may not represent the full range of spectral variability observed in the unknown samples, which would result in reduced classification performance. Ideally, a robust calibration model should be built from reference samples that encompass all sources of variability that can be encountered in the unknown samples (e.g. seasonal, geographical, etc.). However, collecting such a comprehensive calibration dataset was not feasible for known wild-origin Barramundi in this study.

As a result of this poor performance, NIRS of whole otoliths was not pursued any further as a means of resolving Barramundi provenance.

Table 13. *Number of samples available for each provenance-determination method, and for comparison of all four, and only three (excludes whole otolith NIRS) provenance-determination methods.*

	Sectioned otolith NIRS	Whole otolith NIRS	Otolith microchemistry	Genetics parentage analysis	**Overlap for 4 method comparison**	**Overlap for 3 method comparison**
Known hatchery-origin	41	49	49	60	**39**	**39**
Known wild-origin	145	163	163	237	**145**	**145**
Unknown origin	606	19	606	746	**19**	**605**

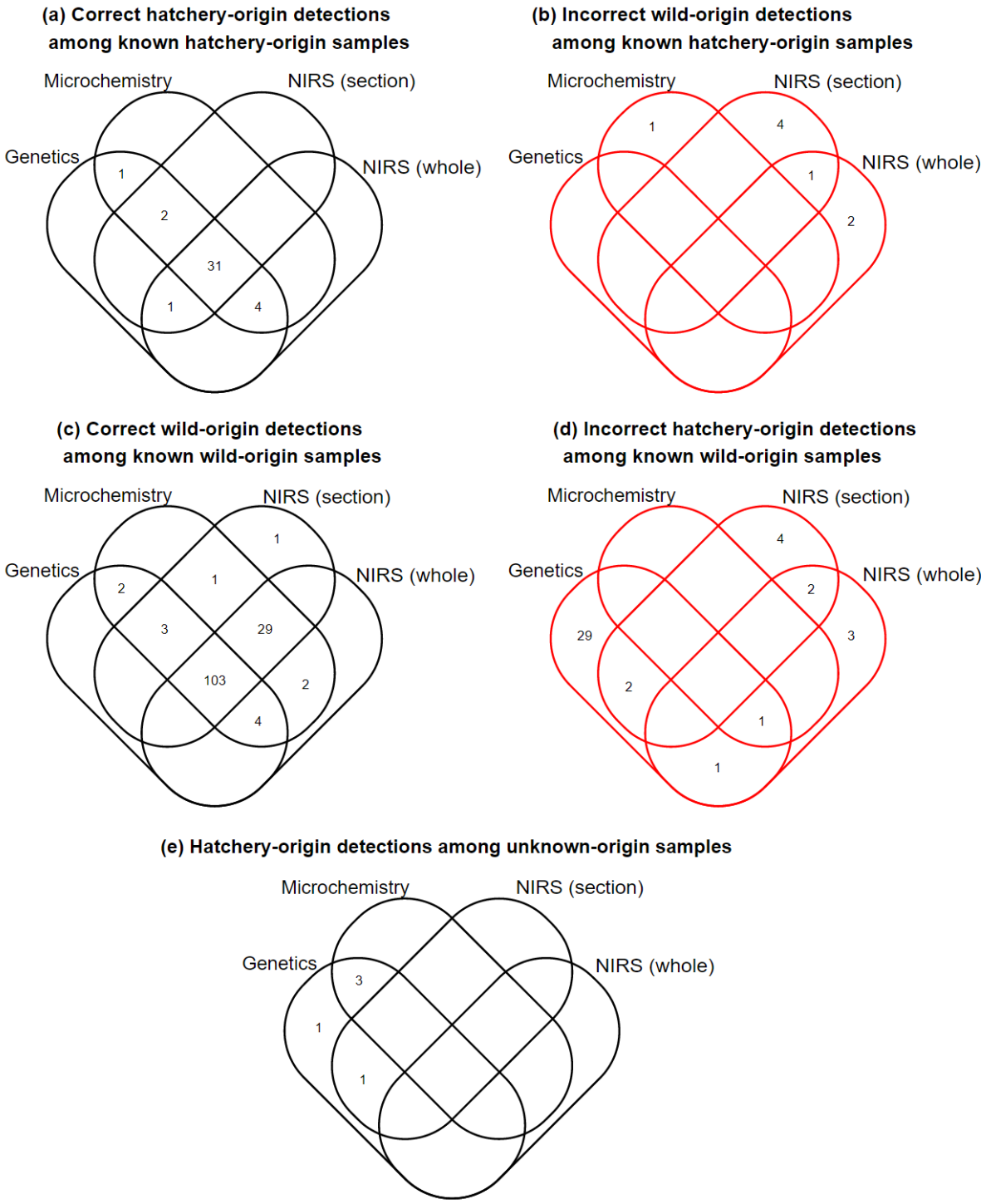

Figure 15. *Venn diagrams illustrating agreement between all four provenance-assignment methods (NIRS of sectioned otoliths, NIRS of whole otoliths, otolith microchemistry, genetic parentage analysis) regarding (a) correct assignment of known hatchery-origin samples as hatchery-origin, (b) incorrect assignment of known hatchery-origin samples as wild-origin, (c) correct assignment of known wild-origin samples as wild-origin, (d) incorrect assignment of known wild-origin samples as hatchery-origin, (e) assignment of unknown-origin samples as hatchery-origin. Total number of samples as per Table 13. Assignment probabilities for each of the hatchery-origin detections in (e) are detailed in Table 14.*

Table 14. *Inter-method comparison of provenance determination on all 19 unknown-origins samples assessed by all four provenance determination methods. Individuals identified as hatchery-origin by at least one provenance determination method are indicated in red. Fish total length is provided in mm. LOD scores ≥ 10 were initially classified as "probable hatchery-origin" but were subsequently revised to "probable hatchery ancestry".*

			NIRS of sectioned otoliths		NIRS of whole otoliths		Microchemistry		Genetics	
Fish ID	**Actual origin**	**Total length**	**Assignment**	**Probability of hatchery-origin**	**Assignment**	**Probability of hatchery-origin**	**Assignment**	**Probability of hatchery-origin**	**Assignment**	**LOD score**
SFLc1451	Unknown	314	Wild	0.000	Wild	0.000	Wild	0.004	Wild	5.89
SFLc1453	Unknown	303	Hatchery	0.518	Wild	0.003	Wild	0.008	Hatchery	12.25
SFLc1454	Unknown	673	Wild	0.012	Wild	0.000	Wild	0.082	Wild	9.21
SFLc1455	Unknown	715	Wild	0.000	Wild	0.000	Wild	0.093	Wild	6.33
SFLc1456	Unknown	703	Wild	0.000	Wild	0.000	Wild	0.272	Wild	8.90
SFLc1457	Unknown	573	Wild	0.000	Wild	0.000	Wild	0.115	Wild	5.31
SFLc1458	Unknown	642	Wild	0.000	Wild	0.000	Wild	0.035	Wild	6.64
SFLc1459	Unknown	783	Wild	0.001	Wild	0.000	Wild	0.073	Wild	3.35
SFLc1460	Unknown	704	Wild	0.000	Wild	0.000	Wild	0.119	Wild	0.00
SFLc1461	Unknown	705	Wild	0.047	Wild	0.000	Wild	0.201	Wild	7.39
SFLc1462	Unknown	645	Wild	0.000	Wild	0.000	Wild	0.122	Wild	2.83
SFLc1463	Unknown	585	Wild	0.004	Wild	0.000	Wild	0.000	Hatchery	12.14
SFLc1464	Unknown	730	Wild	0.010	Wild	0.000	Wild	0.026	Wild	6.79
SFLc1465	Unknown	644	Wild	0.000	Wild	0.000	Wild	0.009	Wild	5.69
SFLc1466	Probable Hatchery	731	Wild	0.000	Wild	0.000	Hatchery	0.968	Hatchery	22.55
SFLc1471	Unknown	269	Wild	0.021	Wild	0.000	Hatchery	0.807	Hatchery	13.82
SFLc1472	Unknown	316	Wild	0.062	Wild	0.000	Hatchery	0.958	Hatchery	11.05
SFLc2001	Unknown	1050	Wild	0.000	Wild	0.000	Wild	0.120	Wild	0.00
SFLc2116	Unknown	642	Wild	0.000	Wild	0.009	Wild	0.019	Wild	2.85

Three method comparison

Inter-method agreement using all overlapping samples from the three provenance-determination methods (NIRS of sectioned otoliths, otolith microchemistry, microsatellite parentage genetics, Table 13) was generally high for classification of known-origin samples (Figure 16a-d). However, the genetic method was particularly prone to incorrectly classifying known wild-origin samples as being hatchery-origin (33/145, ~23%, Figure 16d).

Inter-method agreement was poor when applied to the unknown-origin samples. Just one sample was classified as hatchery-origin by all three methods (Figure 16e). The majority of hatchery-origin assignments were identified by the genetic method only, and without corroboration by the otolith-based methods (102/140, ~73%, Figure 16e). NIRS of sectioned otoliths classified very few samples as hatchery-origin (13/605, Figure 16e), and did not recognise the probable hatchery origin sample (SFLc1466), instead estimating a 0% probability of that individual originating from a hatchery (Table 14). The cause of this error is unlikely to be related to fish size or otolith thickness, as the otolith sectioning process results in a similar thickness otolith section (300 µm) regardless of otolith size. Hatchery detections by the sectioned otolith NIRS method only agreed with the genetic method in 3/13 instances (~23%), and only agreed with the microchemical method in 1/13 instances (~8%) (Figure 16e).

The high levels of disagreement between NIRS and the other methods may be due to the NIRS method detecting otolith variability resulting from macrochemical differences (e.g. proportion of calcium carbonate), rather than microchemical differences (e.g. concentration of manganese). The differences in trace element concentration between the otolith cores of hatchery-origin and wild-origin individuals measured using otolith microchemistry were extremely small (e.g. 0.05 mmol Mn / mol Ca, Figure 11), and therefore may not produce detectable spectral differences using the current NIRS instrument configuration. In addition, several of the major provenance-related otolith trace elements are predominantly deposited as a substitute for calcium (magnesium, strontium, barium) (Campana 1999), and are therefore likely to form similar covalent bonds to what calcium would have, potentially resulting in similar or even identical NIR spectral signatures for otoliths containing a wide range of concentrations of those trace elements. In the absence of a larger collection of known-origin samples with which to attempt further calibration of the NIRS-based model, Barramundi provenance assignments using NIRS of sectioned otoliths are not considered reliable at this time.

The otolith microchemistry approach identified 26 out of 605 samples as being of hatchery-origin, and was the only otolith-based method that successfully identified individual SFLc1466 as hatchery-origin with a high degree of confidence (97%, Table 14). Most microchemical assignments to hatchery-origin were supported by parentage analysis (18/26, ~69%, Figure 16e).

The microsatellite parentage analysis detected a high number of hatchery-origin samples among the unknown-origin fish (122/605, ~20%, Figure 16e). Over-detection of hatchery-provenance using microsatellite parentage analysis of the known-wild samples (33/145, ~23%, Figure 16d) suggests that over-detection of hatchery provenance may be occurring with the unknown-origin samples as well.

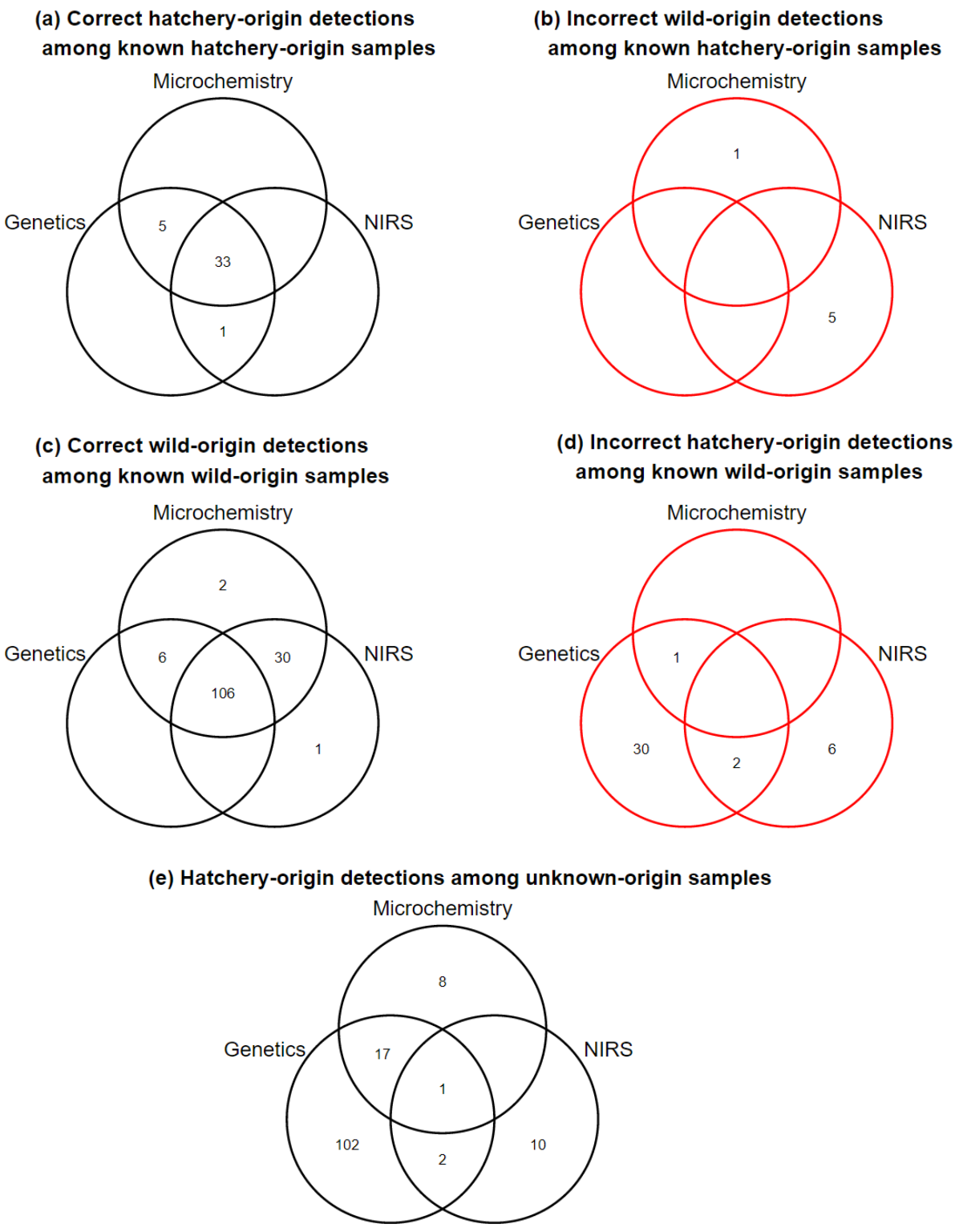

Figure 16. *Venn diagrams illustrating agreement between three provenance-assignment methods (NIRS of sectioned otoliths, otolith microchemistry, genetic parentage analysis) regarding (a) correct assignment of known hatchery-origin samples as hatchery-origin, (b) incorrect assignment of known hatchery-origin samples as wild-origin, (c) correct assignment of known wild-origin samples as wild-origin, (d) incorrect assignment of known wild-origin samples as hatchery-origin, (e) assignment of unknown-origin samples as hatchery-origin. Total number of samples as per Table 13.*

Extrapolation of findings to the 2019 Dry Tropics commercial catch

Contribution of stocked fish to the 2019 Dry Tropics fishery using otolith microchemistry

Extrapolation of the otolith microchemical provenance work to the 2019 Dry Tropics catch estimated that 3.0% (by number) of Barramundi landed were confidently of hatchery-origin (4.1% by weight). A further 1.5% (by number) of Barramundi landed were of uncertain origin (1.4% by weight). Overall, 95.5% (by number) of Barramundi landed were confidently of wild-origin (94.5% by weight) (Figure 17a and b). Otolith core microchemistry only indicates provenance and makes no inferences about animal ancestry. As such, these figures represent the direct, numerical contribution of stocked Barramundi to the 2019 commercial catch in the Dry Tropics.

Hatchery-origin fish appear to be more strongly represented in the larger length classes, and therefore more strongly represented in terms of weight contribution to the fishery (Figure 17a and b). Large fluctuations in the contribution of different year classes to the 2019 fishery are primarily driven by wild-born fish, i.e. variability in wild recruitment (Figure 17c). Stocked fish made up a greater proportion of year-classes in years when climatic conditions were likely to be conducive to poor wild recruitment (i.e. little wet season rainfall, or only minor river flooding, e.g. 2013); stocked fish made up a lesser proportion of year-class abundance when climatic conditions were likely to be conducive to strong wild recruitment (e.g. 2011).

There were no apparent patterns in the contribution of stocked fish to commercial catches across the fishery season (Figure 18, east coast Barramundi season: 1 February to 31 October). Hatchery-born fish were captured from the start of the season in February right through until August. Uncertain provenance fish were captured throughout the year, right up until the end of the Barramundi season in October.

Hatchery-born fish were consistently encountered at larger length-at-ages than their wild-born counterparts (Figure 19), particularly in the youngest age classes (2 to 4 years old). The effect of provenance was statistically significant ($p < 0.001$). Note that both the largest fish captured in 2019 (1200 mm) and the oldest fish captured that year (19 years old) were both wild-born.

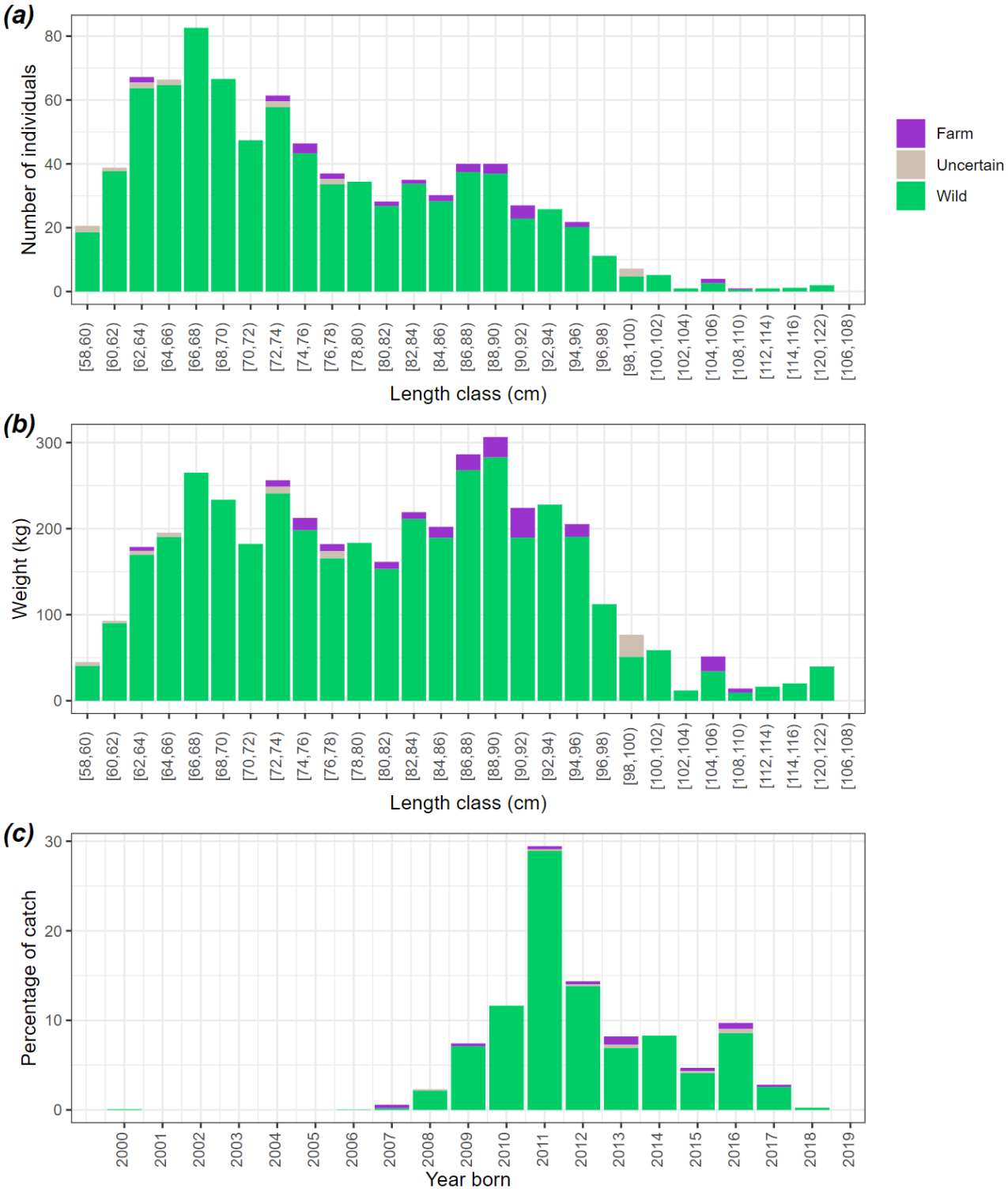

Figure 17. *Contribution of stocked fish to the 2019 Dry Tropics fishery (a) length-frequency, (b) weight, and (c) year class frequency distribution (derived from age-frequency, and summing to 100%) using otolith core microchemistry.*

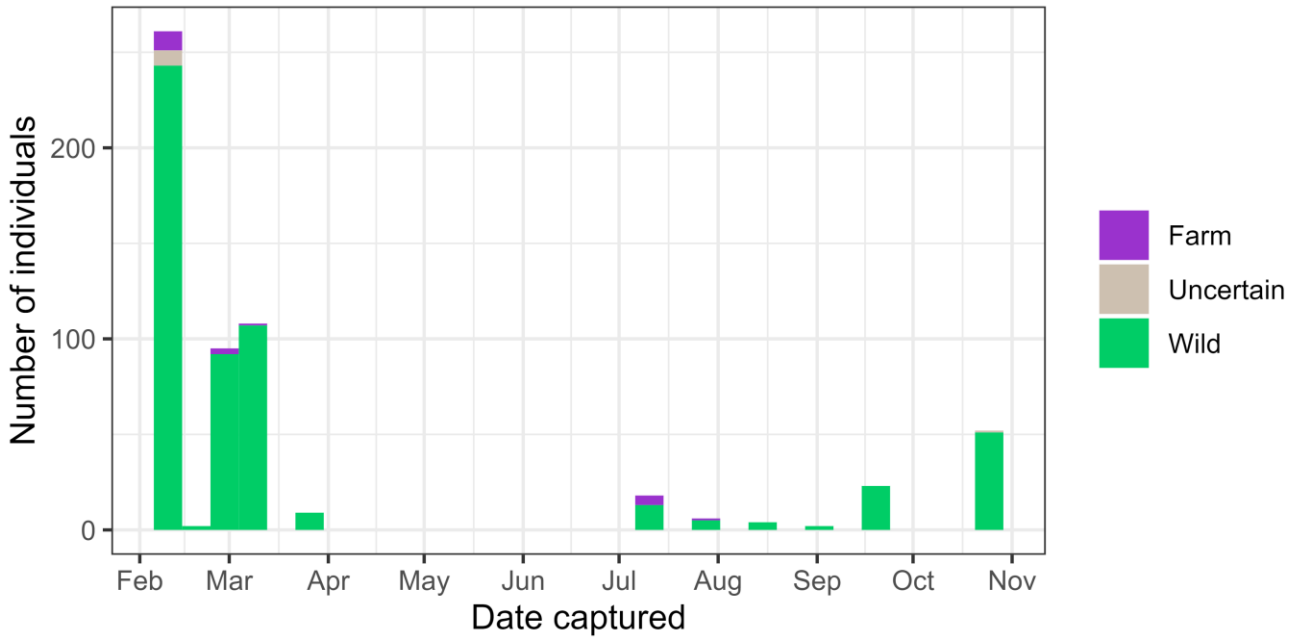

Figure 18. *Contribution of hatchery-born fish to the 580 otoliths from the commercial sector collected throughout the east coast Barramundi season (1 Feb to 31 Oct).*

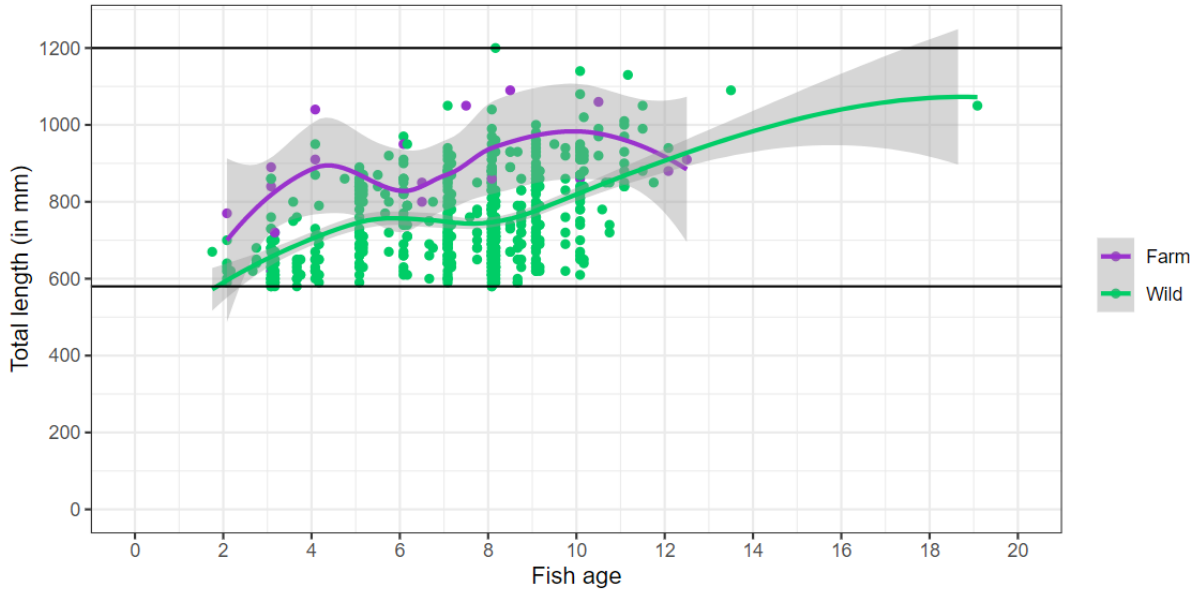

Figure 19. *Length-at-age for commercial sector samples for which both otolith microchemical provenance classification and age data were available (N = 580). For clarity, "uncertain" origin fish are excluded from the figure (N = 9). Trendlines are illustrative smoothers only and not formally fitted growth curves. Horizontal black lines indicate the minimum (580 mm) and maximum (1200 mm) legal size for Barramundi. Differences in length-at-age by provenance are statistically significant ($p < 0.001$).*

Contribution of stocked fish to the 2019 Dry Tropics fishery using parentage analysis

Over-detection of hatchery provenance using microsatellite parentage analysis may be occurring in approximately 17% of cases, likely as a result of introgression. As such, we consider positive hatchery assignments using microsatellite parentage analysis to be indicative of both direct, numerical contribution of stocked Barramundi to the fishery, and indirect, reproductive contribution of stocked Barramundi to the 2019 fishery in the Dry Tropics. For this reason, positive hatchery assignments using microsatellite parentage analysis are hereafter referred to as individuals with "hatchery ancestry".

Extrapolation of the microsatellite parentage analysis work to the 2019 Dry Tropics catch estimated that 20.7% (by number) of Barramundi landed had predominantly hatchery ancestry (22.2% by weight). The remaining 79.3% (by number) of Barramundi landed had predominantly wild ancestry (77.8% by weight). Hatchery-ancestry fish appear to be approximately equally represented across all length classes and year classes (Figure 20). Similarly, there were no temporal patterns in the contribution of hatchery-ancestry fish to the commercial catch throughout the year (Figure 21).

Individuals with hatchery ancestry were typically encountered at slightly larger length-at-ages than their wild-ancestry counterparts (Figure 22), across all age classes. The effect of ancestry was statistically significant ($p = 0.016$). Note that both the largest fish captured in 2019 (1200 mm) and the oldest fish captured that year (19 years old) had wild ancestry.

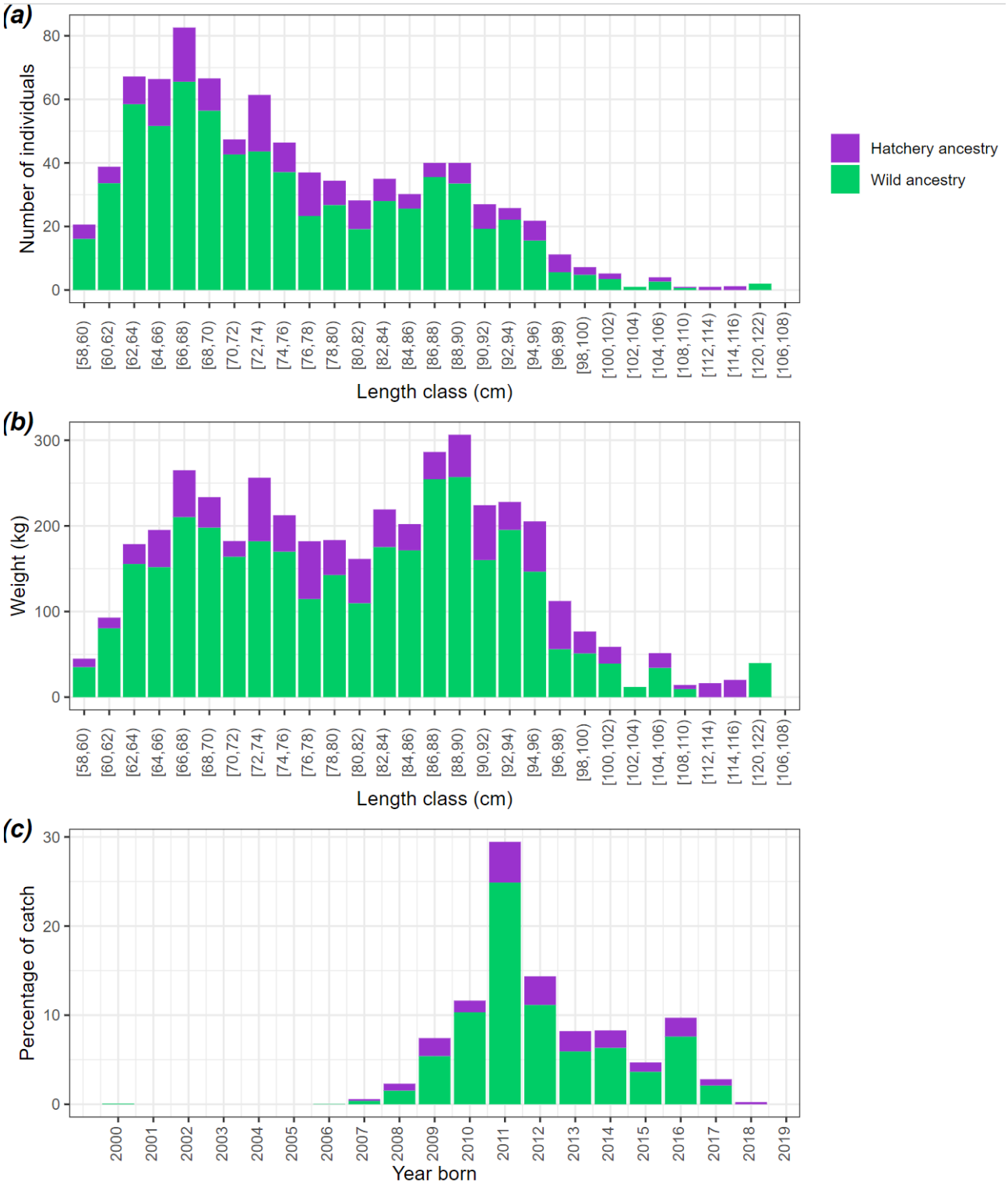

Figure 20. *Contribution of hatchery ancestry fish to the 2019 Dry Tropics fishery (a) length-frequency, (b) weight, and (c) year class frequency distribution (derived from age-frequency, and summing to 100%) using microsatellite parentage analysis.*

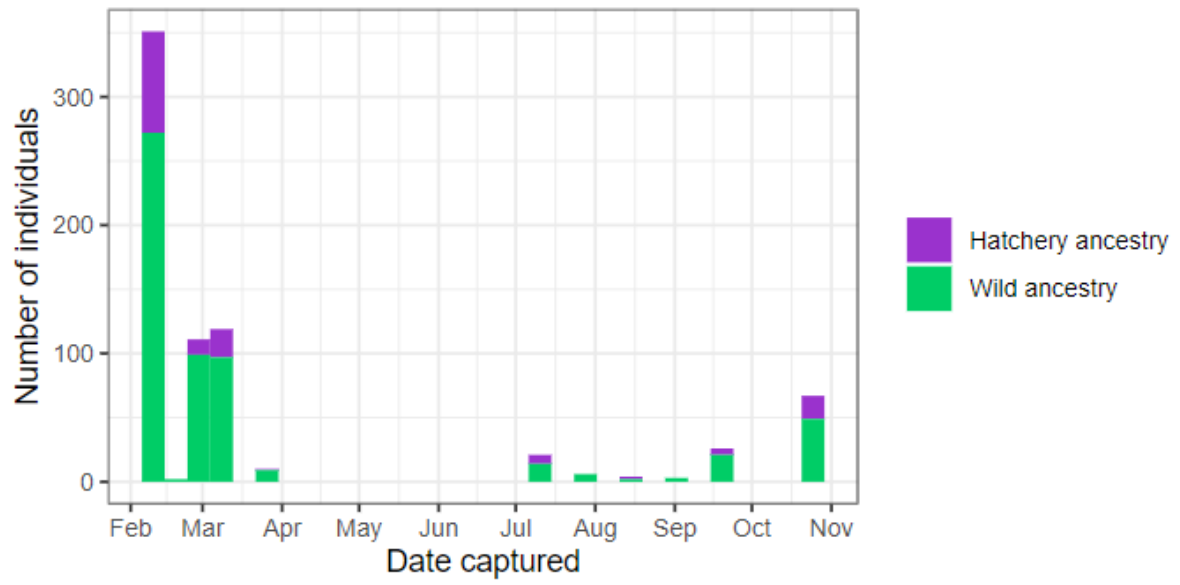

Figure 21. *Contribution of hatchery-ancestry fish to the 720 genetic samples from the commercial sector collected throughout the east coast Barramundi season (1 Feb to 31 Oct).*

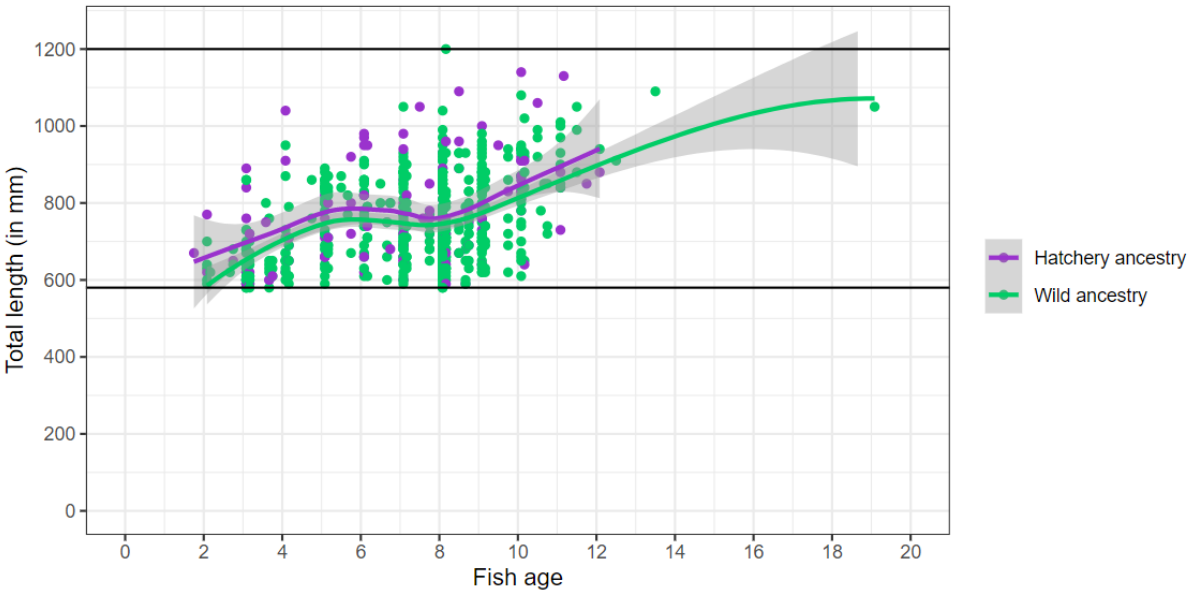

Figure 22. *Length-at-age for commercial sector samples for which both microsatellite parentage analysis provenance classification and age data were available (N = 596). Trendlines are illustrative smoothers only and not formally fitted growth curves. Horizontal black lines indicate the minimum (580 mm) and maximum (1200 mm) legal size for Barramundi. Differences in length-at-age by ancestry are statistically significant (p = 0.016).*

Contribution of juvenile freshwater residency to the 2019 Dry Tropics fishery

Extrapolation of the otolith microchemical juvenile habitat use work to the 2019 Dry Tropics catch estimated that 32.9% (by number) of Barramundi landed had spent at least one full year during their juvenile period (at least one pair of translucent and opaque otolith bands) in a freshwater nursery habitat (38.9% by weight). It was unclear where a further 18.5% (by number) of Barramundi landed had spent their juvenile years (18.8% by weight); these individuals typically displayed multiple brief residency periods in freshwater or low salinity brackish water, but not a full year. The remaining 48.6% (by number) of Barramundi landed in 2019 resided exclusively in saltwater during their juvenile years (42.3% by weight). It is important to note that all legal-size barramundi in the wild-capture fishery are collected in salt or brackish water. The greater contribution of individuals displaying juvenile freshwater residency to the commercial catch weight rather than number is a result of individuals with juvenile freshwater residency being more represented in the larger (and therefore heavier) size classes (Figure 23a and b).

The contribution of juvenile freshwater residency to each year class appears highly variable (Figure 23c). For example, juvenile freshwater residency contributed relatively little to the 2011 year class, which recruited in a strong wet season, but also contributes relatively little to the 2016 year class, which recruited in a poor wet season. Juvenile freshwater residency contributes strongly to both the 2010 year class, which recruited in an average wet season, and the 2012 year class, which recruited in a strong wet season. The mechanisms driving these recruitment patterns merit further investigation, as they appear to be much stronger drivers of recruitment to the fishery than Barramundi stocking in this region. Note that the 2018, 2017, and potentially even 2016 year classes may not be fully recruited to the fishery (580 mm minimum legal size) and therefore the patterns observed in the 2019 catch may not be fully representative of those cohorts. Any formal year-class analyses would benefit from multiple years of sampling to infer consistency in the observed patterns.

Fish displaying juvenile freshwater residency were disproportionately encountered early in the Barramundi season, during the peak wet season period (February), but were nonetheless encountered throughout the year, albeit at lower numbers (Figure 24). Fish displaying juvenile freshwater residency represented a smaller fraction of the catch in the Bowling Green Bay reporting area, indicating limited access to juvenile freshwater habitats in this area. Juvenile freshwater residency was much more common in the Burdekin reporting area, suggesting good access to juvenile freshwater habitats in this area (Figure 24).

Fish with freshwater juvenile residency were consistently encountered at significantly larger length-at-ages than their saltwater juvenile resident counterparts ($p < 0.001$, Figure 25) across all age classes. Almost every Barramundi > 1 m captured in 2019 had resided in freshwater as a juvenile. The oldest fish captured in 2019 (19 years old) was a saltwater juvenile resident.

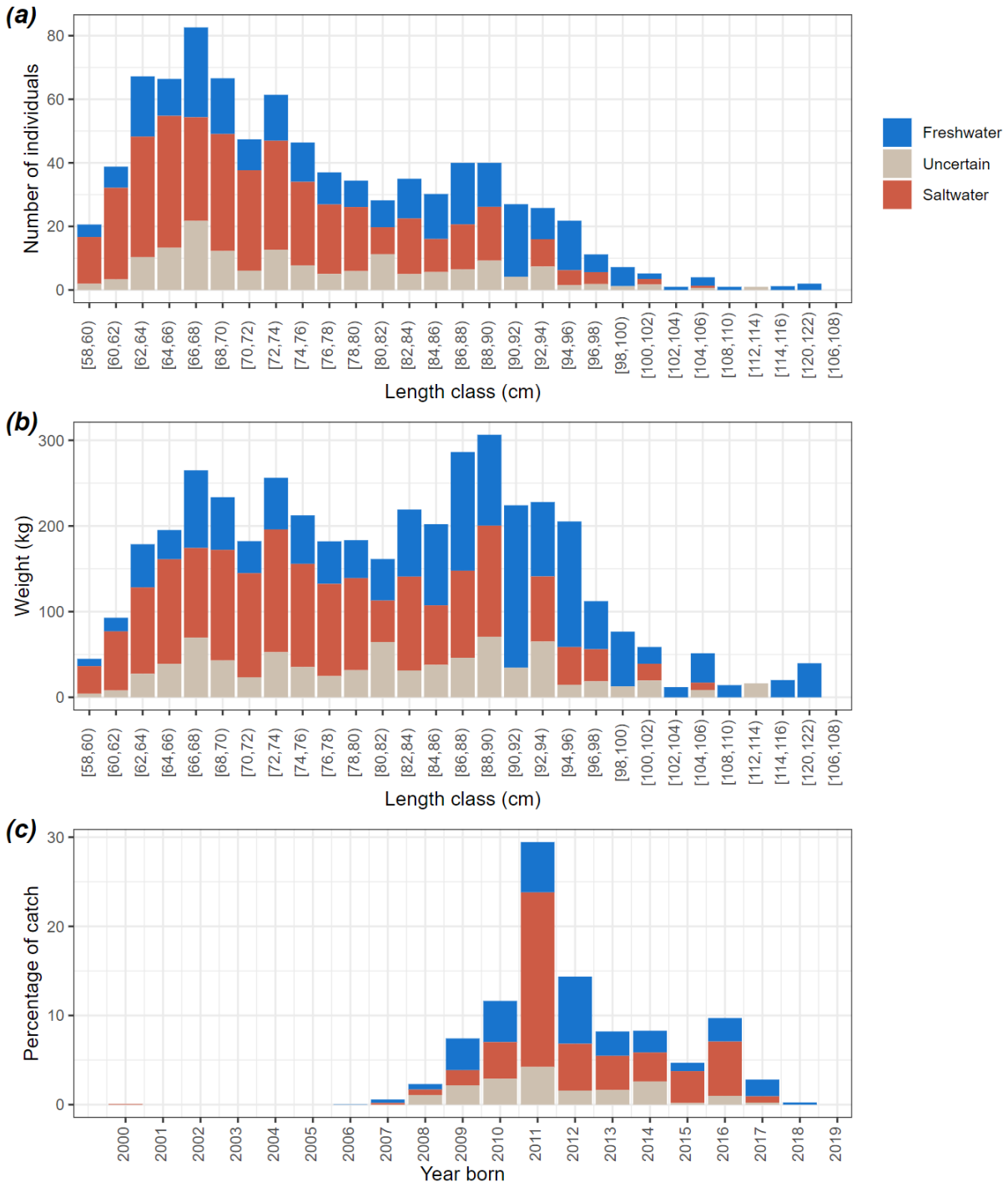

Figure 23. *Contribution of juvenile freshwater residency to the 2019 Dry Tropics fishery (a) length-frequency, (b) weight, and (c) year class frequency distribution (derived from age-frequency, and summing to 100%) using otolith microchemistry.*

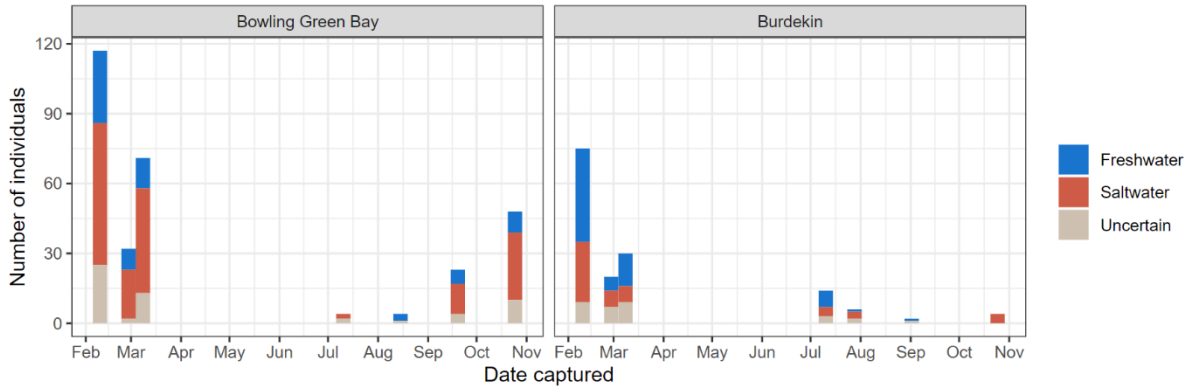

Figure 24. *Contribution of juvenile freshwater resident fish to the otolith microchemistry samples from the commercial sector for the Bowling Green Bay and Burdekin reporting regions only, throughout the 2019 east coast Barramundi season (1 Feb to 31 Oct).*

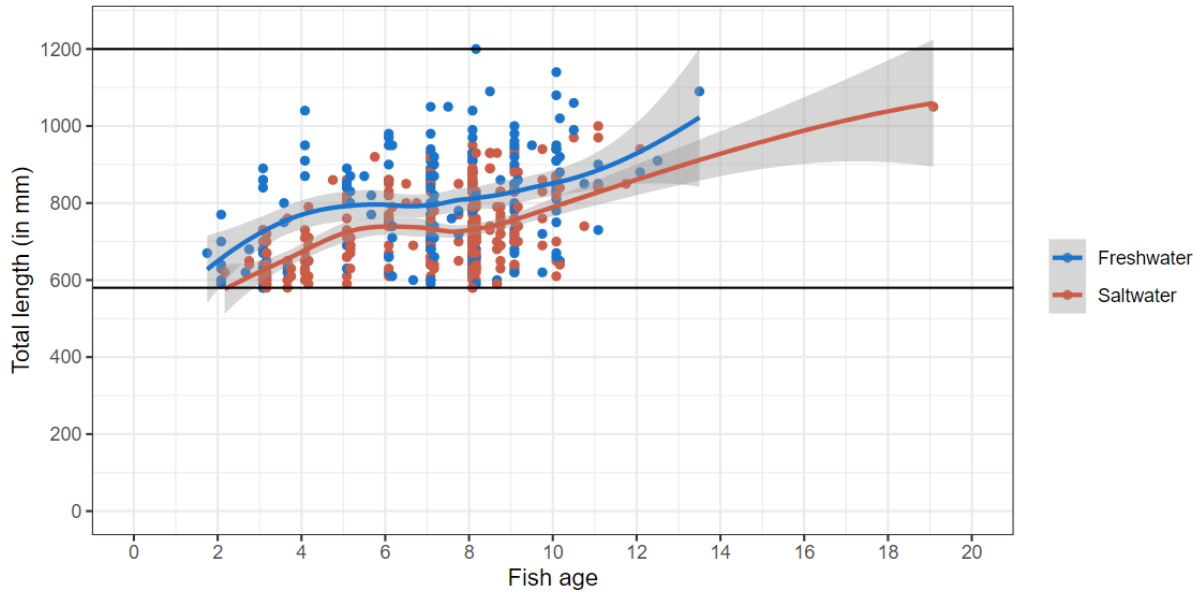

Figure 25. *Length-at-age for commercial sector samples for which both otolith microchemical juvenile residency information and age data were available (N = 579). For clarity, "uncertain" juvenile residency fish are excluded from the figure (N = 104). Trendlines are illustrative smoothers only and not formally fitted growth curves. Horizontal black lines indicate the minimum (580 mm) and maximum (1200 mm) legal size for Barramundi. Differences in length-at-age by juvenile residency are statistically significant ($p < 0.001$).*

Cost-Benefit Analysis

Power analysis

Application of the random forest microchemical classification model under simulation conditions demonstrated rapid improvement in model performance with increasing number of known-origin samples (Figure 26). We recommend a minimum calibration sample size of at least 40 known hatchery-origin samples and at least 60 known wild-origin samples. Increasing sample numbers above those suggested values did not meaningfully improve model accuracy. Where possible, we recommend that hatchery-origin samples be collected from two different cohorts at each farm.

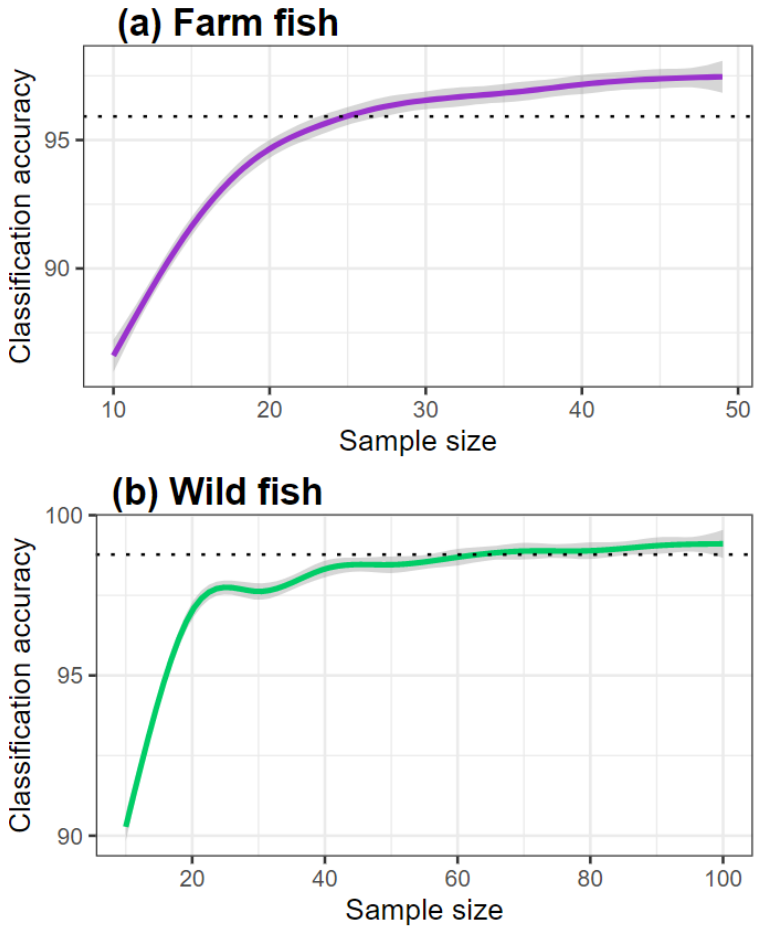

Figure 26. *Provenance model classification error rate for known-origin samples, across a range of simulated minimum sample sizes. Dotted line indicates the class-specific model accuracy achieved using the complete dataset (96% accuracy for N = 49 hatchery-origin samples; 99% accuracy for N = 163 wild-origin samples).*

Similar minimum sample size calculations were not applied to the otolith NIRS models, as the spectral range of known-origin samples available in the current study was inadequate to capture the full range of spectral variability in the population, potentially due to differences in the water chemistry of Barramundi natal environments, or spectral interference resulting from variation in otolith preparation (e.g. differences in otolith thickness, resin thickness, or imperfections in glass slides). In the absence of an objective measure of minimum sample size for the calibration dataset, we tentatively use 650 wild-origin samples and 650 hatchery-origin samples in the cost-benefit analyses presented below (i.e. approximately four times the number of wild-origin samples available in the current study, and equal numbers of wild-origin and hatchery-origin samples). Note that public support, fisheries permits, and animal ethics approval may be difficult to secure for such a large number of samples.

Sample collection methods

The costings provided here do not include the collection of unknown-origin samples (i.e. legal size adult fish). We assumed collection, otolith extraction, and age estimation of unknown-origin samples from the wild-capture marine and estuarine fishery would be carried out as per existing routine Fisheries Queensland monitoring of the commercial fishery. Collection of known wild-origin samples (i.e. sub-legal juvenile fish) by commercial operators (net fishing) was not considered in the cost-benefit analysis, as commercial gear is very size selective and typically does not retain juvenile Barramundi.

A network of volunteer recreational fishers was disproportionately costly when targeting small sample sizes, but was by far the most cost-effective approach for collecting large numbers of juvenile wild-origin samples (Table 2), as it is relatively insensitive to target sample number. However, experience from the current project demonstrates that volunteers may not be able to address all sampling needs due to a range of factors (e.g. interest, availability, site access), particularly if the sampling regime is time-sensitive. Any sampling relying on collection by volunteers will need to be supplemented with targeted sampling via electrofishing and/or charter fishing.

Targeted charter fishing was the most cost-effective method to collect small numbers of samples (N = 60, Table 2), but became increasingly expensive when targeting larger sample sizes (N = 150). In the current study, targeted charter fishing was used to collect samples from brackish and saltwater environments that were not able to be sampled by volunteers or using the available freshwater electrofishing technology. An important benefit of the charter fishing approach is the ability to quickly visit a range of different locations, thereby capturing a wide range of habitats and therefore potential variability in otolith composition. Due to this flexible deployment capacity, we consider targeted charter fishing the most suitable method for collecting small numbers of supplemental samples each year for maintenance of the provenance-determination model.

Boat electrofishing was similarly cost-effective to charter fishing for small sample sizes, primarily due to the greater catch rates achieved with boat electrofishing (Table 2). Boat electrofishing became disproportionately expensive when applied to large sample sizes. It is important to note that although electrofishing can result in high catch rates, it is relatively slow to deploy across a range of sites, and can therefore provide large numbers of samples from a more limited number of locations. As a result, a solely electrofishing-based collection approach may not capture the full range of variability in otolith composition across a region. An estuarine electrofisher (Grassl Model EL65IIGI1) will be available in Queensland in early 2022, which should extend sampling capabilities to a much wider range of potential wild juvenile Barramundi habitats (maximum 22 ppt salinity).

Provenance determination scenarios

When developing a provenance-determination method for Barramundi in a new region, we recommend otolith microchemistry as the most cost-effective and accurate method (Table 15). Genetic parentage analysis using SNPs was also cost-effective, but its accuracy has not been assessed in the current project. For both otolith microchemistry and SNPs, the cost of fieldwork for collection of known wild-origin samples represented the single largest cost. NIRS of whole and sectioned otoliths were prohibitively expensive, primarily due to the cost of fieldwork for collection of the very large number of known wild-origin samples (Table 15). In the case of NIRS of sectioned otoliths, the high cumulative cost of pre-processing large numbers of samples also contributed to the large estimated cost, whereas NIRS of whole otoliths did not incur many pre-processing expenses (Table 15).

For routine monitoring of Barramundi provenance in a region for which a provenance-determination method is already established (i.e. the Dry Tropics), all of the methods were similarly priced (Table 16). Genetic parentage analysis using SNPs was the most cost-effective method to implement once its accuracy has been established for this species and purpose. This is largely due to the minimal number of known-origin samples needed to maintain the model each year, and the minimal amount of sample pre-processing needed. NIRS of whole and sectioned otoliths were slightly more expensive to implement as a routine monitoring tool,

primarily due to the cost of fieldwork for continued collection of known-origin samples to maintain the model each year. NIRS of sectioned otoliths incurred minimal otolith pre-processing expenses, as we assumed that the 400 unknown-origin samples would be pre-processed during routine fishery monitoring activities. This is because unlike otolith microchemistry approaches, otolith NIRS does not require a specially-prepared sectioned otolith surface, but should instead be able to use the same otolith sections already prepared for age estimation for routine fishery monitoring, thereby reducing labour costs. That said, use of standard ageing sections (i.e. including a surface glass coverslip) for NIRS provenance determination is a critical assumption that requires explicit testing to confirm its accuracy and any limitations. Otolith microchemistry was the most expensive method for routine application (Table 16). The biggest components of the cost of microchemistry were from the commercial cost of laser ablation, and the labour required to prepare each sectioned otolith, as both the known and unknown-origin samples need to be pre-processed using a specialised approach.

When applying provenance-determination to a subset of the historic Barramundi otolith collection for the Dry Tropics, NIRS of sectioned or whole otoliths will be by far the most cost-effective methods (Table 17) once a suitable model has been established. This is because there is no need for sample pre-processing, and otolith handling time is minimal. A critical assumption is that NIRS of sectioned otoliths can successfully be applied to otoliths previously-prepared for ageing (i.e. with a glass coverslip). Successful application of either NIRS approach to the historic otolith collection is contingent on successful development of a provenance-determination model for the region of interest, which itself may be prohibitively expensive (Table 15). The genetic SNPs approach had an intermediate cost for application to the historic otolith collection (Table 17). Forensic genetic approaches produce low quantities and poor quality of DNA, that nonetheless can still be useful for analysis (Toomey et al. 2016). It may therefore be possible to extract sufficient genetic material from the historic Barramundi otolith collection to subsequently apply SNPs-based parentage analysis, but this has not been tested in the current study. Otolith microchemistry was by far the most expensive method to apply to the historic otolith collection (Table 17), but is also the most accurate method for provenance determination.

Table 15. *Scenario 1: Estimated cost of model development in a new region, using minimum known-origin sample numbers as determined for each provenance-determination method. Items, rates, and costs as per Table 2 and Table 3.*

		N samples	Rate	Cost	Total cost
NIRS of whole otoliths	Collection of known hatchery-origin fish	650	$20 / fish, $2,122 / trip for 2 trips	$17,244	**$274,063**
	Collection of known wild-origin fish	650	$8,000 per trip for 29 trips	$232,000	
	Otolith extraction	1,300	80 otoliths / day	$5,965	
	Consumables	1,300	$1 / sample	$1,300	
	Otolith handing time	1,300	1 otolith / minute	$1,097	
	Pixel selection	1,300	20 samples / hour	$3,291	
	Consumables, instrument depreciation, software licences	1,300	$6,000 / year	$6,000	
	Data analysis and model development		3 weeks	$7,167	
NIRS of sectioned otoliths	Collection of known hatchery-origin fish	650	$20 / fish, $2,122 / trip for 2 trips	$17,244	**$326,011**
	Collection of known wild-origin fish	650	$8,000 per trip for 29 trips	$232,000	
	Otolith extraction	1,300	80 otoliths / day	$5,965	
	Otolith blocking	1,300	80 otoliths / day	$5,965	
	Otolith sectioning	1,300	30 otoliths / day	$15,906	
	Otolith polishing	1,300	60 otoliths / day	$7,953	
	Otolith mounting	1,300	80 otoliths / day	$5,965	
	Otolith labelling and data entry	1,300	200 otoliths / day	$2,386	
	Consumables	1,300	$12.27 / sample	$15,951	
	Otolith handling time	1,300	1 microscope slide /minute, assume 5 otoliths / slide	$219	
	Pixel selection	1,300	20 samples / hour	$3,291	
	Consumables, instrument depreciation, software licences	1,300	$6,000 / year	$6,000	
	Data analysis and model development		3 weeks	$7,167	
Otolith microchemistry	Collection of known hatchery-origin fish	40	$20 / fish, $2,122 / trip for 2 trips	$5,044	**$46,833**
	Collection of known wild-origin fish	60	$8,000 per trip for 3 trips	$24,000	
	Otolith extraction	100	80 otoliths / day	$459	
	Otolith blocking	100	80 otoliths / day	$459	
	Otolith sectioning	100	30 otoliths / day	$1,224	
	Otolith polishing	100	60 otoliths / day	$612	
	Otolith mounting	100	80 otoliths / day	$459	
	Otolith labelling and data entry	100	200 otoliths / day	$184	
	Consumables	100	$12.27 / sample	$1,227	
	Laser ablation	100	$60 / otolith	$6,000	
	Data analysis and model development		3 weeks	$7,167	
Genetics (SNPs)	Collection of known hatchery-origin fish	63	$20 / fish, $2,122 / trip for 2 trips	$5,504	**$62,086**
	Collection of known wild-origin fish	125	$8,000 per trip for 5 trips	$48,000	
	Tissue sample collection	188	200 samples / day	$345	
	Consumables	188	$6.98 / sample	$1,312	
	JCU commercial charge rate for SNPs	188	$35 / sample	$6,580	
	Labelling + data entry	188	200 samples/ day	$345	

Note: cost of collection of wild-origin fish is via charter (assuming 5 fish/day * 5 days/trip), and adding a 10% buffer to the target sample number to accommodate potential sampling issues/delays encountered while in the field.

Table 16. *<u>Scenario 2:</u> Estimated cost of ongoing model application to 400 unknown-origin samples per year, and model maintenance via collection of 10% known-origin calibration samples per year.*

		N samples	Rate	Cost	Total cost
NIRS of whole otoliths	Collection of known hatchery-origin fish	65	$20 / fish, $2,122 / trip for 1 trip	$5,544	**$40,448**
	Collection of known wild-origin fish	65	$8,000 per trip for 3 trips	$24,000	
	Otolith extraction	130	80 otoliths/day	$596	
	Consumables	130	$1 / sample	$130	
	Otolith handing time	530	1 otolith / minute	$447	
	Pixel selection	530	20 samples / hour	$1,342	
	Consumables, instrument depreciation, software licences	530	$6,000 / year	$6,000	
	Data analysis, model maintenance		1 week	$2,389	
NIRS of sectioned otoliths	Collection of known hatchery-origin fish	65	$20 / fish, $2,122 / trip for 1 trip	$5,544	**$45,373**
	Collection of known wild-origin fish	65	$8,000 per trip for 3 trips	$24,000	
	Otolith extraction	130	80 otoliths / day	$596	
	Otolith blocking	130	80 otoliths / day	$596	
	Otolith sectioning	130	30 otoliths / day	$1,591	
	Otolith polishing	130	60 otoliths / day	$795	
	Otolith mounting	130	80 otoliths / day	$596	
	Otolith labelling and data entry	130	200 otoliths / day	$239	
	Consumables	130	$12.27 / sample	$1,595	
	Otolith handling time	530	1 microscope slide / minute, 5 otoliths / slide	$89	
	Pixel selection	530	20 samples / hour	$1,342	
	Consumables, instrument depreciation, software licences	530	$6,000 / year	$6,000	
	Data analysis, model maintenance		1 week	$2,389	
Otolith microchemistry	Collection of known hatchery-origin fish	8	$20 / fish, $2,122 / trip for 1 trip	$4,404	**$57,571**
	Collection of known wild-origin fish	12	$8,000 per trip for 1 trip	$8,000	
	Otolith extraction	20	80 otoliths / day	$92	
	Otolith blocking	420	80 otoliths / day	$1,927	
	Otolith sectioning	420	30 otoliths / day	$5,139	
	Otolith polishing	420	60 otoliths / day	$2,569	
	Otolith mounting	420	80 otoliths / day	$1,927	
	Otolith labelling and data entry	420	200 otoliths / day	$771	
	Consumables	420	$12.27 / sample	$5,153	
	Laser ablation	420	$60 / otolith	$25,200	
	Data analysis, model maintenance		1 week	$2,389	
Genetics (SNPs)	Collection of known hatchery-origin fish	7	$20 / fish, $2,122 / trip for 1 trip	$2,262	**$29,435**
	Collection of known wild-origin fish	13	$8,000 per trip for 1 trip	$8,000	
	Tissue sample collection	420	200 samples / day	$771	
	Consumables	420	$6.98 / sample	$2,932	
	JCU commercial charge rate for SNPs	420	$35 / sample	$14,700	
	Labelling + data entry	420	200 samples / day	$771	

Note: Otolith pre-processing for NIRS assumes pre-processing of unknown-origin samples is carried out during routine fishery monitoring. The cost of otolith pre-processing for otolith microchemistry is listed for both known and unknown-origin samples due to special requirements of sample pre-processing for microchemical work.

Table 17. *<u>Scenario 3:</u> Estimated cost of model application to a subset of the historical otolith collection (200 samples/year * 15 years of monitoring).*

		N samples	Rate	Cost	Total cost
NIRS of whole otoliths	Consumables	3,000	$1 / sample	$3,000	**$23,904**
	Otolith handing time	3,000	1 otolith / minute	$2,531	
	Pixel selection	3,000	20 samples / hour	$7,594	
	Consumables, instrument depreciation, software licences	3,000	$6,000 / year	$6,000	
	Data analysis and application to historic collection		2 weeks	$4,778	
NIRS of sectioned otoliths	Otolith handling time	3,000	1 microscope slide / minute, 5 otoliths / slide	$506	**$18,878**
	Pixel selection	3,000	20 samples / hour	$7,594	
	Consumables, instrument depreciation, software licences	3,000	$6,000 / year	$6,000	
	Data analysis and application to historic collection		2 weeks	$4,778	
Otolith microchemistry	Otolith blocking	3,000	80 otoliths / day	$13,765	**$309,683**
	Otolith sectioning	3,000	30 otoliths / day	$36,706	
	Otolith polishing	3,000	60 otoliths / day	$18,353	
	Otolith mounting	3,000	80 otoliths / day	$13,765	
	Otolith labelling and data entry	3,000	200 otoliths / day	$5,506	
	Consumables	3,000	$12.27 / sample	$36,810	
	Laser ablation	3,000	$60 / otolith	$180,000	
	Data analysis and application to historic collection		2 weeks	$4,778	
Genetics (SNPs)	JCU commercial charge rate for forensic DNA extraction	3,000	$15 / sample	$45,000	**$155,506**
	JCU commercial charge rate for SNPs	3,000	$35 / sample	$105,000	
	Labelling + data entry	3,000	200 samples / day	$5,506	

Note: external costs-per-sample for laser ablation and SNPs will decrease with very large sample numbers.

Discussion

This project demonstrated that provenance determination using otolith core microchemistry is highly accurate (>98% accuracy). Provenance determination using both whole and sectioned otolith NIRS was not successful in this instance. Provenance confirmation using genetic microsatellite parentage analysis was not possible due to probable introgression, i.e. breeding of stocked fish with the wild population. A cost-benefit analysis indicated that otolith microchemistry is the most cost-effective and accurate approach to apply for provenance-determination in other regions and as a routine monitoring tool in the Dry Tropics.

Extrapolation of the otolith microchemistry method to the 2019 commercial catch in the Dry Tropics region estimated that 3% of the Barramundi landed were hatchery-born and 96% were wild-born, indicating that the fishery is primarily capturing wild-born individuals (the remaining 1% were of uncertain provenance). By contrast, the genetic microsatellite parentage analysis estimated that 21% of the Barramundi landed displayed predominantly hatchery ancestry, and 79% had predominantly wild ancestry. Otolith microchemical profiles also allowed for reconstruction of Barramundi movement history, and indicated that 33% of the Barramundi landed had spent at least one full year during their juvenile period in a freshwater nursery habitat. Productivity of the wild-capture marine and estuarine Barramundi fishery is likely to benefit more from increasing juvenile fish access into suitable freshwater nursery habits, rather than directly through fish stocking.

Objective 1. Accuracy and value of the NIRS approach

We do not consider NIRS of whole or sectioned otoliths to be suitable for provenance detection in Barramundi at this time. The trials using NIRS to predict fish origin based on either sectioned or whole otoliths displayed low accuracy on validation subsets of known-origin samples (Table 4 and Table 5) and low agreement rates with the microchemical and genetic methods on unknown-origin samples (Table 14, Figure 15, Figure 16).

There are a number of reasons why NIRS performed poorly in this instance, some of which can be resolved with further work. In particular, future testing of NIRS for provenance determination would benefit from many more calibration samples (i.e. known-origin fish). The known-origin samples should encompass the full range of biophysical variability that will be encountered in the unknown-origin samples, i.e. differences in the water chemistry of all possible Barramundi natal environments, and the full range of potential spectral interference resulting from variation in otolith preparation (e.g. micron-scale differences in otolith thickness, resin thickness, or imperfections in glass slides). Addressing these limitations is of interest, given the low cost of implementing NIRS monitoring on large datasets (Table 17).

The whole otolith NIRS approach was constrained by the uneven fish size classes between the known hatchery and known wild-origin samples used to build the calibration model (Figure 3), and would benefit from matching the size classes of individuals of each origin. However, the vulnerability of whole otolith NIRS to fish (and therefore otolith) size means that it is unclear how a provenance classification model would perform when used to predict provenance of unknown-origin fish, given that unknown-origin fish will always be three to five times larger than the known-origin samples from which the provenance model can be built.

The sectioned otolith NIRS approach was robust to the uneven fish size classes between the known-hatchery and known-wild origin samples due to direct imaging of the sectioned otolith core, but may have been vulnerable to other sources of error. A major limitation of NIRS analysis is its dependence on the accuracy of the reference material used to develop the statistical calibration model. For example, NIR hyperspectral imaging of sectioned otoliths is assumed to capture reflected NIR light that has penetrated into and reflected from the material of interest. In this instance, that would likely capture the 300 µm (0.3 mm) thick otolith section, as well as the ~50 µm resin beneath it, and the glass microscope slide to which it has been mounted. Micron-scale differences in otolith thickness, resin thickness, or imperfections in the glass slide may be contributing to spectral interference unrelated to fish provenance. Some of these sources of spectral “noise”

may be able to be addressed via advanced data pre-processing techniques such as wavelength selection procedures and spectral wavelength subtraction. Alternatively, one of the best ways to control for the effect of spectral "noise" on model predictive performance is to calibrate the model using substantial numbers of known-origin samples (Table 15), which capture the majority of the biological variability in the material of interest (the otolith) as well as any physical variability in the sample preparation (e.g. otolith section thickness, resin thickness, etc). This reference collection should also be added to over time to continue to capture any new sources of variability (e.g. geographic differences in otolith core composition) (Table 16). A hypothetical calibration dataset of 1,300 known-origin samples was proposed for the cost-benefit analysis, as it was not possible to ascertain a suitable minimum sample size for this component.

Due to confounding variables and low numbers of calibration samples available in the current study, it is unclear if the NIRS configuration used in this study is capable of detecting provenance-related differences in otolith spectra. While NIRS does not quantify otolith composition directly, the major provenance-related differences in otolith composition identified in this study were extremely small (e.g. 0.05 mmol of manganese per mol of calcium, Figure 11). NIRS technology is typically used to identify macro constituents, as its sensitivity limit is about 0.1% for most constituents (Talebi and Armstrong 2020). NIRS technology has been used to detect extremely low levels of certain constituents (i.e. ppm and ppb, Griffiths 2010), but these low levels are typically detected through secondary correlations, rather than detected directly. Detection limits are also influenced by the interaction of organic and inorganic constituents in the sample of interest impacting the spectral characteristics. It is probable that the provenance-related differences in otolith microchemical composition, or their proxies, may fall below the detectability limit of the NIRS hyperspectral instrument used in this study.

It is important to remember that NIRS is a vibrational spectroscopy technique, which detects signatures in absorbance of NIR energy resulting from different types and abundance of covalent bonds in organic molecules. Crucially, several of the major provenance-related otolith trace elements in the current study are predominantly deposited as a substitute for calcium (magnesium, strontium, barium) (Campana 1999), and are therefore likely to be incorporated into the otolith crystal lattice with similar bond energies to calcium, potentially resulting in similar NIR spectral signatures for otoliths containing a wide range of concentrations of those trace elements. Fortunately, other trace elements relating to provenance in this study have previously been shown to bind elsewhere in the otolith, and not only via substitution for calcium in the calcium carbonate crystal (Hüssy et al. 2020). For example, Izzo et al. (2016) demonstrated that both manganese and zinc have an affinity (≥28%) for the otolith protein component, and otolith protein content is typically high in the otolith core (Dove et al. 1996). Rubidium appears to be randomly captured in the otolith crystal lattice (Hüssy et al. 2020). Due to this diversity of trace element concentrations and binding locations, we hypothesise that measurable differences in concentration of these trace elements in the otolith core should result in measurable differences in spectral signatures of the otolith core, given sufficient calibration samples, controlling for all other sources of spectral "noise", and provided the NIRS instrument is sufficiently sensitive.

Objective 2. Accuracy and value of the microchemistry approach

Provenance determination using otolith core microchemistry was highly accurate (>98%) and consistent using known-origin samples (Table 9). It was not possible to objectively assess the accuracy of the microchemical model at assigning provenance to unknown-origin samples due to the lack of clear confirmatory evidence from the genetic microsatellite parentage analysis. However, inter-method agreement for detection of hatchery-origin fish by the otolith microchemistry and microsatellite parentage analysis approaches was reasonable (69%, Figure 16), and the microchemical differences between hatchery-origin and wild-origin samples were stark (Figure 7, Figure 12), consistent through time (Figure 9), and biophysically meaningful (e.g. manganese), which implies that the high accuracy achieved on the known-origin samples likely holds true when the model is applied to unknown-origin samples. This also indicates that provenance determination using otolith core microchemistry in this system and species can be accomplished without the need to invest in and develop a geochemical reference library of water and/or otolith microchemical composition across sites and years.

The otolith microchemistry approach is highly accurate, cost-effective to apply to a new region (Table 15), and is reasonably priced as a routine monitoring tool (Table 16). However, it becomes increasingly costly to apply to large datasets (e.g. historical otolith collections, Table 17). As a result, we consider it better suited for application to small/medium datasets, or to representative subsets from a larger collection. Because of its high statistical power to resolve provenance, a reliable calibration model can be built from a relatively small set of known-origin samples (Figure 26). However, the low incidence of stocked fish detected in the commercial fishery in this region (3%), even following an exceptional flood (February 2019), may make it difficult to detect the presence of any stocked fish in years with average wet seasons. Routine monitoring may therefore still need to apply microchemical provenance detection to somewhat large numbers (e.g. 200-400) of unknown-origin samples in order to produce reliable estimates of the contribution of stocked fish to the fishery over time.

The accuracy of otolith microchemistry data is highly sensitive to correct placement of the laser ablation track, as well as spatiotemporal consistency in water microchemistry. That is, some of the misclassified known-origin samples may be a result of incorrect ablation track placement, while others may be due to true microchemical similarities between hatchery and wild water masses. Close inspection of ablation tracks of all samples will clarify this. However, we suspect that true microchemical similarities do occur between a small number of hatchery-origin and wild-origin samples. In particular, the misclassified hatchery-origin samples in the current study typically originated from a farm that uses seawater pumped in from the local estuary, resulting in farm water chemistry that is more similar to wild Barramundi habitats. Conversely, natural changes in water quality of wild habitats during Barramundi spawning (e.g. particularly clearwater conditions) may result in cohorts of wild-born fish whose otolith microchemistry more closely matches farm-born fish. For this reason, we recommend regular collection of further calibration samples (~10% of the reference collection) to maintain and improve provenance detection models for routine fishery monitoring (Table 16).

Collection of complete otolith microchemical profiles also opportunistically provided the opportunity to collect fish movement history information, in particular information on the prevalence and productivity impacts of juvenile freshwater residency on the Barramundi fishery. This opportunistic data collection allowed us to identify that juvenile residency patterns are a much stronger driver of the productivity of the Dry Tropics Barramundi fishery than fish stocking (Figure 17, Figure 23). This type of fish movement history information is not available from any of the other provenance determination methods tested here (NIRS, genetics).

Objective 3. Method comparisons and agreement

Parentage analysis approach and implications

Microsatellite parentage analysis in this system detected individuals with hatchery ancestry, some of which are likely to have been stocked individuals, while others are likely to be descendants of stocked individuals. This outcome has a number of implications:

(1) Stocked fish are successfully breeding and contributing offspring to the wild Barramundi population in this region.
(2) If stocked fish are successfully contributing genetic material to the wild population, then the genetic composition of stocked fish should be managed to maintain local genetic diversity and persistence of local evolutionary traits.
(3) Microsatellites no longer provide sufficient resolution to determine Barramundi provenance in this system. A higher resolution approach such as Single Nucleotide Polymorphisms (SNPs) would be more suitable.

Population genetic analyses confirmed that the wild locations sampled comprise a single genetic stock (Table 12). Genetic diversity statistics for the six locations sampled in the present research (Table 11) are within expected bounds for Barramundi from Cleveland Bay, Bowling Green Bay, and the Burdekin River (Loughnan

et al., 2019) and indicate that despite the levels of restocking in the region there is limited evidence that genetic diversity has been significantly impacted to date. This is likely due to numerous wild-collected broodstock genetically contributing into the stocked progeny groups each year. If, however, the same broodstock continue to be bred from over many years without replenishment, as is often the case for Barramundi hatcheries, or if F1 generation derived broodstock are used, genetic diversity may be eroded due to a very narrow and highly related gene pool being stocked into natural populations.

Noble et al. (2014) showed that captively-reared Barramundi that had escaped from a fish farm can survive and persist in large numbers for at least 18 months after escaping. This suggests that escaped and/or stocked Barramundi adapt well to the wild environment and thus conceivably have the potential to interbreed with local wild-origin Barramundi. In the present study, 17% of known wild-origin Barramundi had genetic profiles suggesting that they may have ancestral relationships with broodstock in the JCU genetic database. It was not possible to confirm with the microsatellite genetic suite that these fish were F1 progeny resulting from the pairing of a wild-origin and a hatchery-origin parent. However, it is possible that some of the fish sampled were introgressed individuals, and that this lowered the resolution power of the microsatellite marker suite to definitively classify these individuals as either wild-born or hatchery-born fish.

The same 17 microsatellite loci used in the current study have previously been used to look for genetic impacts of Barramundi stocking in the Wet Tropics region (FRDC 2009-040), where the authors did not find evidence of introgression of hatchery genetics in the wild population (Russell et al. 2013). It is possible that introgression had not yet occurred at detectable levels in that system at the time the study was undertaken. Alternatively, it may be that the progeny-simulation approaches used in that study may not have been adequate to detect introgression. In the present study, introgression was suspected in the Dry Tropics fishery as a result of access to multiple lines of evidence for provenance of each sample (collection location, otolith microchemistry, microsatellite parentage analysis). Revisiting the samples collected in the Wet Tropics study, using either otolith microchemistry or a higher-resolution genetic approach (i.e. SNPs), should be applied to confirm whether introgression has/is occurring in that system.

The level of introgression occurring in the stocked locations has implications for the genetic fitness of the local wild population. If stocked fish genotypes are low in genetic diversity, or contain different types of alleles (e.g. if they originate from another genetic stock, or express traits that are deleterious in wild environments), then introgression may result in deleterious genetic diversity shifts that may lead to lowering of fitness within populations. An effective population size (Ne) of 50 broodstock is typically required to keep levels of inbreeding accumulation < 1%, which is the commonly-accepted threshold for breeding and conservation purposes (the “50/500” rule). Given the generation interval of Barramundi is 4 to 5 years, then this Ne should be achieved over 5 years and maintained in stocked progeny to ensure that sufficient genetic diversity is being introduced into the stocked population.

Microsatellites have limited resolution power to identify evidence for introgression compared to genome-wide SNPs panels. Therefore, if the question of introgression is considered important to understand from a fisheries management perspective, it is recommended that recently developed high-resolution SNP arrays (D. Jerry, *unpublished*) be used to screen the Barramundi samples collected in the present study to clarify the level of introgression between hatchery-derived and wild Barramundi. Note that due to their short length, SNPs are also better suited to forensic DNA extraction on historic otolith collections than microsatellites.

Inter-method agreement, accuracy, and reliability

The otolith NIRS approaches had extremely poor agreement with the otolith microchemistry and microsatellite parentage analysis techniques (Figure 15, Figure 16, Table 14), and are not currently suitable for estimating the contribution of stocked fish to the fishery.

The otolith microchemistry approach detected a low number of hatchery-born fish among the unknown-origin samples (3% of the commercial catch), most of which were confirmed by the genetic parentage analysis (69%, Figure 16). Assessment of the objective accuracy of these detections is not possible, however, we consider them reliable due to:

(1) Consistently high accuracy of the otolith microchemistry method on known-origin individuals across different combinations of calibration and validation datasets (Table 9);
(2) Strong and consistent differences in the otolith core microchemistry of hatchery-origin versus wild-origin fish (Figure 12), supported by meaningful biophysical processes (e.g. manganese); and
(3) Implementation of a conservative membership threshold (>60% likelihood of being hatchery-origin, Figure 7).

The microsatellite parentage analysis likely over-detected hatchery provenance in as many as 17% of samples, due to detection of individuals with hatchery ancestry. Note that once the 17% error rate was accounted for, both the otolith microchemistry and genetic microsatellite methods detected similar numbers of hatchery-origin fish in the wild-capture fishery (otolith microchemistry: ~3%, genetic microsatellites: 21% minus 17% = 4%). As a result of this high error rate, we consider the genetic microsatellite parentage analysis method no longer suitable for estimating the direct, numerical contribution of stocked fish to the fishery, but see it as a valuable tool for estimating the combined direct and indirect contribution of fish stocking to the fishery (i.e. stocked fish and their genetic contribution to subsequent generations). The combination of natal origin information from the otolith microchemistry results and genetic ancestry information from the microsatellite parentage analysis provided a more thorough understanding of the consequences of fish stocking in this region than would have been achieved through application of a single provenance determination method. That said, if the goal is provenance detection of fish samples using genetic methods, then alternative approaches that were not tested here (such as SNPs) may be more effective for future work in this region.

Extrapolation of findings to the 2019 Dry Tropics commercial catch

Otolith microchemistry results estimate that 3% of the 2019 commercial Barramundi catch in the Dry Tropics region (4% by weight) was composed of hatchery-born fish that had been stocked, and at some point escaped downstream into the estuary. This indicates that the Dry Tropics wild-capture marine and estuarine fishery is predominantly capturing wild-born fish, even following significant flood events (i.e. February 2019). Stocked fish made up a greater proportion of year-classes spawned when climatic conditions were likely conducive to poor wild recruitment (i.e. little wet season rainfall, or only minor river flooding, e.g. 2013); stocked fish made up a lesser proportion of year-class abundance when climatic conditions were likely to be conducive to strong wild recruitment (e.g. 2011) (Figure 17).

The low incidence of stocked fish in the wild-capture marine and estuarine fishery identified in this study likely represents the upper range of the potential numerical contribution of stocked fish to the Dry Tropics commercial catch. This is because the 2019 East Coast barramundi season began with an exceptional, historic flood event in the Townsville region (23 Jan to 7 Feb), which connected every stocked habitat in the region to major downstream flows, and provided the opportunity for all barramundi to move downstream into the wild-capture fishery.

It is unclear if the low incidence of stocked fish in the wild-capture fishery identified in the current study will be consistent across other regions. Early work using external wire tags estimated that stocked Barramundi contributed to 10 to 15% of the local 580-650 mm size cohort (Russell and Rimmer 1997). These estimates may differ from ours due to significant differences in the study methodologies, in particular: local stocking policy (e.g. river stocking rather than impoundment stocking), the scale of the study area (single river vs. a region containing 3 major river systems), the target size class (580-650 mm vs. all legal size Barramundi), and the methods used (tag returns vs. otolith microchemistry). We hypothesise that Russell and Rimmer's (1997) estimate of the contribution of stocked fish to the wild capture fishery would be more similar to our own 3% if their target population had been the full legal size range of Barramundi (580-1200 mm) and the wider Wet Tropics region fishery.

Much larger estimates of stocked fish contribution to the wild-capture marine and estuarine fishery have occurred following extreme weather events (e.g. Awoonga Dam, Wesche et al 2013, Streipert et al. 2019), but were not observed in the current study despite collection of fish samples immediately following a historic flood event (February 2019). We hypothesise that an important difference between the current study in the

Dry Tropics and the 2011 Awoonga Dam overtopping event is the availability, extent, and accessibility of suitable Barramundi recruitment and/or nursery habitat in each region. It appears that despite extensive and long-term impoundment stocking in the Dry Tropics (3 million potential escapees since 1988, Figure 1), the number of wild-born fish in the Townsville and Burdekin region is so large that it eclipses the number of hatchery-born individuals that may go on to escape downstream. This is likely a result of the excellent and extensive Barramundi recruitment and nursery habitat that occurs across the Burdekin and Haughton floodplains and irrigation scheme. In contrast, historical catch trends indicate that natural recruitment of wild-origin fish in the Gladstone region is likely consistently lower than the Burdekin region (Streipert et al. 2019), potentially as a result of less extensive, accessible, or suitable juvenile Barramundi habitat. Therefore, regardless of the natural or artificially-inflated adult population, recruitment of young Barramundi in the Gladstone region remains constrained by other factors, such as the carrying capacity of local juvenile habitats. This hypothesis explains why, despite the sudden addition of large numbers of stocked fish to the wild-capture marine and estuarine fishery via overtopping of Awoonga dam in 2011, catch rates in the Gladstone region eventually returned to and stabilised at pre-2011 levels (Streipert et al. 2019).

Microsatellite parentage analysis estimates that 21% of the 2019 commercial Barramundi catch in the Dry Tropics region (22% by weight) was composed of fish with hatchery ancestry (Figure 20). This indicates that stocked fish, once they escape from impoundments, can successfully breed in the wild, and are making a sizeable contribution to the genetic composition of the wild Barramundi population in the Dry Tropics region. Management protocols to promote genetic diversity and resilience are described in the Recommendations below.

Otolith microchemical profiles indicate that 33% of the 2019 commercial Barramundi catch in the Dry Tropics region (39% by weight) had spent at least one full year in freshwater as juveniles. A further 19% (by number and by weight) had accessed freshwater more briefly (Figure 23). This indicates that availability and accessibility of freshwater habitats for juvenile Barramundi is extremely important to the Barramundi population and fishery. In addition, juvenile Barramundi that resided in freshwater as juveniles achieved significantly greater lengths-at-age than conspecifics that only had access to saltwater habitats. Management of freshwater resources, habitats, and their connectivity should therefore be a priority for maintaining and potentially growing fishery biomass in this region.

Availability and access to freshwater habitats are critical to Barramundi stocks across Northern Australia (Halliday et al. 2012; Roberts et al. 2019; Robins et al. 2021). Freshwater residency particularly benefits juvenile Barramundi, potentially due to increased food availability, increased feeding opportunity, reduced predation pressure, reduced energetic demands in freshwater lagoons due to absence of tidal currents, or any combination of the above.

The current project contributes to the growing body of evidence on the importance of freshwater resource management for purposes beyond agricultural use, and in particular for estuarine fisheries productivity. In addition, coarse spatiotemporal patterns in the current dataset hint that local Barramundi fishery productivity may be constrained by limited juvenile access to suitable freshwater habitats (e.g. Bowling Green Bay, Figure 24). Managing availability and access to freshwater habitats in this region is important for the sustainability of the local fishery, and for increasing productivity of the fishery into the future.

Objective 4. The most cost-effective method going forward

NIRS of sectioned otoliths can be cost effective to implement on large datasets (Table 16 and Table 17), but did not provide reliable provenance classification in this instance. If a suitable calibration set of known-origin samples can be collected over time, and if the classification algorithm can be trained to account for all possible sources of spectral noise (e.g. resin, glass), then otolith NIRS may have the potential to be quickly and cheaply deployed to assess Barramundi provenance as a routine monitoring tool. However, the calibration samples available in the current study did not produce a suitable predictive model for use in future monitoring or for application to the historic otolith collection.

Otolith microchemistry was cost effective for initial model development on a small number of samples (Table 15), but rapidly became extremely costly when applied at scale (Table 16, Table 17). To balance the high cost and high accuracy of the microchemical provenance classification, we recommend application of otolith microchemistry to representative subsets of a target population. This will reduce implementation costs without significantly reducing model predictive power. A similar suite of trace elements may also be suitable for provenance-determination for other stocked species, particularly those that spawn in wild habitats with distinctly different water microchemistry to hatcheries and farms.

In addition, future work using otolith microchemistry for provenance determination can use a range of approaches to reduce the per-sample costs of both sample preparation and laser ablation. For example, single point ("spot") ablation has successfully been used for provenance determination in other species (e.g. walleyes, *Sander vitreus*, Carlson et al. 2016, golden perch, *Macquaria ambigua*, Zampatti et al. 2021), and can dramatically reduce ablation time and cost. However, single point ablation is more vulnerable to surface contamination and poorly placed ablation location than complete ablation transects, potentially resulting in a higher sample discard rate (A. Sadekov, *pers. comm.*). In addition, trace element distribution is highly heterogeneous, even within a single, continuous growth band (Limburg and Elfman 2017). As a result, the statistical power and reliability of a single datapoint will be lower than that achieved using the hundreds of datapoints generated using ablation transects; a minimum of at least three replicate spots per otolith are therefore recommended for statistical power and accuracy (Di Franco et al. 2014).

The cost of running ablation transects to produce complete microchemical profiles can be reduced by increasing the speed of the ablation track, and/or by reducing the molecular weight range of the target elements. These approaches are unlikely to significantly affect ablation data quality and statistical power in the current study system, given the distinctive microchemical differences observed between hatchery-origin and wild-origin fish using a relatively small number of trace elements.

As a valuable bonus, otolith microchemical profiles provide a lifetime record of fish movement history, and in this study allowed us to determine that extended periods of juvenile freshwater residency are common in Barramundi (33% of the commercial catch). For these reasons, we recommend collection of complete otolith microchemical profiles as a monitoring and assessment tool to identify and inform management of the contributions of juvenile freshwater residency to the wild-capture Barramundi fishery in this and other regions. The complete otolith microchemical profile approach can also be applied to the historic otolith collection to quantify changes to the fishery over time (e.g. changes in patterns of juvenile freshwater residency, potentially associated with habitat modification). Otolith microchemical profiles can likely be used in other species with variable habitat needs (e.g. mullet, *Mugil* spp.) to assess effectiveness of fishways and/or habitat remediation works.

Other cost-saving laser ablation approaches are available, such as the laser "depth-profiling" approach used by Macdonald et al. (2008) to collect trace element concentration data from whole otoliths. This approach dramatically reduces sample processing time and associated expenses (no need for otolith blocking or sectioning, Table 3). To date, the depth-profiling method has only been successfully applied to very small otoliths (age-1 Australian smelt, *Retropinna semoni*, Macdonald et al. 2008; multiple species up to a depth of 140 µm, Warburton et al. 2016), but has the potential to be adapted for larger otoliths. This would involve using a very large laser spot (e.g. 250 µm) to "excavate" material from the whole otolith surface until the otolith core is exposed. A high-resolution microchemical profile can then be collected using laser ablation of the exposed otolith core (A. Sadekov, *pers. comm*.). This modified depth-profiling approach could be extremely cost-effective for provenance determination. Note, the matching otolith from each sample fish will still need to be sectioned if fish age estimation is required.

Genetic approaches to provenance determination are relatively cost effective, even at scale (Table 15, Table 16, Table 17). However, we have established that microsatellite parentage analysis is not suitable in this region due to likely introgression of hatchery genotypes into the wild population, and have yet to test the use of SNPs as an alternative in this system. We recommend a pilot study to confirm the efficacy of SNPs for provenance determination prior to wider implementation. Use of SNPs may also opportunistically provide a

close-kin mark-recapture index from which to infer population demographic information such as abundance and mortality rates (Bravington et al. 2016).

Conclusions

We conclude that:

(1) NIRS of whole and sectioned otoliths was not suitable for provenance determination in Barramundi using the available dataset.
(2) Otolith microchemistry was highly accurate for provenance determination (>98%), and also provided information on patterns of fish movement and habitat use (i.e. juvenile freshwater residency in 33% of commercially-caught Barramundi). The otolith microchemical profiles relating to fish provenance and movement history were clear and likely to hold true across future sampling regions and years. Microchemical approaches are relatively expensive and are best suited to use on small or subsampled datasets.
(3) Microsatellite parentage analysis was not suitable for provenance determination in this system, and instead detected both stocked fish and fish with likely introgressed hatchery ancestry (combined total of 21%). While parentage analysis using microsatellites has been highly successful in prior studies in Barramundi, the extensive stocking history and resulting opportunity for introgression in this region have reduced the discriminatory power of microsatellite-based methods. Alternative genetic approaches such as SNPs may be more effective for future provenance detection work.
(4) The wild-capture marine and estuarine Barramundi fishery in the Dry Tropics region is primarily composed of wild-born fish (>95%). Although Barramundi stocking has minimal numerical contribution to the wild-capture fishery (3%), it is critical to establishing (e.g. Ross Dam) and maintaining (e.g. Burdekin Dam) significant recreational impoundment fisheries which otherwise would not exist.

Implications

(1) The wild-capture marine and estuarine Barramundi fishery in the Dry Tropics region is primarily composed of wild-born fish. This suggests that biomass estimates in the recent stock assessment reflect a predominantly wild-born stock. The low contribution of stocked fish to the wild-capture fishery indicates that future East Coast Barramundi stock assessments are unlikely to significantly benefit from incorporating fish stocking parameters for the Dry Tropics and other regions with similarly high levels of natural recruitment.
(2) Stocked fish represent 3% of the Barramundi fishery, but hatchery ancestry was detected in 21% of the catch, indicating that stocked fish successfully breed and contribute genetic material to subsequent generations. These data suggest that stocked fish provide an enduring contribution to the wild population. The strong representation of hatchery ancestry among the wild-born population highlights the importance of genetic policies for fish stocking to support local genetic diversity and evolutionary traits.
(3) Juvenile access to freshwater habitats is a major driver of the Barramundi fishery (33% by number, 39% by weight). Sustainability of the fishery will require continued juvenile fish access to suitable freshwater habitats into the future. Productivity of the fishery may be enhanced by increasing juvenile fish access to suitable freshwater habitats (e.g. fishways, habitat remediation).
(4) Otolith microchemistry appears to be the most accurate and reliable method for provenance determination in Barramundi, with high potential for use in other regions. It is cost-effective to implement in new regions, and is reasonably priced for implementation as a routine monitoring tool, particularly if applied to representative sample subsets. Collection of complete otolith microchemical profiles for provenance determination also allows for opportunistic estimation of fish movement history data (e.g. juvenile freshwater residency) which otherwise would not be available.

Recommendations

(1) We recommend the use of cross-sectional otolith microchemical profiles for routine monitoring of fish provenance and juvenile habitat use in the Barramundi fishery, using representative sample subsets. This will clarify whether the contributions of stocked fish (3%) and juvenile freshwater residency (33%) identified in the current study are consistent through time. Such monitoring would provide early indications of changes in population dynamics (e.g. increased proportion of stocked fish indicating failure of wild recruitment; reduced proportion of juvenile freshwater residents indicating reduced juvenile habitat availability) and fishable biomass. Targeted application of otolith microchemistry could occur in other regions where stakeholders have expressed concern regarding the contribution of stocked fish to the fishery and/or limitations on juvenile habitat availability.

(2) Although Barramundi stocking has minimal contribution to the wild-capture marine and estuarine fishery, it is critical to establishing (e.g. Ross Dam) and maintaining (e.g. Burdekin Dam) significant recreational impoundment fisheries which otherwise would not exist. As a result, Barramundi stocking may indirectly support wild-capture fisheries by shifting recreational fishing effort away from estuaries and marine environments. Quantifying spatial and temporal changes in recreational fishing effort would be a valuable means of assessing a potential indirect impact of fish stocking on downstream wild-capture fisheries resulting from behavioural and effort shifts.

(3) Consideration should be given to the genetic composition of stocked fish, particularly when stocking into impoundments from which significant numbers of individuals can escape and eventually interbreed with the wild population.
    (a) In order to limit inbreeding accumulation, we recommend stocked fish originate from at least 50 broodstock over a period of 5 years. An effective population size (Ne) of 50 broodstock is recommended to keep levels of inbreeding accumulation < 1%, which is the commonly-accepted threshold for breeding and conservation purposes. Given the generation interval of Barramundi is 4 to 5 years, then this Ne should be achieved over 5 years and maintained in stocked progeny to ensure that sufficient genetic diversity is being introduced into the stocked population.
    (b) In order to conserve local adaptive traits, we recommend that only wild-collected broodstock should be used. We advise that broodstock that have been selected for aquaculture traits should not be used for stocking into impoundments from which significant numbers of individuals can escape and eventually interbreed with the wild population.

(4) Juvenile freshwater residency is a major driver of the Barramundi fishery, but is highly variable through time (i.e. between year classes) and does not seem to correlate to wet season severity. The mechanisms driving annual variation in juvenile Barramundi freshwater residency merit further investigation, as they appear to be much stronger drivers of recruitment to the fishery than Barramundi stocking in this region.

(5) We recommend management policies, as well as incentives for on-ground organisations and landholders to increase suitable freshwater habitat availability and accessibility for juvenile fish, which will contribute to the sustainability of the Barramundi fishery and can potentially be used to increase fishery biomass in this region.

(6) We recommend implementation of a pilot study on Barramundi provenance determination using low-density SNP panels (200-500 SNPs) from both tissue samples and archival otoliths. This will confirm whether SNPs are a suitable tool for high-resolution parentage analysis that can be rapidly deployed as a monitoring tool following events such as dam overtopping, large farm escape events, etc. Use of a high-resolution genetic parentage tool such as SNPs should provide much greater confidence than microsatellites can for identifying wild vs hatchery-born individuals in instances where genetic introgression may be occurring.

Further development

(1) Otolith microchemical profiles may be for useful for determining both provenance and movement history in other stocked species, particularly those that access a range of different habitats at different

life stages (e.g. mullet). Stakeholder interest in stocking a diverse recreational fishing assemblage in Queensland impoundments is growing. Ideally, provenance determination methods such as otolith microchemistry should be established and tested for all stocked finfish prior to the start of large-scale stocking activities. For some species, this may be best achieved by batch microchemical marking of fingerlings prior to stocking (reviewed in Warren-Myers et al. 2018).

(2) Fish habitat availability and accessibility may be a significant driver of recruitment and subsequent biomass patterns in the Barramundi fishery, as well as for other species with similar life histories. We recommend more detailed investigation into the environmental conditions favourable to fish migration. This information can subsequently be used to set thresholds for the volume and timing of freshwater extraction for human use (e.g. agriculture) and for releases of environmental flows in this region.

Extension and Adoption

The project was and continues to be extended and communicated to end users, including fish stocking community groups, the wider recreational fishing community, Barramundi aquaculture facilities, fishery managers, stock assessment scientists, fishery working groups, and the wider fisheries science community.

Extension to fish stocking groups

- Dr J Robins presented the project objectives and plan to attendees at the Queensland Freshwater Fishing & Fish Stocking Workshop in Warwick in Nov 2018.
- Drs S Leahy and J Robins presented project progress to the Townsville Barramundi Restocking Group and the Burdekin Fish Restocking Association at their respective AGMs in Sep 2019.
- Dr S Leahy presented project progress and preliminary results to the Townsville Barramundi Restocking Group and the Burdekin Fish Restocking Association at their respective AGMs, and to the Cungulla Fishing Club at a dedicated event, in Sep 2020.
- Dr S Leahy presented project results and conclusions to the Townsville Barramundi Restocking Group and the Burdekin Fish Restocking Association at their respective AGMs, and to the Cungulla Fishing Club at a dedicated event, in Sep 2021.
- Dr S Leahy presented project results, conclusions, and implications at the Lower Burdekin Wetland Subcommittee meeting in Oct 2021, to attendees of the Queensland Freshwater Fisheries Working Group in Oct 2021, and to attendees of the East Coast Inshore Fishery Working Group in Dec 2021.

Extension to the recreational fishing community

- A start-of-project social media post (Appendix 5. Start-of-project social media post) was released via Fisheries Queensland's Facebook page on 25 Feb 2019 to notify stakeholders of the project's objectives and on-ground activities. The post received 189 "likes", 34 comments, and was shared 37 times, including a share by the owner and manager of a participating aquaculture facility.
- A start-of-project factsheet (Appendix 6. Start-of-project factsheet) was prepared and distributed on-ground to interested recreational fishers in the Townsville and Ayr regions between February and October 2019, and was made available at the About Town Bait & Tackle shop in Ayr.
- An end-of-project factsheet (Appendix 7. End-of-project factsheet) was prepared and distributed on-ground to all project participants and volunteers in Sep 2021.
- An end-of-project social media post is being prepared for release on Fisheries Queensland's Facebook page.

Extension to Barramundi aquaculture facilities

- Dr S Leahy arranged in-person meetings with the managers of each of the participating aquaculture facilities in Feb 2019, and has maintained regular contact with them throughout the project.
- Throughout the project, confidential water chemistry results from each facility were immediately made available to the relevant facility's manager.
- Dr S Leahy provided a digital project progress report to each facility manager in Sep 2019 and Sept 2020, and an in-person progress report in Sep 2021.
- A copy of the accepted FRDC final report will be circulated to each facility manager.

Extension to resource managers

- Dr S Leahy presented project progress and preliminary results to Fisheries Queensland fishery managers and the North Region's Fishery Monitoring team in Oct 2020.
- Dr S Leahy presented project results, conclusions, and implications to Fisheries Queensland fishery managers and the North Region's Fishery Monitoring team in Oct 2021. A recording of this presentation has been circulated to water resource researchers across Queensland Government.
- Dr S Leahy presented project results, conclusions, and implications to the Lower Burdekin Wetland Subcommittee, which includes representatives from local Natural Resource Management groups and the Lower Burdekin Waterboard, in Oct 2021. This project's results validate recent extensive and ongoing work by the Subcommittee member organisations to increase accessibility and suitability of freshwater nursery habitats for juvenile Barramundi in the Burdekin region.
- A copy of the accepted FRDC final report will be circulated to Fisheries Queensland fishery managers.

Extension to the scientific research community

- Dr S Leahy presented the project methods, results, and preliminary conclusions to the international fisheries research community at the World Fisheries Congress in Sept 2021.
- Two scientific manuscripts are in preparation for submission to international journals:
    - Leahy et al, *Multi-method approach to advance provenance determination for stocked fish*, target journal: Canadian Journal of Fisheries and Aquatic Sciences
    - Leahy et al, *Impacts of stocking on recruitment variability in a wild-capture fishery*, target journal: Ecological Applications

Project coverage

- A draft end-of-project media release is being prepared by the DAF communications team, targeting the recreational fishing and scientific communities. It will be released to coincide with submission of the final report.

Project materials developed

The following project materials are included with this report:

- Start-of-project social media post, target audience: recreational fishing community (Appendix 5. Start-of-project social media post)
- Start-of-project factsheet, target audience: recreational fishing community (Appendix 6. Start-of-project factsheet)
- End-of-project factsheet, target audience: recreational fishing community (Appendix 7. End-of-project factsheet)

The following project materials are currently being drafted, and will be published to coincide with submission of the final report:

- End-of-project social media post, target audience: recreational fishing community
- Draft end-of-project DAF media release, target audience: recreational fishing community and scientific community

Appendix 1. Project staff

- Stephen Boyle, AIMS (retired)
- Megan Briede, Fisheries Technician, DAF
- John Cavallaro, Fisheries Technician, DAF
- Julie Goldsbury, Research Officer, JCU
- Peter Graham, Fisheries Technician, DAF
- Steve Grauf, Senior Technician, DAF
- Shirin Hyatt, Fisheries Technician, DAF
- Prof Dean Jerry, JCU
- Dr David Jones, Research Fellow, JCU
- Dr Susannah Leahy, Senior Fisheries Scientist, DAF
- Prof Malcolm McCulloch, UWA
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- Mark McLennan, Senior Fisheries Technician, DAF
- Luke Pavich, Technical Officer, DAF
- Dr Julie Robins, Principal Fisheries Scientist, DAF
- Dr Aleksey Sadekov, Research Fellow, UWA
- Tonia Sankey, Fisheries Technician, DAF
- Dr Richard Saunders, Fisheries Scientist, National Institute of Water and Atmospheric Research (NIWA), New Zealand
- Michelle Sellin, Fisheries Technician, DAF
- Dr Brett Wedding, Principal Scientist, DAF
- Olivia Whybird, Senior Fisheries Biologist, DAF
- Dr Samuel Williams, Senior Fisheries Biologist, DAF
- Dr Carole Wright, Senior Biometrician, DAF
- Jayden Zieth, Fisheries Biologist, DAF

Appendix 2. Intellectual property

No intellectual property has been generated by the project

Appendix 3. References

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Appendix 4. Pilot study

Methods

Otolith preparation

A pilot sample of 8 hatchery-origin, 18 wild-origin, and 4 unknown-origin otoliths (total N = 30 otoliths) were prepared for trial microchemical and NIRS analysis to identify ideal spectral settings, placement of ablation track, and target trace elements and isotopes. Subsampled otoliths were selected to capture both within- and among-site variation in microchemical conditions. To that end, the 8 hatchery-origin samples were composed of 2-3 otoliths from across three Barramundi farms, and the 18 wild-origin otoliths were composed of 1-2 otoliths from across 13 different collection locations.

For each sample, the left sagittal otolith was blocked in clear casting resin. The right otolith was blocked if the left otolith was damaged or unavailable. All plasticware used was washed with 10% nitric acid for 24 hours and rinsed with Milli-Q water (Millipore Corporation). Blocked otoliths were sectioned transversely through the core (i.e. primordium) using a low speed saw lubricated with Milli-Q water to produce two 300 µm thick sections exposing the otolith core. The section that best captured the otolith core ("primary section") was prepared for spectroscopy and microchemical analysis. The primary section was rinsed in analytical grade ethanol to remove surface contaminants potentially introduced by the saw. Primary sections were stored in acid washed plastic vials until mounting, and mounted on microscope slides using clear casting resin; air dried slides were stored in individual plastic bags. Secondary sections were prepared for age determination by mounting on microscope slides using clear casting resin and a glass coverslip.

NIRS data collection and analysis

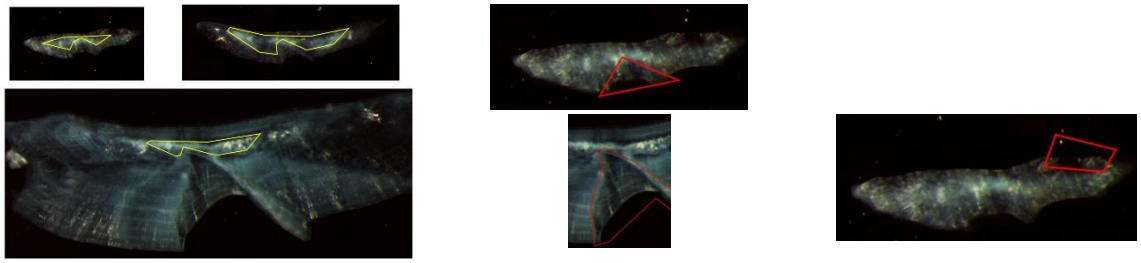
The pilot sample was used to assess NIR hyperspectral imaging systems on a small population of sectioned otoliths, encompassing juvenile samples of known origin (N = 8 hatchery-origin, N = 18 wild-origin) and adult otoliths from unknown origin (N = 4). Hyperspectral imaging captures a full image of the sample, providing a grid of pixels across the entire sample, resulting in both spectral and spatial information about the sample item simultaneously at each pixel of the sample image (the three-dimensional hyperspectral cube containing the data consists of two-dimensional spatial images with additional spectral information). Each spatial pixel contains the spectral information relating to the chemical attributes of substances at the corresponding spot (grid location) on the hyperspectral image, providing an opportunity for a detailed image analysis of the whole sample and detection of localised effects. The disadvantage of hyperspectral systems is the large accumulation of data which requires significant time for image acquisition and relatively complicated procedures for offline image analysis.

Two NIR hyperspectral camera systems were utilised to capture the spectral reflectance characteristics of the slide mounted otolith sections. The systems utilised were: (1) a Resonon Pika XC hyperspectral camera (Resonon Inc., USA) covering the spectral region of 400-1000 nm range; and (2) a Resonon NIR-320 hyperspectral camera (Resonon Inc., USA) covering the 900-1700 nm range.

Spectra were collected in diffuse reflectance mode using a single 100 watt halogen light source (as the incident NIR energy) mounted at 45° to the camera on a benchtop stage with motorised sample platform (Figure 5). Frame rate and integration times were adjusted to avoid light saturation, while maximising lighting to enhance spectral information. Spectral data was collected using the Spectronon Pro program Version 2.122 (Resonon Inc., USA) utilised by the Resonon camera.

Spectral data pixels from the otolith core were selected and spectrally averaged in the Spectronon Pro program Version 2.122 (Resonon Inc., USA) and then exported into the commercially available software package The Unscrambler Version X 10.3 and X 10.5 (CAMO, Oslo, Norway) for data analysis. Care was taken in the spectral data pixel selection process to avoid both the sulcus and otolith edge effects as these contain

materials from the end of the fish's life, and would contaminate the spectral signature of the start-of-life otolith core area (Appendix Figure 1).



(a) (b) (c)

Appendix Figure 1. *Example of (a) otolith core area selection; (b) avoiding sulcus and (c) edge effects.*

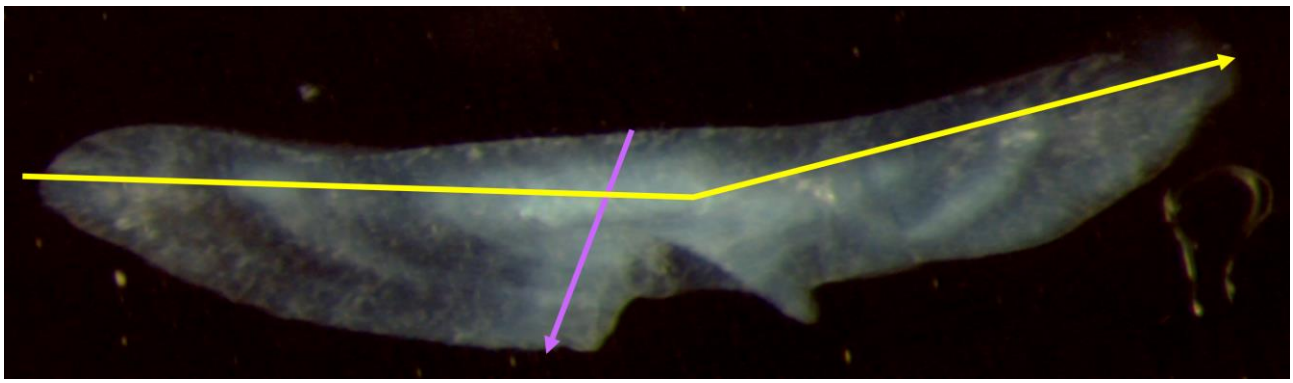
Qualitative classification was undertaken using principal components linear discriminant analysis (PC-LDA), together with various mathematical pre-processing methods, wavelength selection and outlier elimination to develop the predictive model. PC-LDA is a classification technique in which the number of groups and the samples that belong to each group are pre-defined (Otto, 1999, Naes et al., 2002). This technique produces a number of orthogonal linear discriminant functions that maximise the separation between the groups, yet minimises the variance within groups. To overcome the requirement of LDA that the number of samples in the calibration set is larger than the number of variables, the data dimensionality is reduced using principal component analysis (PCA) prior to running the LDA. Three separation methods within LDA were investigated: linear, quadratic and mahalanobis distance in combination with and without a standard deviation (sd) weighting process. Spectral pre-processing transformations were applied where necessary to enhance the spectral features and included Savitsky-Golay (SG) smoothing, SG first and second derivatives, multiplicative scatter correction (MSC) and standard normal variate (SNV).

The 26 known-origin otoliths were utilised in the classification model and were categorised into: hatchery-origin (n=8) and wild-origin (n=18). Model selection was based on obtaining a high percentage correct for both sample groups which may not necessarily correlate to the highest overall percentage correct. The high level of classification achieved on the pilot sample (88%) confirmed proof-of-concept.

Microchemical data collection and analysis

A pilot trial was used to assess different laser ablation transects and a wide range of trace element concentrations and isotopic ratios on a small population of sectioned otoliths, encompassing 26 samples from known-origin juvenile fish (8 hatchery-origin and 18 wild-origin juveniles) and 4 otoliths from unknown-origin legal size fish. The aim of the pilot trial was to identify the most appropriate laser ablation transect placement and most relevant trace elements and isotopic ratios to measure for all subsequent sectioned otoliths in this project.

Each otolith section was imaged dry under a microscope at 16x magnification. Recommended vertical and horizontal polyline transects for laser ablation were then identified for each otolith (Appendix Figure 2). The vertical ablation transect is standard for microchemical analysis of fish movement histories, but in some instances can miss the primordium, which contains important information on the microchemical environment experienced at the start of a fish's life. The horizontal ablation transect is significantly longer and therefore contains more data on the first year of a fish's life, but is also more expensive to ablate.



Appendix Figure 2. *Example hatchery-origin otolith section prepared for NIRS hyperspectral imaging and laser ablation. Includes recommended vertical (purple) and horizontal (yellow) polyline transects for laser ablation. Arrows indicate direction of ablation.*

Otolith sections were analysed for trace element composition and Strontium isotopes using LA-ICP-MS at the University of Western Australia (Perth, WA, Australia). An Analyte G2 laser ablation system was coupled with an ElementXR Sector-field ICP-MS for trace element measurements and with a Neptune Plus Multicollector ICP-MS for Strontium isotope measurements. Prepared otolith slides were ultrasonicated to remove any surface contaminants.

Ablation occurred in a Helium-flushed chamber mixed with argon and nitrogen. For each otolith, the horizontal and vertical polyline ablation transects were pre-cleaned three times using pre-ablation tracks with a spot size of 50-150 µm and a pulse rate of 12 Hz. Strontium isotopes were quantified first, followed by 19 trace elements and calcium (Appendix Table 1). Background measurements were collected for 60 seconds before and after each otolith ablation track, and standards were analysed every 30-50 minutes throughout each session to correct for any short-term instrument drift.

Strontium isotope composition was collected using a 25 µm by 100 µm rectangular laser "slit" with a speed of 10 $\mu m \cdot s^{-1}$, a pulse rate of 10 Hz, and a fluency of 2 $J \cdot cm^{-2}$. Strontium isotope data was calibrated against two in-house standards: e-Blue (low Strontium standard) and PAR (high Strontium standard). Strontium isotope external reproducibility was 0.705937 ± 0.000042 for the eBlue standard and 0.7130154 ± 0.0000076 for the PAR standard.

Trace element composition was collected using a 40 µm by 60 µm rectangular laser "slit" with a speed of 6 $\mu m \cdot s^{-1}$, a pulse rate of 10 Hz, and a fluency of 2 $J \cdot cm^{-2}$. Trace element data were calibrated against a National Institute of Standards (NIST) 614 standard for internal precision, a NIST 612 secondary standard for external precision, and an in-house UWAC carbonate standard. Mean relative standard deviation (RSD) for each trace element with each standard are reported in Appendix Table 1.

Data reduction was carried out using Iolite software (Paton et al. 2011). Strontium isotopic ratios are expressed as a ratio of Strontium-87 to Strontium-86 ($^{87}Sr/^{86}Sr$). Trace element concentrations are expressed as a ratio to calcium in $mmol \cdot mol^{-1}$.

Strontium isotopic ratio data were collected along both the horizontal and vertical ablation tracks for all 30 trial otoliths. Trace element concentration data was collected along only the horizontal ablation track for each of the 30 trial otoliths (Appendix Table 1). Trace element concentration data is expressed as a ratio to calcium to account for variable deposition rates of the calcium carbonate otolith structure, but is referred to by the trace element name only for convenience in this report.

Ablation data for each otolith, each microchemical variable (trace elements and strontium isotopes), and each ablation transect were visually inspected for errors and to identify any preliminary patterns. Patterning in the microchemistry of the 30 trial otoliths were examined using Principal Component Analysis (PCA) of otolith core microchemistry, first using the full set of microchemical markers, and then using a subset of most informative microchemical markers.

Preliminary classification of the 26 known-origin Barramundi was carried out using a Random Forest approach, which is a machine learning classification method. Random Forest approaches are increasingly used to classify otolith microchemistry datasets as they do not assume normal distribution of predictor variables among sampling units the way Linear Discriminant Analysis does, and cross validation is included in model development due to Random Forest's bootstrapping approach.

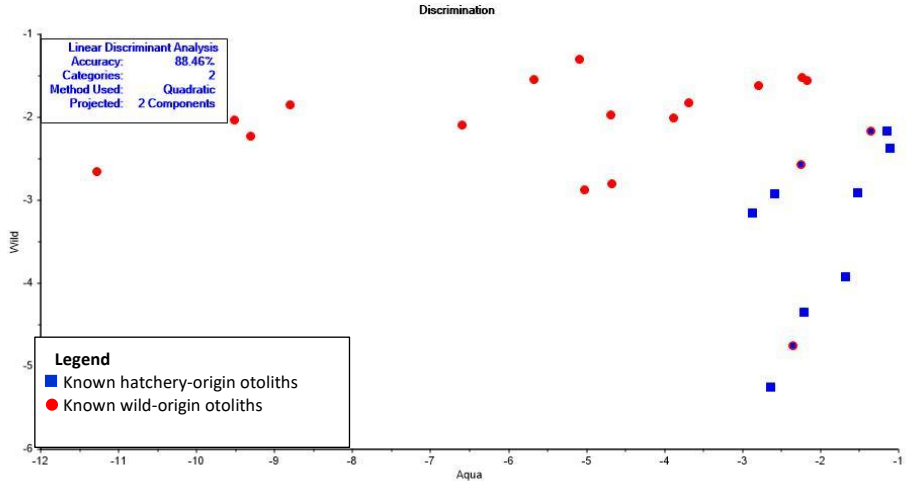
Appendix Table 1. *Microchemical variables (trace elements and isotopic ratios) measured for the 30 trial otoliths, including mean relative standard deviation (RSD) for each trace element against each standard.*

Microchemical variables	Ablation data location	Mean RSD for NIST 614 (internal precision)	Mean RSD for NIST 612 (external precision)	Mean RSD for UWAC
Aluminium (Al)	Horizontal track	0.1%	0.9%	33.5%
Barium (Ba)	Horizontal track	0.5%	1.2%	2.4%
Boron (B)	Horizontal track	0.5%	4.2%	4.7%
Cadmium (Cd)	Horizontal track	0.9%	2.9%	36.2%
Copper (Cu)	Horizontal track	0.8%	1.6%	13.7%
Iron (Fe)	Horizontal track	0.4%	1.8%	18.9%
Lead (Pb)	Horizontal track	0.2%	1.8%	13.7%
Lithium (Li)	Horizontal track	1.1%	1.2%	7.7%
Magnesium (Mg)	Horizontal track	0.5%	1.3%	4.1%
Manganese (Mn)	Horizontal track	0.3%	0.9%	10.3%
Nickel (Ni)	Horizontal track	0.5%	4.9%	12.2%
Phosphorus (P)	Horizontal track	0.2%	7.4%	15.6%
Potassium (K)	Horizontal track	0.2%	1.9%	10.4%
Rubidium (Rb)	Horizontal track	0.7%	2.5%	17.2%
Strontium (Sr)	Horizontal track	0.3%	0.8%	1.7%
Sulphur (S)	Horizontal track	3.7%	4.6%	10.5%
Uranium (U)	Horizontal track	0.2%	1.3%	2.6%
Yttrium (Y)	Horizontal track	0.3%	1.3%	14.9%
Zinc (Zn)	Horizontal track	0.9%	3.0%	29.4%
Strontium isotopes ($^{87}Sr/^{86}Sr$)	Horizontal and vertical tracks	N/A	N/A	N/A

Results and Conclusions

NIRS

A range of pre-processing methods and wavelength selections were trialled. From the preliminary analysis, PC-LDA using a pre-processing method of a 25-point Savitzky-Golay smoothing with a 25-point first derivative Savitzky-Golay produced the most promising classification model with the small data set. The model correctly classified 100% of the known hatchery-origin otoliths (8/8), and 83% of known wild-origin otoliths (15/18), with an overall correct classification of 88% utilising two principal components (Appendix Figure 3). These preliminary results demonstrate that NIRS has great potential as a rapid, objective, non-invasive predictive tool to estimate fish origin from sectioned otoliths.



Appendix Figure 3. *LDA plot of predicted fish origin, wild in red and hatchery in blue.*

Microchemistry

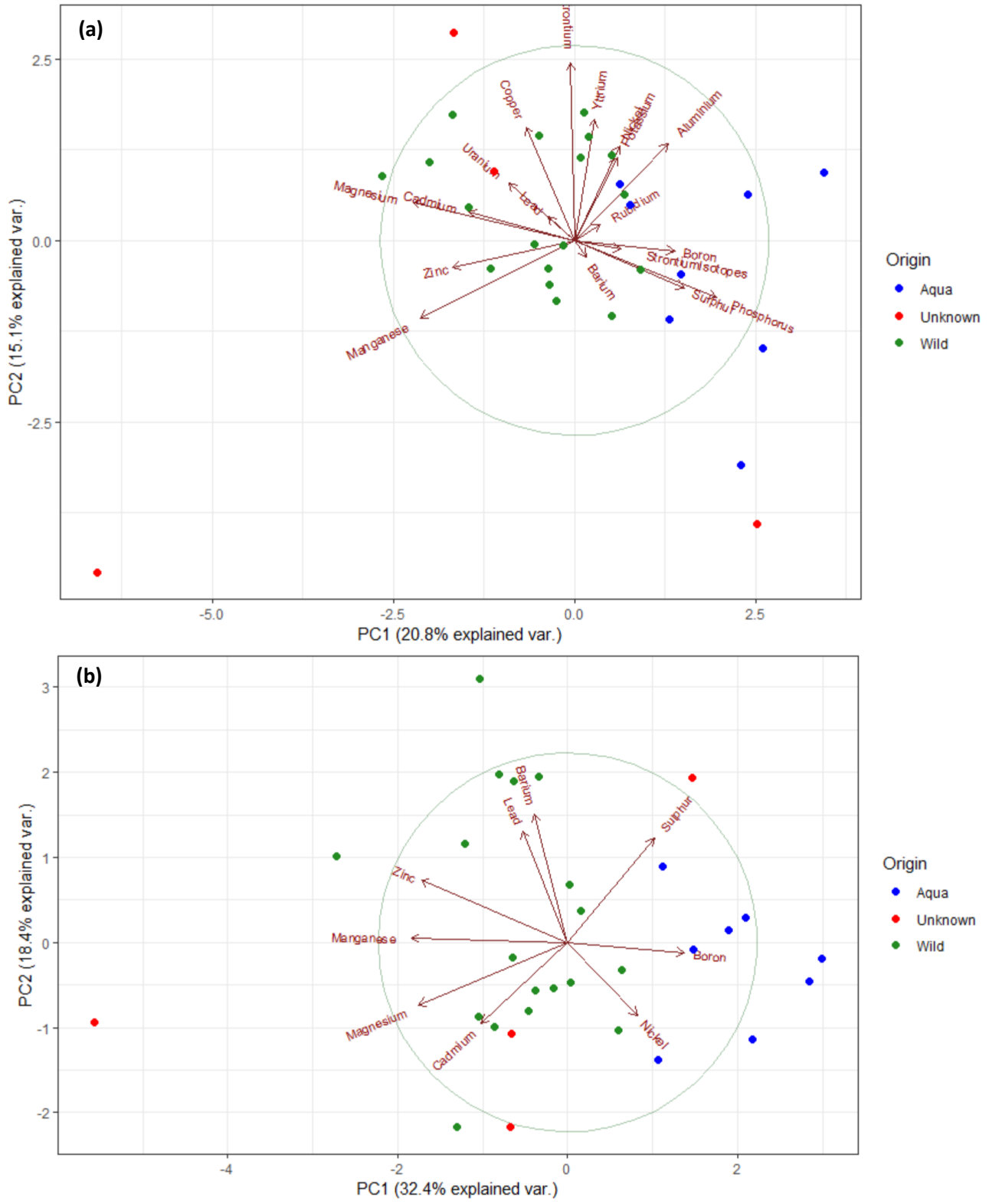
Microchemical analysis of the pilot sample of 30 otoliths identified the vertical edge-to-edge track as the ideal ablation transect, as it was equally likely as the horizontal transect to consistently ablate the otolith core area, and had the added benefit of capturing the complete life history of the fish, rather than just the first months of life. If used on a larger dataset, this can provide supplemental information on the timing of Barramundi movement from impoundments into the wild-capture fishery. In addition, a vertical ablation transect is considerably shorter than the horizontal transect, resulting in reduced ablation time and associated expense. For these reasons, all subsequent samples were ablated along the vertical transect.

The full set of trace element concentrations and strontium isotopic ratios was inspected for each fish in order to identify the otolith core location in the ablation timeseries data. The mean trace element concentrations and mean strontium isotopic ratios of the otolith core were then calculated for each individual and used in subsequent Principal Component Analysis (PCA) and Random Forest (RF) analyses. Iron and lithium concentration data were removed prior to PCA and RF analyses, as iron concentration is used to detect surface contamination of the otolith sample, and lithium detection can be inconsistent and vulnerable to processing error (A. Sadekov, *pers. comm.*).

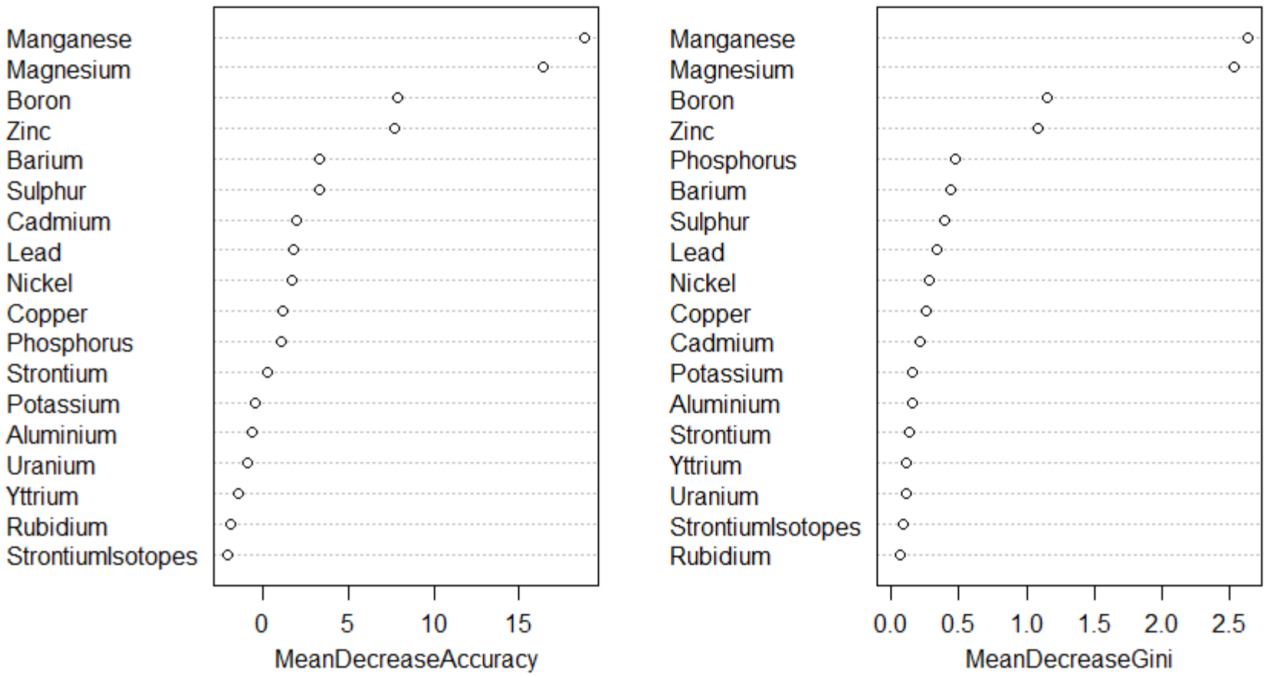
PCA was carried out on the mean concentrations of the remaining 17 trace elements and strontium isotopes in the otolith cores of the pilot samples (Appendix Figure 4). PCA clearly resolved hatchery-origin from wild-origin fish, and indicated that three of the unknown-origin samples grouped with the known-wild samples, and one of the unknown-origin samples grouped with the known-hatchery samples (Appendix Figure 4a).

A RF classification algorithm was subsequently applied to the 26 known-origin samples, and resulted in a mean prediction error rate (OOB estimate) of 4% (i.e. classification accuracy of 96%), with all 18 wild-origin fish correctly classified in every bootstrap replicate, and 7 out of 8 hatchery-origin fish correctly classified. Inspection of the ablation track of the one misclassified hatchery-origin fish indicated that that individual's ablation track did not capture the complete otolith core area.

Assessment of the cross-validated predicted performance of the model indicated that the RF model's error rate would be unchanged if the number of predictor variables was reduced from 18 (17 trace elements plus strontium isotopes) to nine. The RF model was therefore reapplied using only the nine most informative predictor variables (as identified by both the "mean decrease in accuracy" score and "mean decrease in the Gini impurity index", Appendix Figure 5). Separation of the samples in the PCA improved considerably when using this reduced number of predictor variables (Appendix Figure 4b). RF model output indicated that strontium isotopes did not significantly improve provenance determination for Barramundi. As a result, strontium isotopic ratios were not collected for subsequent samples in this project.



Appendix Figure 4. *Resolution of the core areas of each of the 30 trial otoliths using a principal component analysis (PCA) using (a) all trace element concentrations and isotopic ratio datasets (PC1 + PC2 = 36.1% explained variance), (b) a reduced list of microchemical variables resulting from the Random Forest simplification procedure (PC1 + PC2 = 50.8% explained variance).*



Appendix Figure 5. *Contribution of each predictor variable to the Random Forest classification model performance, as assessed by the "mean decrease in accuracy" score, and the "mean decrease in the Gini impurity index".*

Appendix 5. Start-of-project social media post

Appendix Figure 6. *Screenshot of the start-of-project social media post by Fisheries Queensland, including metrics of public engagement (comments, shares, and likes).*

Appendix 6. Start-of-project factsheet

Barramundi origins – FRDC Project 2018/047

The challenge

Since 2010, at least 4 million barramundi fingerlings have been released in Queensland. Whilst some perish, many survive and then migrate downstream when dams and weirs overtop during floods, and are subsequently caught in the wild-harvest fishery. It is unclear how much and how often these stocked fish contribute to the wild-harvest fishery, which makes it difficult to assess and manage the sustainability of the barramundi fishery.

The aim of this project is to develop a cheap and effective method to identify the contribution of stocked barramundi to current and historic catches to support assessment of the sustainability of the Queensland barramundi fishery.

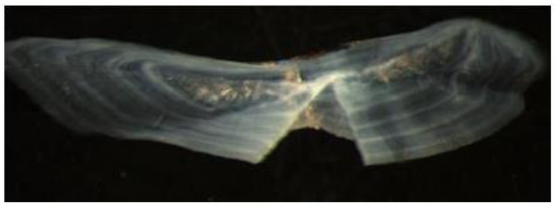
How will we achieve this?

The project will use the Dry Tropics area of Queensland near Townsville (Cattle Creek to Cape Upstart), as a case study to determine the most cost-effective way to identify the origins of barramundi.

The project will test the effectiveness of two different methods of identifying stocked versus wild barramundi: (1) otolith microchemistry, and (2) otolith spectroscopy using near infrared. Both approaches rely on the chemically stable core of fish otoliths, fish "ear stones" which grow like tree rings as the fish grows throughout its life. Otolith chemistry reflects the chemical conditions of a fish's environment throughout its life.

Otoliths are already collected from the commercial and recreational fisheries as part of routine monitoring of the barramundi fishery in Queensland. Results from the current project could therefore be applied to the long-term monitoring of the barramundi fishery to identify the historical and current contribution of stocked fish to the fishery.

The project will be carried out in estuaries and freshwater systems between Cattle Creek and Cape Upstart.



A sectioned barramundi otolith used for routine fish ageing, and used for otolith microchemistry and spectroscopy in this project.

Identification of barramundi origins during this project will be confirmed using DNA analyses. The project will run over the next two years.

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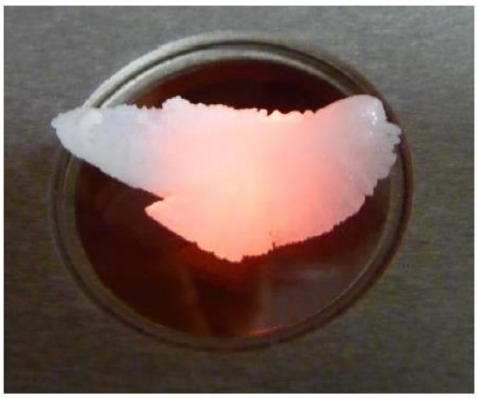
Appendix Figure 7. *Screenshot of the first page of the two page start-of-project factsheet distributed to stakeholders and interested members of the general public.*

DAF staff measuring barramundi and preparing to extract the otoliths.



A barramundi otolith being scanned with the near-infrared spectrometer.

Who is involved?

The project is a joint effort between stakeholders that have an interest in sustainable barramundi fishing. It is funded by the Fisheries Research and Development Corporation (FRDC) in collaboration with researchers from Queensland DAF and James Cook University.

The genetic work will be supervised by Dr Dean Jerry (James Cook University) and will include a DNA parentage analysis. The near infrared spectroscopy work will be supervised by Dr Brett Wedding (DAF) and is a novel application of this technique to determining fish providence. Dr Julie Robins (DAF) will supervise the otolith microchemistry work which relies on hatchery-origin fish experiencing different water chemistry and food sources to wild-origin fish.

How can you help?

The project will sample small wild barramundi (<401 mm total length) over the course of the 2019 and 2020 barramundi seasons with the assistance of local fishers under the supervision of DAF staff and with the necessary permits. Samples will also be collected from the commercial net fishery in collaboration with the Fisheries Queensland Monitoring program. Hatchery-spawned fish within the Dry Tropics area will also be sought with the assistance of local hatcheries and stocking groups such as the Townsville Barramundi Stocking Society Inc. and the Burdekin Fish Restocking Association.

Contact details

For further information contact:

- Dr Susannah Leahy (Fisheries Scientist) at or Susannah.Leahy@daf.qld.gov.au,

or

- Dr Julie Robins (Senior Fisheries Biologist) at or Julie.Robins@daf.qld.gov.au.

Small barramundi (less than 30 cm).

Appendix Figure 8. *Screenshot of the second page of the two page start-of-project factsheet distributed to stakeholders and interested members of the general public.*

Appendix 7. End-of-project factsheet

Barramundi origins – FRDC Project 2018/047

The challenge

More than 4 million barramundi fingerlings have been released in dams and weirs in Queensland since 2010. When impoundments overtop during floods, some of these stocked fish can move downstream into estuarine and marine environments. It is unclear how much and how often stocked barramundi contribute to the estuarine and marine catch, which makes it difficult to assess the sustainability of the barramundi fishery.

The goal of this project was to develop a cheap and effective method to identify the contribution of stocked fish to current and historic barramundi catches. This will inform assessment of the sustainability of the Queensland barramundi fishery.

What did we do?

We tested the effectiveness of three different methods of identifying stocked versus wild barramundi:

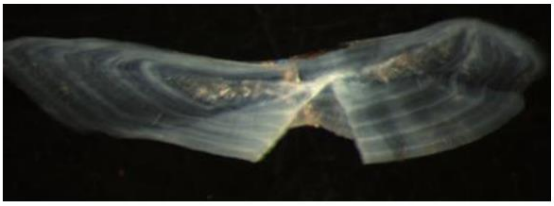
(1) otolith microchemistry,
(2) otolith hyperspectral imaging, and
(3) genetic analysis.

Otoliths are fish ear bones which grow like tree rings as a fish grows older. Otolith microchemistry reflects the environments a fish has lived in throughout its life.

Fish samples were provided by participating barramundi aquaculture facilities, and juvenile fish were collected from the wild by 30 keen volunteers registered with the project.

Otoliths were also collected from the commercial and recreational fisheries as part of routine monitoring of the barramundi fishery in Queensland. Microchemical "fingerprints" developed using the known-origin juvenile barramundi were applied to the 2019 monitoring collection of commercially-caught barramundi.

The project was carried out in estuaries and freshwater systems between Cattle Creek and Cape Upstart.



A sectioned barramundi otolith used for routine fish ageing, and used for otolith microchemistry in this project.

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THE UNIVERSITY OF WESTERN AUSTRALIA
FRDC
Queensland Government

Appendix Figure 9. *Screenshot of the first page of the two page end-of-project factsheet distributed to stakeholders and interested members of the general public.*

DAF staff measuring barramundi and preparing to extract the otoliths.

What did we find?

We found distinct and consistent patterns in the otolith microchemistry of fish born on barramundi farms compared to wild fish. The otolith microchemical fingerprints allowed us to correctly classify fish as farm-born or wild-born with 98% accuracy. The otolith hyperspectral method was not successful in this instance.

We used the high-accuracy otolith microchemical information to calculate that 4% of the 2019 barramundi catch in the Dry Tropics (Townsville and Burdekin regions) was composed of stocked fish that had moved downstream. This indicates that the barramundi fishery in this region is primarily catching wild-born fish.

The genetic analysis indicated that 22% of the 2019 barramundi catch carried genes from stocked fish. This confirms that stocked fish successfully breed and contribute offspring to the wild population. The genetic contribution of stocked barramundi to the fishery highlights the importance of responsible fish stocking to support local genetic diversity and evolutionary traits.

Otolith microchemical data also indicated that at least 39% of the 2019 barramundi catch had spent their juvenile years in freshwater environments such as lagoons or weirs, regardless of whether they were stocked or wild-born. Barramundi that spent their juvenile years in freshwater environments grew significantly faster than those that remained in estuaries.

Who was involved?

The project was a joint effort between stakeholders that have an interest in barramundi. It was co-funded by the Fisheries Research and Development Corporation (FRDC) in collaboration with researchers from Queensland's Department of Agriculture and Fisheries (DAF), James Cook University, and the University of Western Australia.

Juvenile barramundi (less than 30 cm).

The genetic work was led by Dr Dean Jerry (James Cook University). Hyperspectral work was led by Dr Brett Wedding (DAF). Dr Susannah Leahy (DAF) led the otolith microchemistry work.

The project leaders are especially thankful for the support provided by members of the Townsville Barramundi Restocking Group and the Burdekin Fish Restocking Association, as well as the barramundi aquaculture facilities that provided samples for the project.

Where can I get more information?

The final report for this project will be available online at www.frdc.com.au/project/2018-047 from March 2022.

For more information contact Dr Susannah Leahy (Fisheries Scientist) on or Susannah.Leahy@daf.qld.gov.au

Appendix Figure 10. *Screenshot of the second page of the two page end-of-project factsheet distributed to stakeholders and interested members of the general public.*