

**Influence of freshwater flows on recruitment and growth of Barramundi and Giant mud crab in the Northern Territory: a biochronological approach**



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**February 2021**

**FRDC Project No 2015/012**

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FRDC Project No.: 2015-012

2021

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# Acknowledgments

This research was funded by the Fisheries Research and Development Corporation (FRDC), Project 2015/012, with support from Charles Darwin University (CDU) and the Northern Territory Department of Primary Industries and Resources (DPIR). The authors gratefully acknowledge FRDC research portfolio managers Nicole Stubing and Skye Barrett and the Northern Territory Research Advisory Committee for their ongoing support and interest in the project. We thank Chris Izzo for constructive and insightful comments on earlier versions of the report and Leah Fergusson for administrative support. Staff from DPIR (Fisheries Division) played an instrumental role in the project, particularly with regards to the collection, dissection and preparation of fish otolith and crab ossicle samples. Among the many contributors from DPIR, we are especially grateful to Bryan MacDonald, Quentin Allsop, Wayne Baldwin, Nathan Crofts, Chris Errity, Sean Fitzpatrick, Kurtly Harvey and Graham Schultz. We thank staff from the NT Department of Environment and Natural Resources (Water Resources Division) including Tim Bond, Simon Cruickshank, Peter Dostine, Bridie Velik-Lord, Mardi Miles and Michelle Rodrigo, for their insights into water management in the NT and for assistance with the development of water abstraction scenarios for the barramundi modelling. Staff and students at CDU's Research Institute for the Environment and Livelihoods provided productive discussions and feedback on the project, and we thank Roanne Ramsey and Tahlia Timms for CDU for their invaluable administrative support. Finally, we gratefully acknowledge the large number of commercial and recreational fishers who contributed fish and crab samples used in our analyses.

# Executive Summary

The research presented in this report was led by Charles Darwin University and conducted in collaboration with the University of Melbourne and the Fisheries Division of the Northern Territory (NT) Department of Primary Industries and Resources (DPIR). A novel statistical modelling framework based on age and growth data from otolith analyses was used to examine relationships between recruitment and growth of Barramundi and a range of climatic and river hydrology variables in four rivers in the NT. We found strong evidence that the magnitude of Barramundi recruitment was linked to the strength of the Australian Monsoon Index. This relationship offers potential to predict future fisheries yields three years in advance based on observed meteorological phenomena. We used river-specific hydrology analyses to predict the effects of water abstraction on Barramundi recruitment and growth. Scenario analyses predicted strong negative effects of water abstraction (>30% in several scenarios) on Barramundi recruitment, with the predicted effects variable among river systems. Results of the analyses were communicated to DPIR and the NT Department of Environment and Natural Resources (DENR) via a series of presentations to promote their use in future fisheries management and water resource planning.

The second component of the report describes an evaluation of the utility of gastric ossicles (calcified mouthparts) for direct age estimation in the Giant mud crab. Originally, we intended to use growth increments in gastric ossicles using the methods of Leland and Bucher (2017; FRDC 2014/011) to undertake analyses similar to those described above for Barramundi. However, we identified significant issues in the early stages of the project which raised concerns regarding the technique's suitability for Giant mud crab and, potentially, other species of crustacean. In light of these concerns, the emphasis of this project component was shifted towards a comprehensive assessment of the use of gastric ossicles for direct ageing of Giant mud crab. Based on this assessment, we conclude that considerable further research on method validation is required before direct ageing of crustaceans is adopted in fishery research and monitoring. In the meantime, we caution against the use of this age estimation technique for crustaceans.

## Background

The development and future expansion of northern Australia's economic potential is firmly on the agenda of Federal and State/Territory governments. Fresh water is both an enabler and a constraint to future economic development, and water management decisions need to balance a range of economic, environmental and social/cultural interests. There is considerable interest in expanding both agricultural and mining activities in Northern Australia, which will require additional water abstraction and storage infrastructure. Whilst such developments provide opportunities for these industries, the consequent increase in water use will alter river flows and may impact riverine and estuarine ecosystems and the fisheries (and other values) they support. The aims of this project were to employ biochronological analyses (i.e., analyses of calcified structures) to improve our understanding of relationships between fishery productivity and river hydrology, and to predict the likely effects of increased water abstraction on fisheries in northern Australia.

## Aims/objectives

### *Barramundi*

We used otolith (fish earstone) increment analyses to explore correlations between Barramundi recruitment and growth and a range of environmental variables (including key aspects of climate and river hydrology). These detailed analyses identified environmental factors that were most strongly correlated with recruitment and growth and explored their interactions using an integrative modelling approach. The information generated from these analyses was then applied to model the outcomes of different water management scenarios. The results were provided to water managers to support decisions relating to water resource development in the NT.

### *Giant mud crab*

We conducted a comprehensive assessment of the use of sectioned gastric ossicles for direct ageing in Giant mud crab. Specifically, we tested whether putative annual age estimates from gastric ossicles can be reliably reproduced, if age estimates are compatible with previous studies, and if ossicle growth is cumulative across ontogeny. Based on these analyses, we present recommendations regarding the suitability of gastric ossicles as a means of ageing crustaceans in fishery research and monitoring.

## Methodology

### *Barramundi*

Sectioned otoliths from 3,586 Barramundi collected from the Daly, Mary, Roper and McArthur rivers in the NT were used to estimate age and measure annual increment widths. Age data were used to back-calculate the year of birth for each fish in order to calculate year class strength (YCS). Otolith increment widths were used to estimate year-specific growth rates for each fish. Time series of YCS and growth were estimated using a generalised linear mixed model (GLMM) formulation of catch curve regression. A two-stage process was adopted for the YCS and growth analyses that consisted of a regional model, which combined data from the four rivers, and river-specific models that treated the data for each river separately. We used the outputs of the river-specific YCS models to explore the potential impacts of flow abstraction on Barramundi recruitment and growth in the Daly River and Roper River by conducting water abstraction scenario models for three hydrologically distinct (dry, average, wet) years.

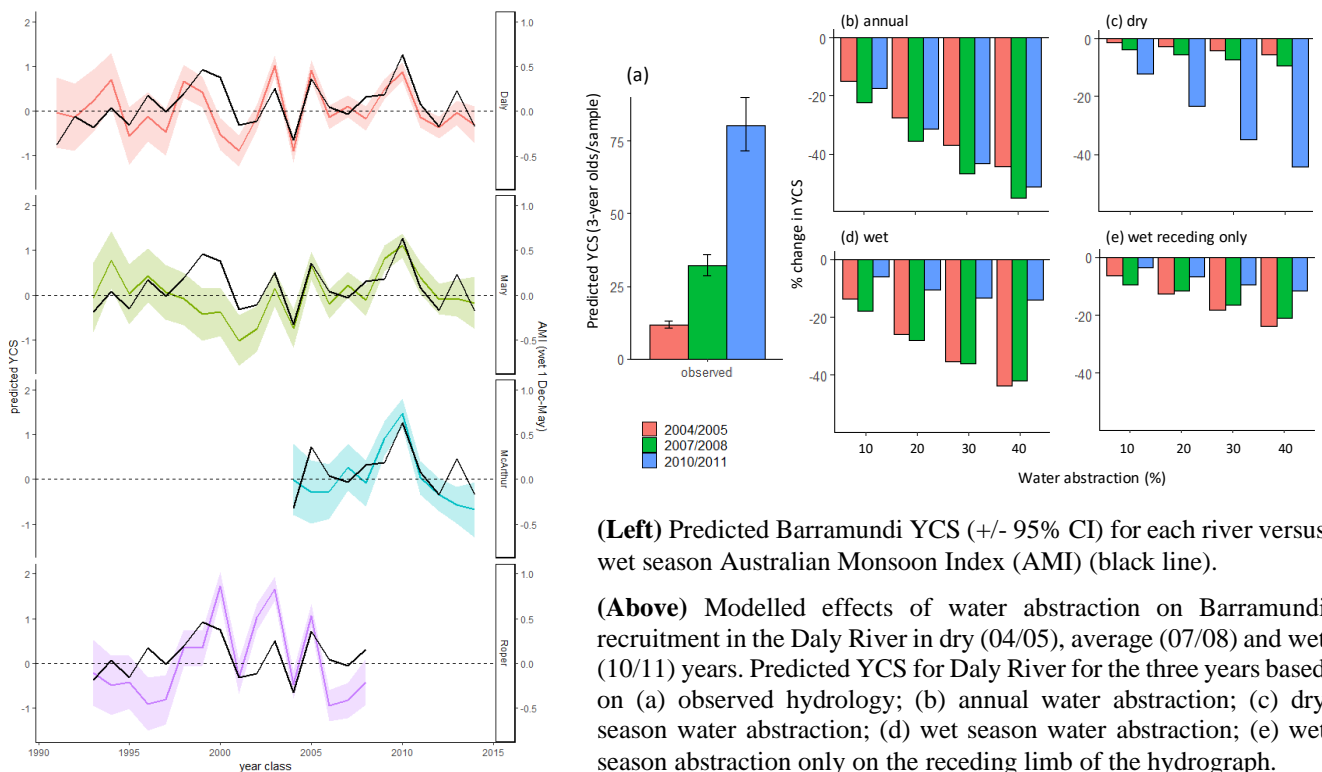
### *Giant mud crab*

Zygocardiac ossicles were removed from the gastric mills of 210 Giant mud crab sourced from commercial fishers from the Adelaide, Roper and McArthur rivers. The ossicles were sectioned and examined using a stereo microscope. Age was estimated by counting the number of pairs of opaque and translucent bands using the methods of Leland and Bucher (2017; FRDC project 2014/011). Samples were analysed to examine the consistency of increment counts along the length of the ossicle and a series of analyses conducted to examine various aspects of ossicle growth morphology. Based on these analyses, a model of ossicle growth was developed and used to identify and explore issues associated with the use of ossicles as a direct ageing technique for crustaceans.

## Results/key findings

### *Barramundi*

Year class strength (YCS) was strongly correlated with the Australian Monsoon Index (a measure of wet season strength) across all rivers in the regional analysis. River-specific analyses demonstrated strong relationships between YCS/growth and both climate and hydrology. However, these relationships were complex and variable among rivers. Scenario modelling predicted that high levels of water abstraction during both the wet and dry seasons would have strong impacts on YCS in the Daly and Roper rivers.





### *Giant mud crab*

Important issues regarding the suitability of using sectioned gastric ossicles for ageing Giant mud crab were identified, including: 1) generally poor readability; 2) potential for systematic age underestimation; 3) minor variations in sample preparation likely to affect age estimates; and 4) evidence that ossicles are shed during the moult cycle or extensively reworked across ontogeny. Based on this evidence, we conclude that counts of bands in gastric ossicles do not provide reliable age estimates for Giant mud crab.

### **Implications for stakeholders**

#### *Barramundi*

A key finding of the project was the strong correlation between YCS and wet season Australian Monsoon Index across all four rivers. Identification of a single climatic index that can predict YCS at the regional scale has the potential to facilitate forecasting of harvestable biomass over a 3-year horizon (i.e., the time taken for recruits to enter the fishery). Incorporation of this index into fishery models could improve the efficacy and timeliness of management for NT Barramundi fisheries and may also have applications for other fisheries and jurisdictions in tropical Australia. Our finding that a high proportion of variance in YCS can be explained without reference to spawning stock size also raises important questions regarding the use of stock-recruitment functions for modelling Barramundi populations.

Water resource managers were consulted during the development of the water abstraction scenarios to ensure their relevance to current and future decision-making processes. The model outputs showed a strong effect of water abstraction on Barramundi YCS and these results were communicated to managers via a series of meetings and presentations. The project team aims to maintain ongoing dialogue with water managers to facilitate incorporation of the project outputs into future water resource planning.

#### *Giant mud crab*

Based on our analyses, we caution against the use of gastric ossicles for direct ageing of Giant mud crab and other crustaceans unless further research adequately addresses the issues raised in the current study.

### **Recommendations**

#### *Barramundi*

1. Our results provide empirical evidence of the detrimental effects that future water resource development may have on Barramundi fisheries. Consideration of this information is necessary to ensure that community expectations are met with regards to the sustainable development of northern Australia's water resources.
2. The models developed in this project have the potential to enhance the accuracy of stock assessment models for Barramundi by accounting for environmental recruitment drivers;
3. The utility of the Australian Monsoon Index as a forecasting tool for tropical fisheries should be explored for other fisheries in northern Australia.
4. Further research is required to develop our understanding of stock-recruitment relationships in Barramundi populations in order to refine population models.

#### *Giant mud crab*

1. The morphological growth characteristics and non-cumulative growth of gastric ossicles in Giant mud crab suggests that caution should be applied to direct age estimates based on gastric ossicle increment counts unless further detailed research validates the method.
2. Detailed research is needed regarding the physiological processes leading to band (putative growth increment) formation in Giant mud crab and other Australian crustaceans.
3. Any future examinations of the retention of calcified structures across the moult cycle should account for the potential reabsorption of fluorochrome dyes from moulted gastroliths and exuviae.

### **Keywords**

Barramundi, Giant mud crab, northern Australia, water management, flow abstraction, recruitment, year class strength, otoliths, gastric ossicles, ageing

# 1. Introduction

## 1.1 Background and Need

The development and future expansion of northern Australia's economic potential is firmly on the agenda of both Federal and State/Territory Governments (Australian Government 2015; NT Government 2017). Freshwater is often seen as both an enabler, and a constraint, to future economic development opportunities, with water management decisions required to balance economic, environmental and social interests (COAG 2004).

In northern Australia, there is much interest in developing water resources through water abstraction<sup>1</sup> and storage infrastructure to support growing agriculture and mining industries (CofA 2015). However, extracting water from these wet-dry tropical rivers can reduce the total discharge and affect the timing of flows, potentially impacting natural riverine and estuarine resources including fisheries (King *et al.* 2015, Warfe *et al.* 2011; Chan *et al.* 2012, Robins *et al.* 2005, 2006; Burford *et al.* 2010). Water resource development in the NT is increasing, with water allocations in some planning areas approaching or exceeding the 20% maximum sustainable yield limit recommended under the NT *Water Act 2016*.

River discharge is known to have an important influence on the productivity of estuaries and near-shore coastal waters, potentially affecting the production and catches of coastal fisheries (Loneragan and Bunn 1999, Robins *et al.* 2005; Meynecke *et al.* 2006). For example, Barramundi (e.g. Robins *et al.* 2005; Tanimoto *et al.* 2012) and Giant mud crab catches (Meynecke *et al.* 2010, 2012) may be positively related to hydrological metrics (e.g. total wet season flows), leading to predictions that the abundance of both species may be negatively impacted by increased water extraction (Tanimoto *et al.* 2012, Bayliss *et al.* 2008; 2014).

Previous studies suggest that the size of the predicted effect is dependent on both water abstraction levels and the hydrological characteristics of the system under consideration (Bayliss *et al.* 2014). However, the specific aspects of river hydrology driving these relationships remain unclear and it is therefore difficult to predict how specific alterations to flow management regimes will affect fisheries. Thus, despite the value of Barramundi and Giant mud crab fisheries to local and regional economies, our ability to provide confident predictions on the impacts of water abstraction to guide water management in northern Australia remains limited.

The current project was conceived based on concerns raised by NT Government water managers, fisheries managers and other stakeholders regarding the potential impacts of future water development on river flows and coastal fisheries in the NT. Although the broad relationship between river hydrology and Barramundi fisheries from previous research is well recognised, water resource managers identified an urgent need for more specific and quantitative information on the nature of these relationships to guide operational water management into the future.

The goal of the project is to meet this need by applying biochronological techniques to quantify the relationships between climate, river hydrology and the productivity of Barramundi and Giant mud crab fisheries in the Northern Territory. We developed quantitative models of these relationships using empirical data from four key river systems and then tested a range of water abstraction scenarios to explore their potential impacts on downstream fisheries. This approach has the potential to significantly improve understanding of the effect of water regime changes - including water resource development and climate change - on these fisheries and to contribute to informed decision-making about future water allocation in northern Australia.

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<sup>1</sup> The term 'abstraction' refers to the removal of water from the natural environment for human usage.

## 1.2 Application of Biochronological Analysis

The capacity to estimate the age of organisms plays a fundamental role in models used in the assessment and management of natural resources, including fisheries. Many plants and animals contain hard structures that have distinctive growth marks which can be counted and measured to derive estimates of age and growth (e.g. tree growth rings, otolith rings in fishes) (Stokes 1996; Crook and Gillanders 2013). A fundamental characteristic of these hard structures is that new material is added cumulatively to the structure as it grows, and this material is preserved through development in a way that allows the growth marks to be reliably observed and measured (Stokes 1996; Campana 2001). Biological information recorded in the hard parts of animals and plants over time is known as a “biochronology”.

Three main sources of information can be extracted from animal hard parts, including 1) life-history and demographic information from structural features such as growth-increment counts and widths; 2) environmental and physiological records from structural features and isotopic and elemental composition, and 3) genetic data from the organic fraction or attached tissue (Morrongiello *et al.* 2012). The aims of the current study are to apply a biochronological approach to analyse structural features (growth-increment counts and widths) in the otoliths of Barramundi *Lates calcarifer* and gastric ossicles of Giant mud crab *Scylla serrata* to examine the influence of environmental factors (primarily river hydrology) on the growth rates and age structure of populations of these species in the NT.

Several recent studies have used otolith biochronologies to address questions relating to fisheries and water resource management. In a study of Estuary perch *Percolates colonorum* in eastern Victoria, Morrongiello *et al.* (2014) used otolith microstructural analysis to model the effects of river hydrology and large-scale climatic variables on year class strength and growth (using otolith increment distances). Based on biochronologies that spanned 39 years for recruitment and 46 years for growth, they found that recruitment was strongly related to high freshwater inflows during the spawning season. Individual fish displayed age-dependent growth and highest rates were observed at younger ages in years characterized by warm temperatures, and to a lesser degree, greater magnitude freshwater flows into the estuary. Likewise, Barrow *et al.* (2017) showed how the growth of a commercially important fish was positively affected by freshwater inflows to coastal waters.

Biochronological analyses provide direct empirical information on fishery productivity in response to environmental change without some of the key assumptions that may apply to other modelling approaches (e.g., constant stock-recruitment relationships in population models e.g., Bayliss *et al.* 2008; Tanimoto *et al.* 2012). Combined with recent innovations in the statistical approaches used to analyse biochronological data (e.g., Morrongiello and Thresher 2015), biochronological analysis represents a powerful tool for developing our understanding of how environmental and intrinsic (within-population) factors influence fishery productivity.

## 1.3 Study Species

### 1.3.1 Barramundi (*Lates calcarifer*)

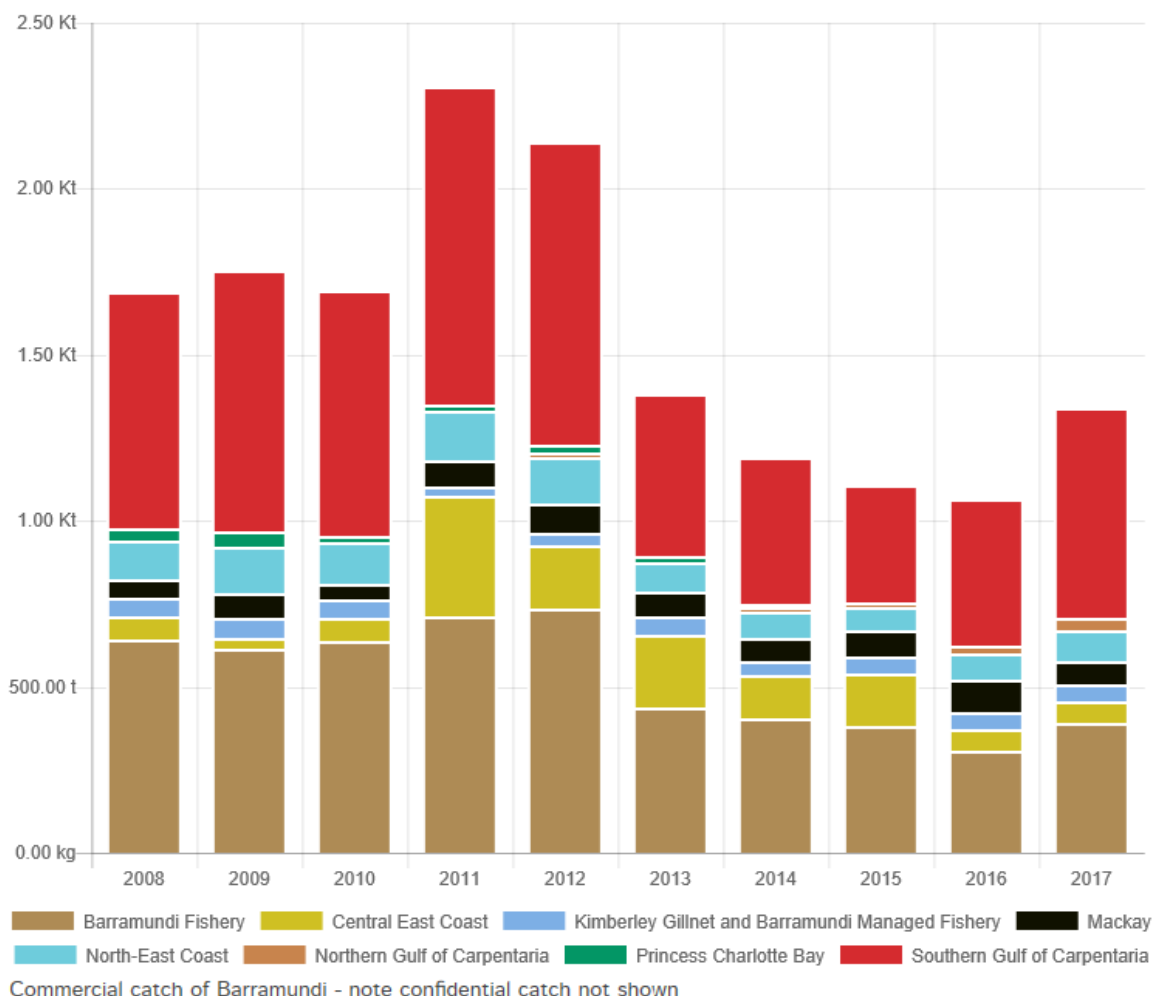
The Barramundi *Lates calcarifer* (Figure 1.1) is a large perciform fish that occurs in coastal marine and fresh waters throughout the Indo-West Pacific region, including northern Australia (Grey 1987).

The maximum recorded size of Barramundi is ~1.5 m and up to 35 years of age, although in Northern Territory rivers they reach ~1.3 m and live for up to ~18 years. Barramundi are a much-prized food fish and support major commercial and recreational fisheries, as well as a significant and growing aquaculture industry. Total landings of wild fish from commercial fisheries in Australia have ranged from ~1,100-2,200 tonnes pa from 2008-2017



Figure 1.1: Barramundi *Lates calcarifer*

(Figure 1.1). Barramundi are an important resource for Aboriginal people in Australia, with a total take of 110 tonnes recorded in 2000 in the NT (Saunders *et al.* 2016).

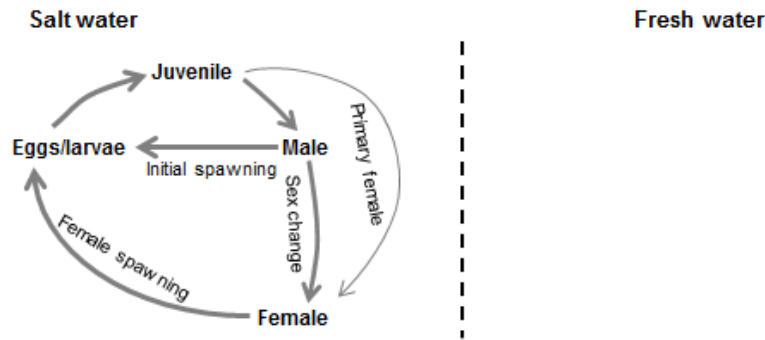


**Figure 1.2: Commercial Barramundi catch for 2008-17 in Australia. Reproduced from Saunders *et al.* (2018).**

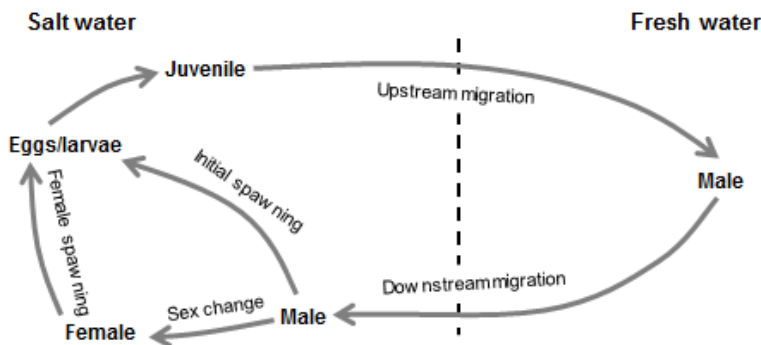
The biology of Barramundi is complex, with high levels of variation in life history characteristics occurring among individuals and populations (Davis 1987; Grey 1987; Crook *et al.* 2017). Barramundi are sequential, protandrous hermaphrodites, developing initially as males at 3–5 years of age and then into females at 4–8 years of age (Davis 1987). In Australia, Barramundi are generally considered catadromous<sup>2</sup> because spawning occurs in saline coastal waters and large numbers of juvenile fish have been observed migrating upstream into freshwater habitats (Moore and Reynolds 1982). However, more recent evidence has shown that there is considerable within-population variation in migration strategies, with a proportion of fish never entering freshwater and others inhabiting freshwater habitats for many years (Pender and Griffin 1996; Milton and Chenery 2005, Cappel *et al.* 2005). Based upon an integration of previous information on Barramundi biology, Crook *et al.* (2017) proposed a conceptual model that categorises Barramundi populations in Australia into three migratory contingents (Figure 1.3).

<sup>2</sup> Catadromous species migrate into freshwater as juveniles and mature in freshwater before migrating back to the marine environment to spawn. Catadromy is a form of ‘diadromy’, which is a broad term describing fish that migrate between freshwater and marine habitats at specific phases of the life history.

### 1) Estuarine



### 2) Catadromy, sequential hermaphroditism



### 3) Catadromy, delayed female spawning

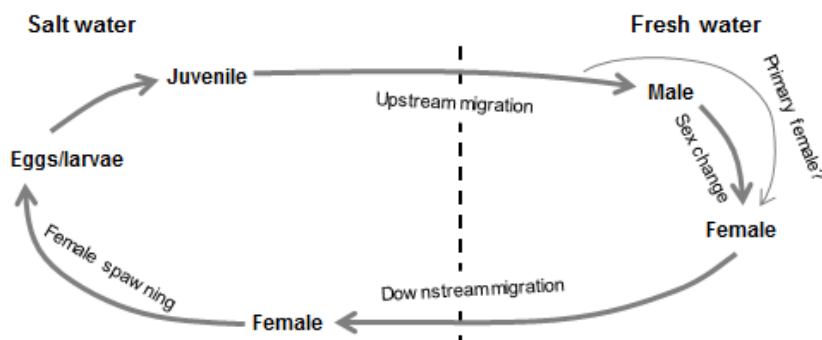


Figure 1.3: Schematic of migratory contingents in Barramundi. 1) The ‘estuarine’ contingent consists of non-diadromous individuals that undertake their life cycles (including sequential sex change) within coastal or estuarine habitats.; 2) The ‘catadromy, sequential hermaphroditism’ contingent migrate into fresh water as juveniles and then move at the onset of male maturation (3–5 years of age) back to coastal waters, where they spawn initially as males and then later as females. 3) The ‘catadromy, delayed female spawning’ contingent consists of individuals that migrate into fresh water as juveniles and remain within fresh water without returning to coastal waters to spawn as males. Sexual inversion from male to female (or possibly direct female development) in these fish occurs within fresh water and an eventual downstream migration to saline water is undertaken at a large body size (reproduced from Crook *et al.* 2017).

## 1.3.2 Barramundi and River Hydrology

River hydrology has long been considered to have a strong influence on the productivity (i.e., recruitment and growth) of Barramundi populations (Griffin 1994). Several studies have attempted to quantify these relationships by analysing correlations between Barramundi landings from commercial or recreational fisheries versus various descriptors of climate and river hydrology (Robins *et al.* 2005; Bayliss *et al.* 2008, 2014; Balston 2009a, b; Meynecke *et al.* 2006; 2014; Meynecke and Lee 2011; Halliday *et al.* 2012; Jerry *et al.*

al. 2013). The premise of these approaches is that landings represent a measure of fishery productivity (i.e., that landings are positively correlated with recruitment and population size) (Meynecke *et al.* 2014). Such studies have almost invariably reported positive relationships between fishery productivity and river flows (or rainfall or climatic indices as proxies for flow), but the nature of these relationships is generally complex and geographically variable.

One of the issues associated with using fisheries catch data to examine the influence of river hydrology on Barramundi productivity is that multiple factors operate simultaneously to influence catch (including factors influencing catchability and fishing effort). This makes it difficult to isolate the specific aspects of river flow that affect population dynamics. For example, several studies have reported strong positive correlations between effort-adjusted Barramundi landings and total annual or seasonal discharge within the same year (Bayliss *et al.* 2008, 2014; Meynecke and Lee 2011). Such correlations cannot be attributed to increased recruitment because Barramundi do not enter the fishery until they are at least 2-3 years of age (Loneragan and Bunn 1999). Instead, non-lagged correlations between river flow and effort-adjusted fishery catch appear likely to be driven primarily by increased catchability (Loneragan and Bunn 1999; Meynecke and Lee 2011). Suggested mechanisms to explain the greater catchability of Barramundi associated with high flows include increased downstream migration of mature fish from freshwater habitats into the estuary, higher encounter rates with passive gear (gill nets) due to more frequent and extensive movements, and increased congregation of fish in specific habitats - such as the outflows of floodplain run-off creeks - that can be targeted by fishers (Loneragan and Bunn 1999; Robins *et al.*, 2005; Bayliss *et al.* 2008; Balston 2009a; Halliday *et al.* 2012).

In addition to non-lagged correlations, many studies have reported lagged correlations between river flow and fishery landings (e.g., Robins *et al.* 2005; Bayliss *et al.* 2008; Balston 2009a, b; Meynecke and Lee 2011; Meynecke *et al.* 2014). For example, in a study of Barramundi fisheries in Queensland, Balston (2009b) reported positive correlations between fisheries landings and large-scale climatic variables lagged by 1, 2, 3 and 4 years (i.e., catch in a given year was correlated with conditions that occurred 1-4 years previously). In contrast, Meynecke and Lee (2011) reported positive responses between Queensland Barramundi landings and wet season rainfall both within the same year and lagged by two years. In the Daly River (NT), Bayliss *et al.* (2008) reported correlations between Barramundi catches and wet season flows at 0, 2 and 3-year time lags. Although these analyses of Barramundi catch data consistently report positive relationships between freshwater flows and Barramundi landings, the existence of potentially interacting lagged and non-lagged effects, the inconsistency of the periodicity of lagged effects across studies and issues of variable catchability and fisher behaviour make it difficult to isolate the specific aspects of river hydrology that influence fishery productivity from this type of analysis.

An alternative way of exploring relationships between Barramundi productivity and river hydrology is to quantify variability in recruitment over time using otolith ageing data (year class strength analysis). Unlike analyses of fisheries landings, this approach is not directly influenced by changes in catchability or fisher behaviour and can be used to examine the abundance of annual cohorts in relation to the environmental conditions to which they were exposed. Year class strength (YCS) analysis relies on several key assumptions, including that: 1) the age structure of the population is determined by recruitment; 2) the age structure of the sample is representative of fish recruited at the study site (i.e., not influenced by immigration or stocking); and 3) that age can be accurately determined so that fish can be accurately allocated to year classes (Staunton-Smith *et al.* 2004).

Staunton-Smith *et al.* (2004) examined YCS of Barramundi in the Fitzroy River in Queensland based upon 3 years of sampling representing 11 cohorts (1990 to 2000) using ages estimated from annual growth increments in otoliths. They examined relationships between seasonal flow metrics (total river discharge per season) and found significant positive correlations between YCS and river flow in the spring, summer and autumn of spawning. Halliday *et al.* (2010) conducted a similar study in the Fitzroy River using independent samples collected over a different 5-year period (representing cohorts from 1990 to 2002) and found the same broad relationships between YCS and river flow as reported by Staunton-Smith *et al.* (2004). Halliday *et al.* (2012) also conducted YCS analyses on the Mitchell and Flinders rivers in Queensland and the Daly and Roper rivers in the NT. They reported correlations between YCS and rainfall or flow metrics in all four systems, although the nature of these relationships was highly variable among the rivers.

Another approach that has been used to examine the influence of river flows on Barramundi fishery productivity is analysis of growth rates using mark-recapture data. Robins *et al.* (2006) used tag-recapture data from 1,168 Barramundi from the Fitzroy River, Queensland, to calculate growth rates using a version of the von Bertalanffy growth function that incorporated the effects of freshwater flow. Robins *et al.* (2006) found that growth rates of adult Barramundi were positively correlated with total seasonal discharge into the estuary. Due to the nature of the data, Robins *et al.* (2006) were not able to determine whether any lagged effects existed between river flow and growth rate.

### 1.3.3 Modelling flow-response scenarios

The above-mentioned studies demonstrate that Barramundi fishery productivity in northern Australia is affected by rainfall and river flows, but the nature of these relationships appears variable both temporally and spatially. In the context of future water resource development, a major challenge is to utilise and interpret this information to make quantitative estimates of the potential impacts of anthropogenic changes to river hydrology on fisheries.

As part of a broader quantitative ecological risk assessment for the Daly River, Bayliss *et al.* (2008) applied a Bayesian network approach to generate predictions of Barramundi fisheries yield under scenarios of altered river hydrology. They used a significant correlation between recreational catch per unit effort (CPUE) of Barramundi and wet season flow from 1985 to 2005 in the Daly River as a basis for models to predict the effects of 20% and 50% extractions of wet season flows. Bayliss *et al.* (2014) also used regression approaches to predict the effects on Barramundi catches and year class strength of changes to flow regimes in the Flinders and Gilbert Rivers in Queensland. These models predicted significant negative effects on Barramundi fishery landings of several flow extraction scenarios.

Tanimoto *et al.* (2012) used an age- and length structured population model to test a series of flow abstraction scenarios for the Fitzroy River in Queensland. Modelled outputs were used to generate predictions regarding key fisheries indicators (biomass, spawning stock, maximum sustainable yield, annual catch) under a series of alternative river discharge and climate change scenarios. While such population models are a powerful tool to support water resource management, they rely on several key assumptions that may limit their realism and broader applicability. For example, expected recruitment of Barramundi in the models of Tanimoto *et al.* (2012) is based on the spawning stock size of the previous year derived from a Beverton-Holt stock-recruitment function, adjusted by the effect of anomalies in total summer discharge (i.e. December to February) and total spring discharge (i.e. September to November). These adjustments were derived from the results of Staunton-Smith *et al.* (2004) and Halliday *et al.* (2011).

Models that incorporate stock-recruitment functions rely upon a consistent and well-parameterised relationship between spawning stock size and recruitment - for which there is little direct evidence for Barramundi and many other species (Lobon-Cervia 2013). Given the complexity of relationships between river hydrology and Barramundi recruitment evident from previous studies, such assumptions may not be realistic or applicable to other geographic areas (Griffin 1994). Thus, while population models provide a very useful approach to generating flow-response predictions, the assumptions and parameter estimates underlying these models require critical appraisal, and the realism and transferability of model outputs requires field validation across locations and over time.

### 1.3.4 Giant mud crab (*Scylla serrata*)

The Giant mud crab *Scylla serrata* (Figure 1.4) is a large Portunid crab that occupies estuary-associated mangrove and mud flat habitats across the Indo-Pacific region, including South Africa, south-east Asia, Australia, Fiji and Samoa.

In Australia, their range extends from Exmouth Gulf in Western Australia, through to NT and Queensland to the southern coast of New South Wales (Knuckey 1999). Giant mud crabs reach ~280 mm carapace width (CW) and 3 kg in weight (males) and females between 80-120mm CW. Size at functional maturity is ~135 mm CW for females and ~150 mm CW for males (Knuckey 1999). Giant mud crabs support valuable fisheries in throughout the Asia-Pacific region. In Australia, important commercial and recreational fisheries exist in Queensland, New South Wales and the NT, with the national annual catch generally exceeding 1,500 tonnes (Grubert and Lee 2013; Grubert et al. 2018; Figure 1.5). Indigenous catches are also significant in the NT, although estimates are not recent (69 tonnes in 2000/01; Grubert et al. 2016).



Figure 1.4: Giant mud crab *Scylla serrata*.

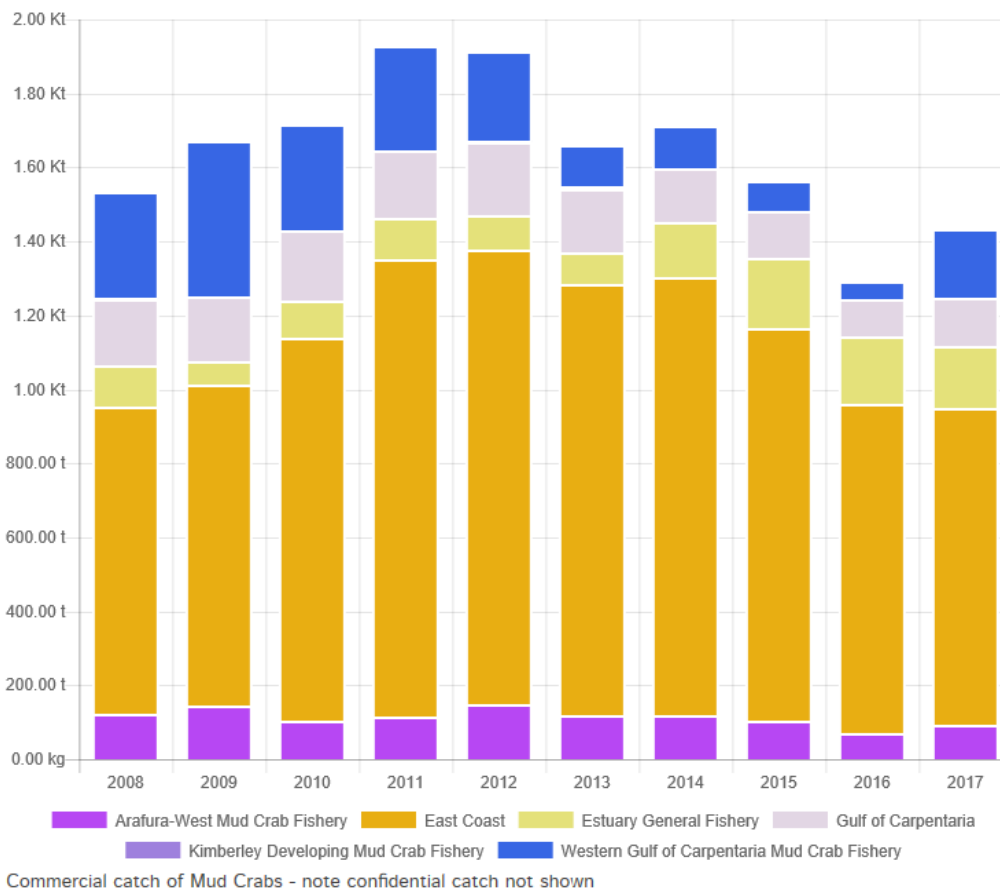


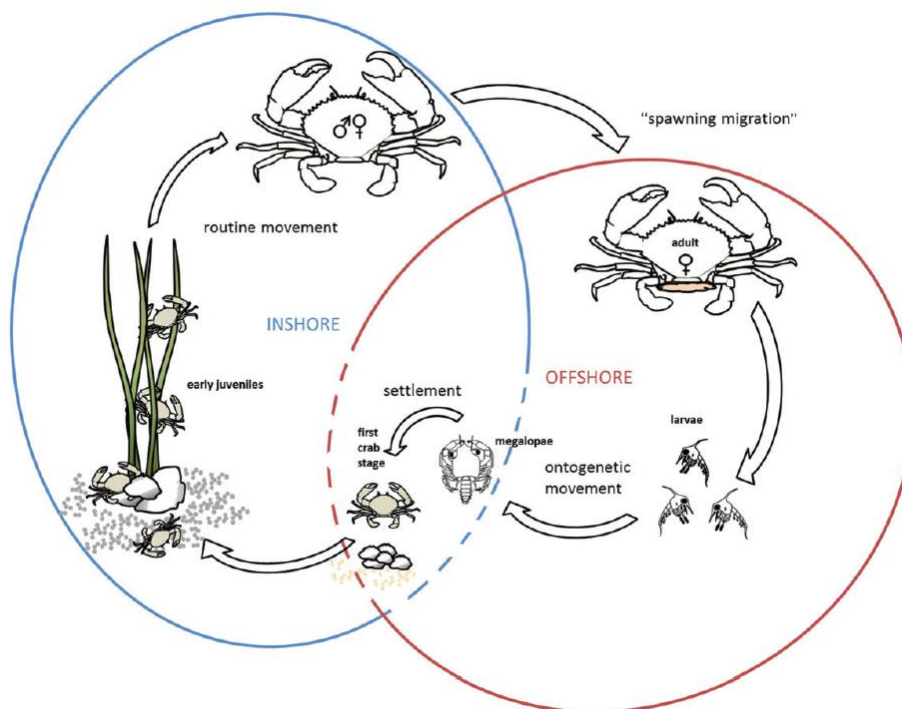
Figure 1.5: Commercial Giant mud crab catch for 2008-17 in Australia. Reproduced from Grubert *et al.* (2018).

Giant mud crabs have two distinct life phases in inshore and offshore habitats (Figure 4). Juvenile crabs usually reside in the intertidal zone, while some adults move to deeper sub-tidal regions where spawning also occurs. Those in the intertidal zone often burrow into the mud as the tide changes (Davenport and Wong, 1987), perhaps to keep cool and avoid predation at low tide (Moser *et al.* 2005). Reproduction occurs in the warmer wet season months, November to March, with potentially two mating seasons per year (Heasman *et al.* 1985). Mating only occurs when the female has a soft shell after moulting (Knuckey 1996), and a spermatophore is then deposited into the female and stored until the ova are ready for fertilization. This



usually occurs from October to November, and catches are usually lower during this time. Mated females then migrate offshore to deeper waters, where spawning occurs in batches of 2-5 million eggs (Quinitio *et al.* 2001).

Hatchlings develop through five larval stages over four weeks to become megalopae (Quinitio *et al.* 2001), before migrating back to benthic inshore habitats where they settle and moult into juvenile crabs (Shelley 2008 cited in Meynecke *et al.* 2010). As Giant mud crabs undergo such defined ontogenetic movements between inshore and offshore habitats, it is perhaps not surprising that the larval and juvenile stages are thought to be the most heavily influenced by environmental factors such as salinity and temperature (Nurdiani and Zeng 2007). This responsiveness to environmental variability is thought to be a primary reason for the high variation in Giant mud crab commercial catch rates through space and time (Meynecke *et al.* 2010, 2012a).



**Figure 1.6: Life cycle of Giant mud crab showing ontogenetic shift between inshore and offshore phases (reproduced from Meynecke *et al.* 2014).**

### 1.3.5 Giant mud crabs and river hydrology

Several studies have reported relationships between freshwater flows and commercial catches of Giant mud crab (Loneragan and Bunn 1989; Robins *et al.* 2005; Meynecke *et al.* 2010; 2012a), although these relationships are less consistent than for Barramundi (Bayliss *et al.* 2014). Loneragan and Bunn (1989) reported strong correlations between commercial catches of Giant mud crab and total summer discharge in the Logan River, Queensland. Robins *et al.* (2005) examined correlations between Giant mud crab commercial catch and seasonal river flows in the Fitzroy River region of Queensland and found that catch adjusted for year was negatively correlated with autumn flow and summer rain, but was positively correlated with autumn rain lagged by two years.

Meynecke *et al.* (2010, 2012a) subsequently explored relationships between climate drivers and Giant mud crab catch and found that ~30-40% of catch variability was explained by the Southern Oscillation Index (SOI), with strong catches related to La Niña phases which are associated with increased rainfall and higher temperatures over northern Australia. Temperature was a less important driver of annual and seasonal Giant mud crab catches in northern Australia, and the link between Giant mud crab catches and river flows (with either a six month or one-year lag) was particularly strong in the NT, but variable in Queensland, NSW and WA (Meynecke *et al.* 2010). Whilst these previous studies provide evidence of a link between Giant mud

crab catches and river hydrology, the use of commercial catch data means that these assessments may be influenced by effort and catchability issues as discussed previously for Barramundi. At present, there is little empirical information regarding the effects of river hydrology and other environmental factors on growth and recruitment for Giant mud crab.

## 1.4 Study objectives

The aims of the current study were to apply a system-specific, empirical approach using biochronological data to explore correlations between fishery productivity and environmental variables (including key aspects of climate and river hydrology). These detailed analyses were used to identify the environmental factors that most strongly influence recruitment and growth and to explore their interactions using an integrative modelling approach.

The information generated from this analysis was then applied to model the outcomes of water management scenarios that incorporate different levels of change to river hydrology. Water resource managers from the Northern Territory Department of Environment and Natural Resources were consulted during the development of the models and scenarios, and the outputs and models have been made available as tools to support water resource allocation decisions into the future.

The specific objectives of the study are:

1. Undertake ageing and biochronological measurement of Barramundi otoliths and Giant mud crab ossicles.
2. Develop rigorous, regionally specific empirical models to quantify the relationships between fisheries productivity and various metrics of freshwater inflow using advanced statistical techniques.
3. Provide research findings to industry and government for a scientifically defensible and robust basis for decisions on the future use of water in northern Australia in relation to fisheries resources.

Summaries of the Methods, Results and Discussion and Conclusions for each of the two project components (Barramundi, Giant mud crab) are provided in the following sections. Full details of each component have been prepared as scientific manuscripts.

**Note:** objectives relating to the Giant mud crab component were changed due to concerns that arose in relation to the suitability of using gastric ossicles for direct age estimation. Rather than examining the effects of environmental variables on Giant mud crab fisheries as originally intended, the focus of this component shifted to an assessment of the direct ageing technique. Whilst this shift in focus did not allow us to address the original objectives for Giant mud crab, the final outcome has important implications for future research and management of crustacean fisheries.

## 2. Influence of freshwater flows on recruitment and growth of Barramundi<sup>3</sup>

### 2.1. Background

As outlined in Section 1.3.2, a broad range of scientific evidence has demonstrated the importance of climate and river hydrology as drivers of productivity in Barramundi fisheries across northern Australia. Nonetheless, critical gaps in our understanding of hydrology-fishery relationships continue to limit the integration of this evidence into water policy development. One of the key issues is the wide range of climatic and hydrological factors associated with variability in Barramundi catches and recruitment in previous studies. Meynecke *et al.* (2014), for example, reported 11 statistically significant correlations between climatic and hydrological variables and effort-adjusted catch in the commercial Barramundi fishery in Princess Charlotte Bay, Queensland. In the same study, YCS analyses of five rivers across northern Australia (Fitzroy, Mitchell, Flinders, Roper, Daly) identified 14 climatic and hydrological variables that were significantly correlated with Barramundi recruitment. The large number of climatic and hydrological variables that have been associated with fishery catches and recruitment likely reflects complexity in the relationships between hydrology and population processes; but it can also be at least partially attributed to high levels of covariance among the climatic and hydrological variables that have been examined in previous analyses.

This component of the current project uses biochronological data collected from four rivers of the NT (Daly, Roper, Mary, McArthur) to explore correlations between Barramundi year-class-strength (YCS) and growth (otolith increment widths) and a range of environmental and intrinsic variables within an integrated modelling framework. These analyses are used as a basis to identify the key climatic and hydrological drivers of recruitment and growth in these systems, and to explore interactions among these factors. The relationships generated from the models are then applied to generate quantitative estimates of the outcomes of different water abstraction scenarios (ranging from 10% to 40% abstraction rate per season/annum) in low, moderate and high discharge years.

### 2.2. Methods

#### 2.2.1. Sample collection and preparation

Otoliths from 3,586 Barramundi caught in the Daly, Mary, Roper and McArthur rivers between 2007 and 2018 were sourced from the Fisheries Division of DPIR (Figure 2.1, Table 2.1). All samples were captured between February and October (i.e. dry season only) by commercial gillnetting, recreational angling, scientific gillnetting or electrofishing, encompassing 39 unique sampling events.

Sagittal otoliths were dissected from the fish, embedded in epoxy resin, and a transverse section of ~200 µm thickness taken through the core using a slow-speed saw. Sections were then mounted on microscope slides using resin and a clear cover slip placed over the section. The samples used in the study are held in an archive maintained by the Fisheries Division of DPIR.

Prior to the analysis, otolith sections were examined in detail by members of the project team and a standardised protocol for conducting the increment measurements was developed using the approach outlined by Morrongiello *et al.* (2014). In addition, a batch of 50 otolith sections was sent for processing to Fish Ageing Services (FAS) in Portarlington, Victoria. Age estimates and interpretations of increment locations provided by FAS were used to further refine and cross-check our increment counts and

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<sup>3</sup> At the time of writing, the results of this chapter were under review for publication as: Crook, D.A., Morrongiello, J.R., King, A.J., Adair, B.J., Grubert, M.A., Roberts, B.H., Douglas, M.M. and Saunders, T.M. (*in review*). Environmental drivers of recruitment in a tropical estuarine fishery: monsoonal effects and vulnerability to water resource development. *Ecological Applications*.

measurements. Increment width was used as a proxy for age-dependent somatic growth, with longer fish also having wider otolith radii (explaining 45% of length variation; Supplementary Figure 1).

For the final growth increment measurements, two experienced readers examined the transverse sections using a Leica M125 compound microscope fitted with a Leica DFC295 digital camera (running Leica Application Suite X software, Life Science) under reflected light at approximately x25 magnification. All ageing was conducted following the Barramundi ageing protocol developed by the Queensland Department of Primary Industries and Fisheries (DPIF 2005).

Age was estimated by counting the number of paired opaque and translucent zones between the otolith core and the growing edge (Figure 2.2), where one year of growth was the combination of one translucent and one opaque zone (Stuart and McKillup 2002). Marginal increments were visually classified as narrow, intermediate or wide as per Ewing *et al.* (2003). A readability rating between 1 (poor) and 5 (excellent) was assigned to every otolith section examined. We excluded from further analysis fish that were scored 2.5 or below (211 fish), as measured increments from these fish were not considered sufficiently reliable.

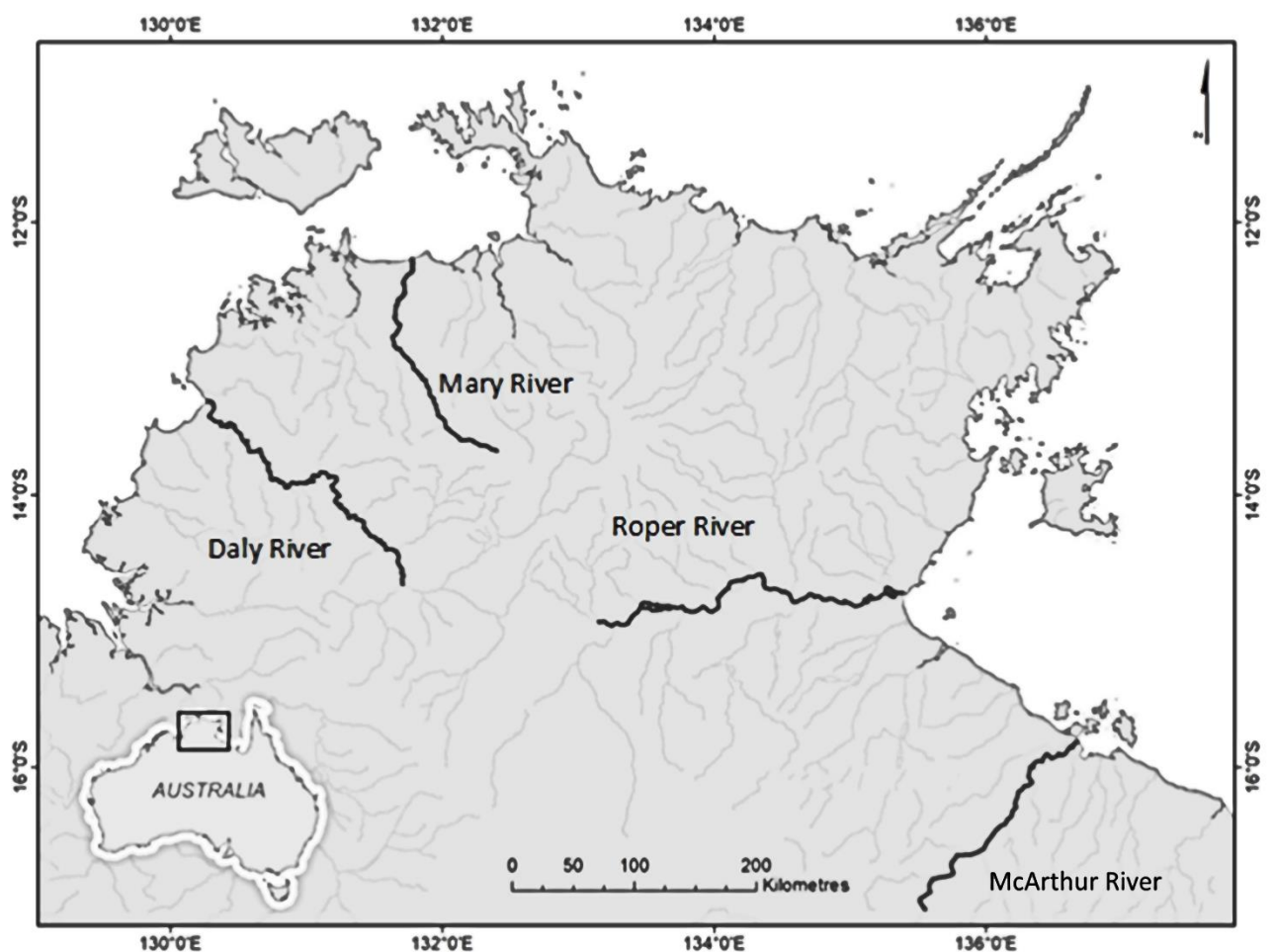
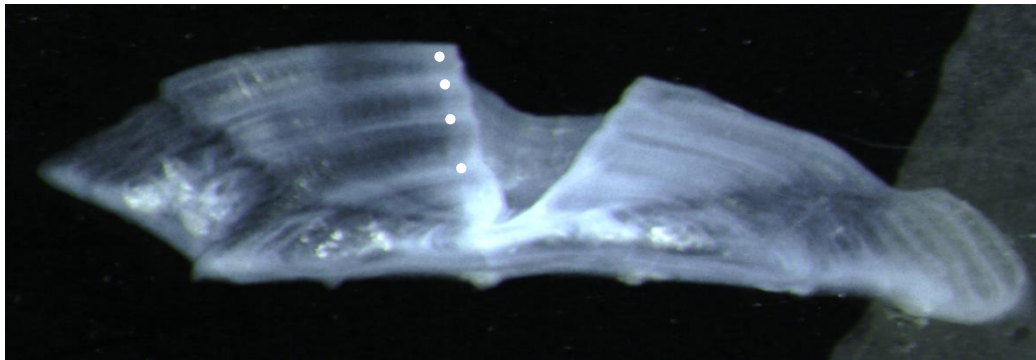


Figure 2.1: Map showing the locations of the four study rivers in the Northern Territory. Reproduced from Crook *et al.* (in review).

**Table 2.1: Summary of samples used in models to estimate river-specific recruitment and annual growth histories. Year class strength (YCS) models used all fish aged 3 and older, growth models used all fish aged 2 years and older. See text below for justifications. Adapted from Crook *et al.* (in review).**

River	Sample years	Capture methods	Age range	# YCS	Year classes	Year class range	# Growth	Growth years	Growth year range
Daly	2007-11; 2013-17	Commercial gillnet, angling, electrofishing	1-16	2110	24	1991-2014	1842	22	1995-2016
Mary	2010-13; 2015-17	Commercial gillnet, scientific gillnet, angling, electrofishing	1-17	363	22	1993-2014	310	22	1995-2016
Roper	2008-11	Commercial gillnet, angling	2-15	722	16	1993-2008	672	15	1996-2010
McArthur	2015-17	Commercial gillnet, angling, electrofishing	2-11	90	11	2004-2014	80	9	2007-2015



**Figure 2.2. Sectioned Barramundi otolith viewed under reflected light. Four annuli are marked with dots and the margin is classified as intermediate (some translucent material laid down after the marginal increment). This section was assigned a readability score of 5 (excellent).**

### 2.2.2. Age assignment

We assigned Barramundi a 1<sup>st</sup> October birth date following the otolith ageing validation study of Stuart and McKillop (2002). Assignment of an October birth date differs from the approach of Staunton-Smith *et al.* (2004) and Halliday *et al.* (2012), who used a 1<sup>st</sup> January birth date. Use of different birth dates does not affect the assignment of fish to the appropriate year class but needs to be considered when comparing the results of the current study with previous studies. For example, a fish born during the September 2000 to February 2001 spawning season (i.e., the 2000/01 wet season) would be assigned to the 2000 year-class in our study, whereas it would be assigned to the 2001 year-class according to Staunton-Smith *et al.* (2004) or Halliday *et al.* (2012).

Examination of the marginal increment classifications across our sample of >3,500 fish suggested a peak in narrow increments and a large decline in wide increments in August (Figure S2): 1<sup>st</sup> August was therefore assigned as the putative date of annual increment formation. This differs somewhat from Stuart and McKillop (2002) and Staunton-Smith *et al.* (2004), who suggested that increment formation peaks in October in Barramundi from the Fitzroy River, Queensland. However, the assessment of Stuart and McKillop (2002) was based on very low numbers of fish collected from July to September (n=3) and Staunton-Smith *et al.* (2004) only examined marginal increment formation in October and February, making it difficult to draw conclusions about the timing of peak increment formation from their data.

Based on an annual increment formation date of 1<sup>st</sup> August, increment counts were adjusted where necessary (see O’Sullivan 2007) to ensure that fish collected at different times of year were assigned to the appropriate year class according to the below criteria:

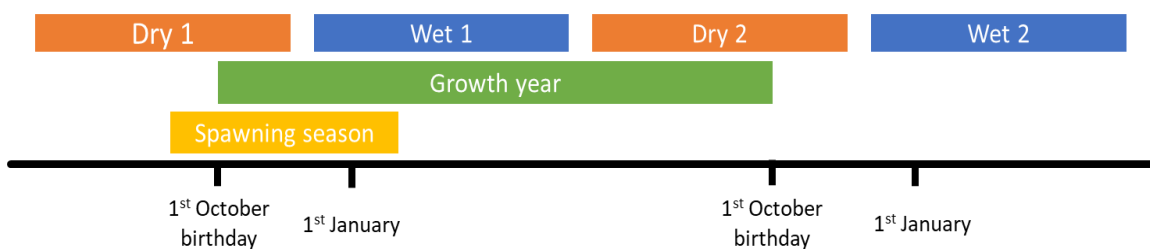
- *Narrow margin*: add 1 year to age if fish was sampled in February or March. No adjustment for other sample dates (April to October).
- *Intermediate margin*: add 1 year to age if fish was captured before August (February-July). No adjustment for other sample dates (August to October).
- *Wide margin*: add 1 year to age for all sampling dates.

For example, a fish collected in March 2015 with four annuli and a narrow margin would be assigned to the 2010 year-class ( $2015 - [4 + 1] = 2010$ ), which corresponds to the 2010/11 spawning season. Further information on the rationale for these adjustments is provided in Appendix 4.

### 2.2.3. Environmental variables

We explored how regional climatic and local environmental parameters affected inter-annual variation in Barramundi year class strength (YCS) and growth across four time periods: preceding dry season (dry 1), wet season (wet 1), following dry season (dry 2) and following wet season (wet 2) (Fig. 2.3). We defined the start and end of wet and dry seasons based on hydrology (Table 2.2) as this allowed for dynamism in variables not afforded by fixed calendar dates. These periods were included in the modelling framework based on *a priori* expectations that conditions during the periods may strongly influence growth and recruitment (see Table 2.2). For each individual and time period, we developed a series of intrinsic (i.e. fish specific) and extrinsic (i.e. environmental) variables (Table 2.3). Intrinsic variables included: fish age in years for each increment (*age*) and *age-at-capture* (see Morrongiello and Thresher [2015] for a discussion of this term). The average Australian monsoon index (*AMI*) was calculated over the wet season (Dec - May) as a regional extrinsic variable because it represents a broad-scale climatic driver of climate and rainfall across northern Australia (Marshall and Hendon, 2015).

Local extrinsic variables were related to each river's hydrology and the thermal regime. Hydrological variability was captured by including the total amount of flow during a defined period (*totalF*) and measures of wet and dry season duration (*wetDur* and *dryDur* respectively) (Table 2.3). The effect of temperature on growth was explored using seasonally calculated growing degree days. Degree days are the time integral of water temperature within an optimal band which is related to an ectotherm's cumulative metabolism (Trudgill *et al.*, 2005) and have successfully been linked to fish growth (Morrongiello *et al.*, 2011, Neuheimer and Taggart, 2007, Venturelli *et al.*, 2010). Suitable flow data was not available for the Mary River, so river-specific relationships were not modelled for this system. River-specific modelling was attempted for the McArthur River; however, the numbers of samples available for analyses in this system were low ( $n = 80-90$ ) and the models failed to converge. Consequently, river-specific models for YCS and growth are presented for the Daly and Roper rivers only.



**Figure 2.3: Relationship between Barramundi year classes, growth years and periods over which environmental variables were calculated. Reproduced from Crook *et al.* (in review).**

**Table 2.2: Description of the time periods used in the year class strength (YCS) and growth analyses. Reproduced from Crook *et al.* (in review).**

Time period	Model	Description
Dry1	Growth	The period of low flow experienced at the beginning of a growth year, and is defined by the first and last time daily flow passes below each river's 30-year daily flow Q40, outside of the wet season as defined below
Wet1	YCS & growth	The period of high flow experienced by young-of-year individuals or encompassed by a growth year, and is defined by the first and last time daily flow is above each river's 30-year daily flow Q30
Dry2	YCS & growth	The period of low flow experienced in the latter part of the growth year or as a young-of-year individual, and is defined as per Dry1 flow thresholds
Wet2	YCS	The period of high flow experienced by one-year-old individuals, and is defined as per Wet1 flow thresholds

**Table 2.3: Description of predictor variables used in the analysis of Barramundi YCS and growth, the time periods for which they were generated, and the data source. Adapted from Crook *et al.* (in review).**

Variable	Type	Time Period	Description	Data source
Age	Intrinsic	1-4	Age (years) at which increment formed	Otolith
Age-at-capture (ACC)	Intrinsic	1-4	Age in years at time of capture (2–18)	Otolith
Australian monsoon index (AMI)	Regional extrinsic	1*,4*	Monsoon index defined as land area-averaged rainfall anomalies north of 25S	Australian Bureau of Meteorology <a href="http://poama.bom.gov.au/project/s2s/monsoon/monsoon.html">http://poama.bom.gov.au/project/s2s/monsoon/monsoon.html</a> (Marshall & Hendon, 2015)
Total flow ( <i>totalF</i> )	Local extrinsic	1-4	Total flow (ML period <sup>-1</sup> ) for each river	NT Government water data portal <a href="https://nt.gov.au/environment/ water/water-information-systems/water-data-portal">https://nt.gov.au/environment/ water/water-information-systems/water-data-portal</a>
Wet season duration ( <i>wetDur</i> )	Local extrinsic	1, 4	Length of wet season (days), ranging from first to last time Q30 flow was surpassed	NT Government water data portal <a href="https://nt.gov.au/environment/ water/water-information-systems/water-data-portal">https://nt.gov.au/environment/ water/water-information-systems/water-data-portal</a>
Dry season duration ( <i>dryDur</i> )	Local extrinsic	2,3	Length of dry season (days), ranging from first to last time flow went below Q40, outside of the wet season	See above
Wet season degree days ( <i>wetDD</i> )	Local extrinsic	1	Cumulative sum of daily water temperature within 27–36°C during a season. This range spans temperatures where growth rate is at least 90% of its maximum (Norin <i>et al.</i> , 2014). Water temperature (°C day <sup>-1</sup> ) was estimated using a regression relationship between mean air temperature (derived from daily minimum and maximum air temperature records during period of interest) and available water temperature data from Daly River ( $y = 6.4003 + 0.8281 * \text{mean air temp}$ ; $r^2=0.76$ )	Australian Bureau of Meteorology ( <a href="http://www.bom.gov.au/climate/data/stations/">http://www.bom.gov.au/climate/data/stations/</a> ) and Banyan Farm water quality sampling site Bureau of Meteorology station numbers for each river: Daly River: 14938 and 14901 Roper River: 14612 and 14299 McArthur River: 14704 and 14723
Dry season degree days ( <i>dryDD</i> )	Local extrinsic	2,3	As above	

# 1: wet1; 2: dry1; 3: dry2; 4: wet2, see Table 2.1 and Figure 2.3 for detail; \* wet season AMI is standardised as 1 Dec to 31 May across all rivers to facilitate direct comparison in YCS analysis.

The otolith ageing analysis provided 611 observations of catch-at-age across the four rivers (Table 2.1). We examined age-frequency plots for each of the 39 sampling events and found that fish younger than age-3 were under-represented; most likely because they were not fully vulnerable to the sampling gear (see Maceina 1997). This being the case, we omitted 104 young fish (< age-3) from subsequent YCS analyses. Missing age classes within these river-specific bounds were assumed to be reflective of weak year classes, assigned a zero catch, and retained for analysis (Morrongiello *et al.*, 2014).

Time series of year class strength for each river were estimated using a generalised linear mixed model (GLMM) formulation of catch curve regression (Maceina, 1997), developed by Morrongiello *et al.* (2014). The slope of the catch-at-age regression provides an estimate of total mortality. Positive and negative residuals around this relationship are then assumed to reflect strong and weak year classes respectively (Jenkins *et al.* 2015, Staunton-Smith *et al.* 2004). GLMMs are ideally suited to the estimation of year class strength from age frequency data as random intercepts can account for the repeated observations of year class abundance across sampling events, and Poisson or negative binomial distributions best reflect the underlying count nature of the data (Morrongiello *et al.* 2014).

We adopted a two-stage process for our YCS analyses. First, we combined data from all rivers (regional YCS model) and modelled catch as a function of the fixed effects *Age-at-capture* (present in all models), River and Capture method. We tested whether mortality rate (*Age-at-capture* slope) differed among rivers and capture methods. Further, we included a random intercept and *Age-at-capture* slope for each of the 39 sampling events. These interaction terms allowed us to correct for any biases in gear selectivity or spatial variation in mortality rates. We included river-specific random intercepts for year class to capture variation in catch not attributable to sampling artefacts, and an offset term to condition individual age-specific catches on the total number of fishes collected at that event. A Poisson model suffered from over-dispersion (ratio = 1.237,  $\chi^2 = 637$ ,  $\text{rdf} = 515$ ,  $p < 0.001$ ), so we used a negative binomial distribution for all subsequent models.

We compared models with and without a random age-at-capture slope for sample event, and then models with all combinations of fixed effects with Akaike's Information Criterion corrected for small sample size (AICc, Burnham and Anderson 2002). We then extracted the year class random intercepts as estimates of YCS for each river. Temporal trends were removed from each YCS time series using a first-order autoregressive model that included a linear term for year (Buonaccorsi *et al.* 2001; Koenig, 1999). Zero-lag cross correlations were performed between each residual YCS series to estimate overall levels of spatial synchrony. Then, we calculated 11-year running correlations between the residual YCS series of different rivers to explore temporal patterns in spatial synchrony. The significance of 11-year running correlations was determined using a Monte Carlo simulation, where 1000 permutations of the data were taken and running correlations calculated. The highest correlation of each trial was then used to determine the critical value above which correlations are significant at the 0.05 level. This approach avoided inflated type I error associated with multiple comparisons of correlation significance. We investigated the importance of AMI calculated over the wet1 and wet2 periods on Barramundi YCS by adding linear and quadratic (including interaction with river) terms to the optimal regional intrinsic effects model.

We then conducted river-specific analyses by separating the data for each river and exploring the environmental drivers of YCS (river-specific YCS models). River-specific GLMMs always included an age-at-capture by method interaction, an offset term for sample size, random intercepts for sample event and year class, and a random age-at-capture slope for each sample (see Results). We compared models including combinations of totalF, wetDur and dryDur (linear and quadratic) calculated over wet1, dry2 and wet2 time periods.

#### 2.2.4. Growth rate analysis

Otolith growth increments were measured from 2,904 Barramundi (Table 2.1). We excluded the first year of growth for each fish due to difficulties identifying the core and because potential differences in spawning time could mean that this increment represented between ~9 and ~13 months of growth. Thus, our data represented between 2 and 16 years of growth for individual fish. Variation in Barramundi annual growth increments were analysed using mixed effects modelling (Morrongiello and Thresher 2015), first to all growth data across rivers (regional growth model), then within each river separately (river-specific growth



models). All models included a random intercept for *FishID* (unique identifier for each fish) which induced a correlation among increments within fish to account for any individual-specific differences in growth.

Regional models included a random intercept for *river:growth.year* (unique identifier for each year within a river) which induced a correlation among increments from different fish living in the same river that were deposited in the same year. This term accounted for potential temporal growth differences induced by common environmental effects. River-specific models just included a random intercept for *growth.year*. Preliminary analyses indicated that otolith increment width exponentially declined with fish age and so we linearised this pattern by taking the natural log of both variables (e.g. Barrow *et al.*, 2018, Morrongiello *et al.*, 2014). We tested (using AICc) whether there were individual-specific differences in the age-growth relationship by including a random age slope on *FishID*. The random slopes model performed best ( $\Delta\text{AICc}=206.2$ ) and this structure was carried forward for all subsequent regional and river-specific growth models.

We developed a series of increasingly complex intrinsic effects model structures that included as fixed effects combinations of *Age*, *age-at-capture*, *River* and their interactions. The *Age-at-capture \* River* interaction accounts for potential biases in estimated growth rates associated with differential mortality or sampling regimes through time within each river (see Morrongiello *et al.* 2012). *Age* and *age-at-capture* were scaled  $[(x - \bar{x})/s]$  to facilitate model convergence and interpretation of interactions. These models were fitted with maximum likelihood (ML) and compared using AICc. The best model was refit using restricted maximum likelihood (REML) and the river-specific growth year BLUPs were extracted to explore spatial synchrony in growth (using the same approach as outlined for YCS). We added AMI (linear and quadratic), calculated over the wet 1 period, to the optimal regional intrinsic growth model to investigate *River* and age-specific effects of this regional climate driver on Barramundi growth.

We then separated data by river and explored the environmental drivers of growth variation (river-specific growth models). These river-specific linear mixed models always included *Age* and *Age-at-capture* (see Results). Within each river we compared growth models that included combinations of totalF, wetDur and dryDur (linear and quadratic), and their interaction with *Age*, calculated over wet 1, dry1 and dry2 time periods.

All YCS models were fit using the *glmer.nb* function and all growth models fit using the *lmer* function from the *lme4* package. Model selection was performed using the *MuMIn* and *bbmle* packages. TotalF variables were natural log transformed to satisfy models assumptions and all continuous predictor variables were scaled to facilitate model convergence and interpretation of interaction terms (where present). Parameter estimates and 95% credible intervals were derived from the posterior distribution of the fixed effects in the best model using 1000 model simulations generated by the *arm* package, and model predictions generated using the *effects* package. All analyses were performed in R 3.5.1.

## 2.3. Results

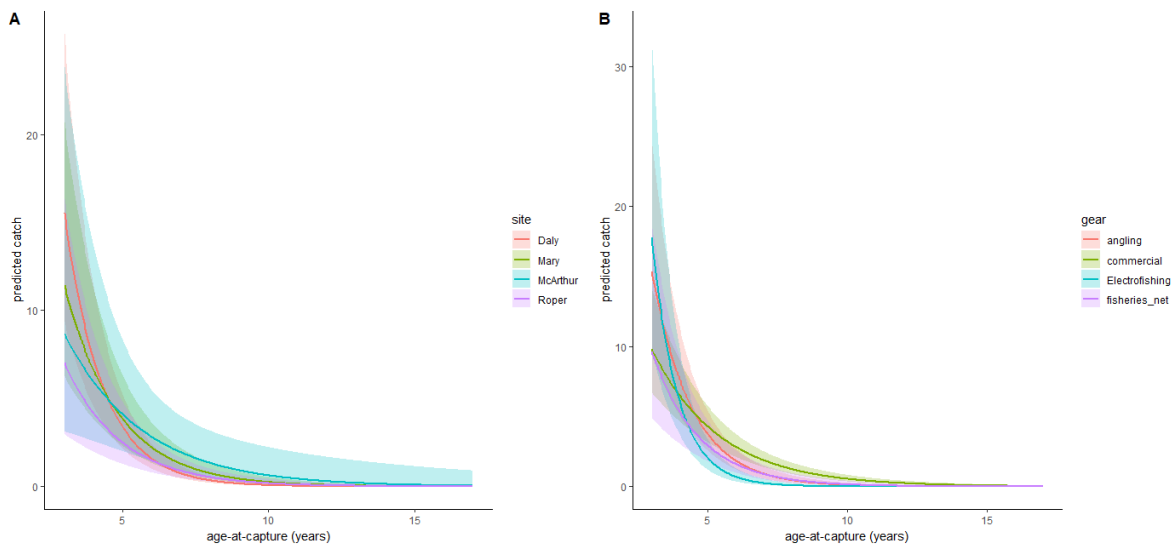
### 2.3.1. Year class strength

#### ***Mortality rate estimation***

The slopes of the catch-at-age regressions were examined to estimate total mortality among samples, rivers and collection methods. We found evidence for sample-specific differences in mortality rate (i.e. mortality or gear selectivity varies through space and time;  $\Delta\text{AICc}=4.2$ ) and so retained the random age-at-capture slope in the overall intrinsic model. Mortality rate also differed among rivers and collection methods (Table 2.4). Daly River fish experienced highest mortality rates (steeper age-at-capture slope) than the other three rivers (Table 2.5; Figure 2.4A). Estimated mortality rates for angling and scientific gillnets were similar. The mortality curve derived from the commercial fishery was shallower due to an increased prevalence of big fish in these catches, whereas the mortality curves from electrofishing samples was steeper due to the inclusion of smaller and, thus, younger fish in the samples (Table 2.5; Figure 2.4B).

**Table 2.4: Results of model selection exploring sources of mortality rate variation for Barramundi year class strength across the four rivers. Shown are the degrees of freedom (df), log likelihood (logLik) and the  $\Delta AICc$  values for each fixed effect structure. The best model is highlighted in bold.**

Model	df	logLik	$\Delta AICc$
<b>age-at-capture * River + age-at-capture * method</b>	<b>19</b>	<b>-777.491</b>	<b>0</b>
age-at-capture * method	13	-785.285	2.807
River + age-at-capture * method	16	-784.499	7.588
age-at-capture	7	-798.944	17.638
age-at-capture + method	10	-796.2	18.357
age-at-capture * River	13	-793.922	20.081
age-at-capture * River + method	16	-791.01	20.611
age-at-capture + River + method	13	-795.693	23.623
age-at-capture + River	10	-798.854	23.666



**Figure 2.4: Age-dependent catch of Barramundi as a function of A) river and B) capture method. The slopes of these lines reflect different mortality rates for each river and capture method.**

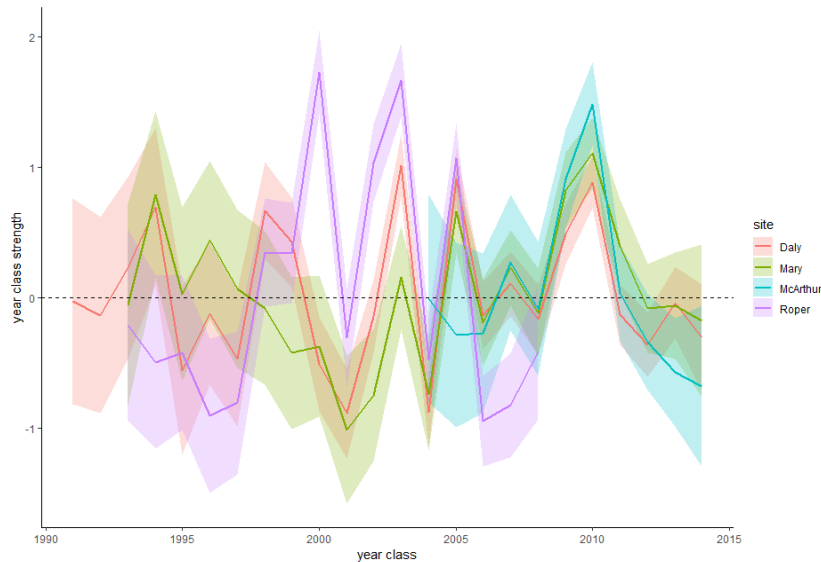
**Table 2.5: Parameter estimates ( $\pm 95\%$  confidence intervals) for best fixed effects model explaining sources of mortality variation in Barramundi across four rivers.**

<b>Intrinsic fixed effects</b>	
<b>Parameter</b>	<b>Estimate (95% CI)</b>
intercept (Daly, angling)	-6.075 (-6.822, -5.315)
Age-at-capture (Daly, angling)	-3.403 (-3.992, -2.837)
River (Mary, vs. Daly)	1.115 (0.276, 1.994)
River (McArthur, vs. Daly)	1.941 (0.6, 3.173)
River (Roper, vs. Daly)	0.794 (-0.123, 1.595)
Age-at-capture (Mary, vs. Daly)	0.899 (0.222, 1.573)
Age-at-capture (McArthur, vs. Daly)	1.604 (0.483, 2.743)
Age-at-capture (Roper, vs. Daly)	0.993 (0.256, 1.693)
Method (commercial, vs. angling)	1.43 (0.728, 2.245)
Method (electrofishing, vs. angling)	-2.281 (-3.921, -0.642)
Method (fisheries gillnet, vs. angling)	0.208 (-0.907, 1.451)
Age-at-capture (commercial, vs. angling)	1.191 (0.63, 1.874)
Age-at-capture (electrofishing, vs. angling)	-1.537 (-2.819, -0.249)
Age-at-capture (fisheries gillnet, vs. angling)	0.411 (-0.567, 1.475)
<b>Random effects</b>	
<b>Variance component</b>	<b>Standard deviation</b>
River:Year Class	0.797
Sample	0.453
Age-at-capture sample	0.36

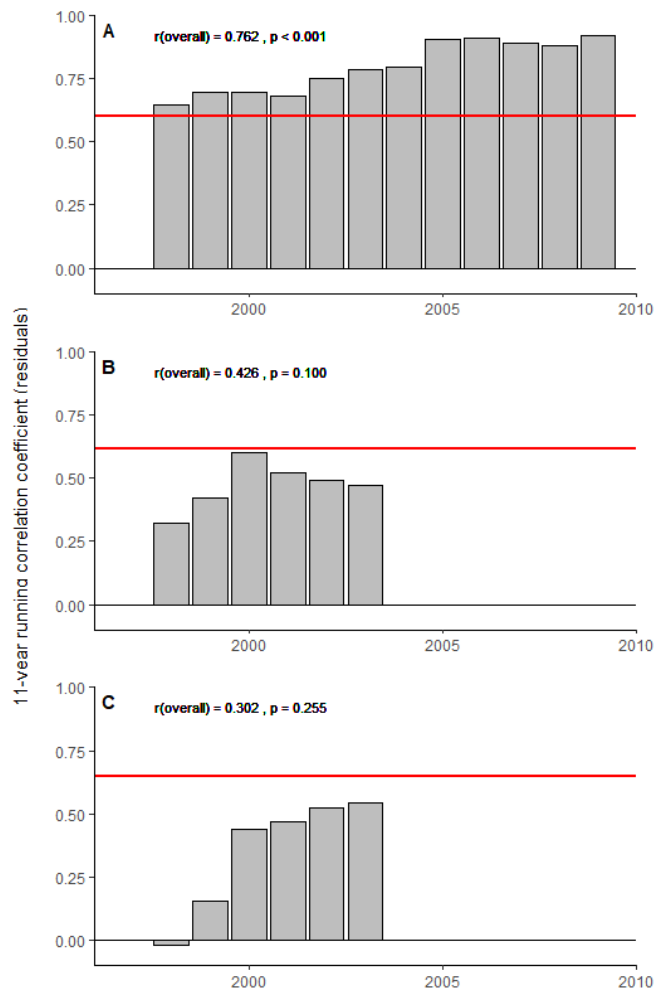
### **Regional patterns**

Barramundi year class strength displayed inter-annual variation within and between rivers (Figure 2.5). Our analyses indicated that 2009/10 and 2010/11 were strong year classes for the Daly, Mary and McArthur rivers. Likewise, both 2003/04 and 2005/06 were strong years classes for the Daly and Roper rivers. Both 2000/01 and 2002/03 were strong year classes for the Roper River.

Across all data, Daly River and Mary River YCS were significantly and positively correlated and this synchrony increased through time (Figure 2.6A). Daly River and Roper River YCS were positively, but not significantly, correlated (Figure 2.6B), whilst there was negligible correlation between Mary and Roper River YCS (Figure 2.6C). Daly River and McArthur River YCS were weakly correlated ( $r = 0.458$ ,  $p = 0.157$ ) and there was some evidence of a stronger relationship between Mary River and McArthur River YCS ( $r = 0.512$ ,  $p = 0.107$ ). Roper River and McArthur River only shared five years of YCS data and there was no evidence of a correlation between these rivers ( $p = 0.496$ ).



**Figure 2.5: Estimated index of year class strength ( $\pm$  standard error) for four rivers derived from catch curve regression. Dotted horizontal line represents the long-term mean year class strength index across rivers (model fixed effect intercept).**



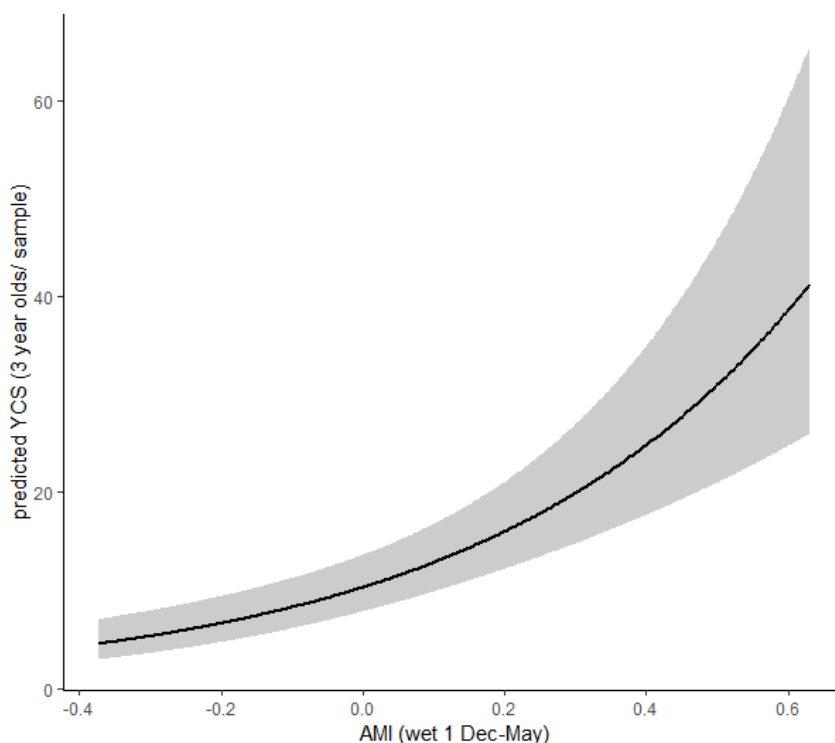
**Figure 2.6: 11-year running correlations between A) Daly and Mary river YCS index, B) Daly and Roper River YCS index, and C) Mary and Roper River YCS index. Running correlations above the solid red line are significant at the 95% level.  $r(\text{overall})$  is the Pearson's correlation coefficient across the whole period. Note, 11-year running correlations could not be calculated with the nine years of McArthur River YCS.**

Wet season Australian Monsoon Index in the year of spawning (wet1 AMI) was the best extrinsic predictor of regional Barramundi YCS (Table 2.6), with YCS increasing when associated with stronger monsoonal indices ( $\beta$  [ $\pm 95\%$  CI] = 0.542 [0.370, 0.718]; Figure 2.7). The fixed effects in the best model explained ~87% of the variance (marginal  $r^2 = 0.867$ ) in the catch-at-age data across the four rivers. Linear regression analyses between wet1 AMI (explanatory variable) and YCS (response variable) across the whole data series showed that wet1 AMI explained the highest proportion variance in YCS in the Daly River ( $r^2 = 0.370$ ,  $p = 0.002$ ), followed by the McArthur River ( $r^2 = 0.318$ ,  $p = 0.071$ ), the Roper River ( $r^2 = 0.271$ ,  $p = 0.039$ ) and the Mary River ( $r^2 = 0.247$ ,  $p = 0.012$ ).

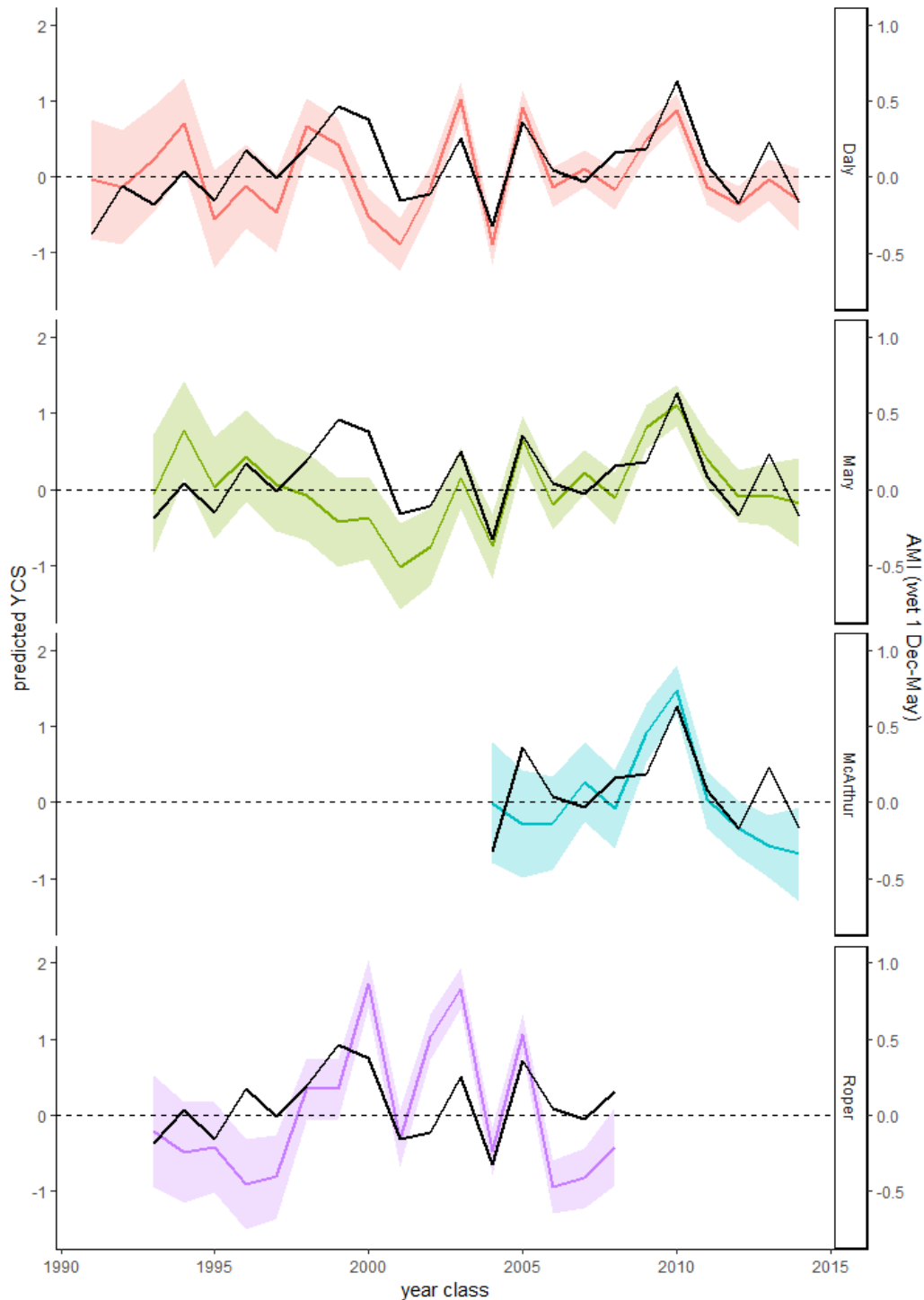
Further examination of the AMI-YCS relationships for each river showed that the strength of the AMI-YCS relationships was highest in the more recent years of each time series (Figure 2.8). This appears to be due to increased model error resulting from under-representation of older age classes in the earlier parts of time series. When the regression analysis was repeated using only data for the most recent 10 years of each series, the proportion of variance explained by AMI increased considerably in all four rivers: Daly River,  $r^2 = 0.710$ ,  $p = 0.002$ ; McArthur River,  $r^2 = 0.412$ ,  $p = 0.046$ ; Roper River,  $r^2 = 0.308$ ,  $p = 0.096$ ; Mary River,  $r^2 = 0.574$ ,  $p = 0.011$ . Correlation coefficients ( $r$ ), which have been reported in previous studies (Halliday *et al.* 2011; Meynecke *et al.* 2014) and are presented here for comparative purposes, were as follows for the last 10 years of each time series: Daly,  $r = 0.843$ ; McArthur,  $r = 0.642$ ; Roper,  $r = 0.555$ ; Mary,  $r = 0.574$ .

**Table 2.6: Model selection exploring importance of wet season AMI in describing regional patterns in year class strength. Shown are the top three models with degrees of freedom (df), AICc and  $\Delta$ AICc for each model structure. The best model is highlighted in bold. Reproduced from Crook *et al.* (in review).**

Model	df	logLik	$\Delta$ AICc
intrinsic	19	-777.49	24.41
<b>+ wet1 AMI</b>	<b>20</b>	<b>-761.68</b>	<b>0</b>
+ wet1 AMI + wet2 AMI <sup>2</sup>	22	-764.27	0.84
+ wet1 AMI <sup>2</sup> + wet2 AMI <sup>2</sup>	23	-761.57	1.97



**Figure 2.7: Predicted regional Barramundi year class strength (YCS,  $\pm 95\%$  CI) as a function of wet1 Australian monsoon index (AMI). YCS is shown as the number of 3-year old fish predicted per sample. Reproduced from Crook *et al.* (in review).**



**Figure 2.8: Predicted Barramundi year class strength index (YCS +/- 95% CI) for each river and wet season 1 Australian Monsoon Index (wet1 AMI) (black line). Reproduced from Crook *et al.* (in review).**

### ***River-specific patterns***

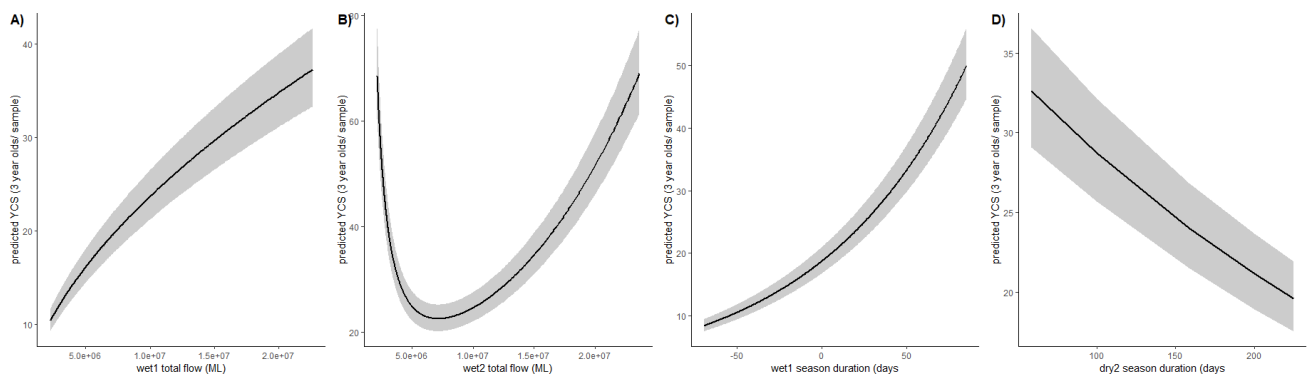
The addition of local extrinsic variables improved the fit of both the Daly and Roper river-specific YCS models (Table 2.7). Daly River YCS increased with wet1 total flow and wet1 duration, and displayed a curvi-linear relationship with wet2 total flow, where YCS was highest in low and high flow years (Table 2.8, Figure 2.9). Roper River YCS increased, albeit with greater uncertainty, with Wet 1 and Dry total flow, and Wet 1 and Wet 2 duration (Table 2.7, 2.8, Fig. 2.10). The marginal  $r^2$  was 0.955 for the best Daly River YCS model and 0.894 for the best Roper River model.

**Table 2.7: Comparison of best extrinsic factor YCS models including hydrological variables from the wet and dry season in the Daly and Roper rivers. Shown are the degrees of freedom (df), and  $\Delta$ AICc values for each model structure. The best model for each river is highlighted in bold. Reproduced from Crook *et al.* (in review).**

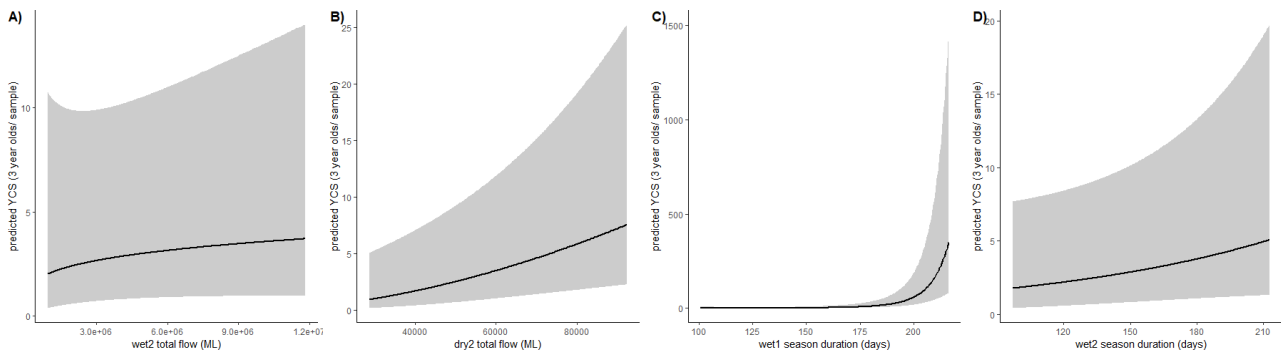
Model	df	logLik	$\Delta$ AICc
<i>Daly</i>			
intrinsic	11	-382.41	11.63
<b>+ wet1 totalF + wet2 totalF<sup>2</sup> + wet1 wetDur</b>	15	<b>-372.13</b>	<b>0</b>
+ wet1 totalF + wet2 totalF <sup>2</sup> + wet1 wetDur <sup>2</sup>	16	-371.08	0.19
+ wet1 totalF + wet2 totalF <sup>2</sup> + wet1 wetDur + dry2 dryDur	16	-371.52	1.08
<i>Roper</i>			
intrinsic	9	-158.31	42.32
<b>+ dry2 totalF + wet2 totalF + wet1 wetDur<sup>2</sup> + wet2 wetDur</b>	<b>16</b>	<b>-126.12</b>	<b>0</b>
+ dry2 totalF <sup>2</sup> + dry2 dryDur + wet1 totalF <sup>2</sup> + wet2 totalF + wet2 Dur <sup>2</sup>	17	-124.99	1.42
+ dry2 totalF <sup>2</sup> + wet2 totalF <sup>2</sup> + wet1 wetDur <sup>2</sup>	15	-129.2	2.62

**Table 2.8: Parameter estimates ( $\pm$ 95% confidence intervals) for best river-specific extrinsic effects models explaining sources of mortality variation in Barramundi. Reproduced from Crook *et al.* (in review).**

Daly		Roper	
Parameter	estimate (95% CI)	Parameter	estimate (95% CI)
intercept (angling)	-6.573 (-7.562, -5.642)	intercept (angling)	-6.387 (-8.081, -4.633)
Age-at-capture (angling)	-3.584 (-4.376, -2.866)	Age-at-capture (angling)	-2.108 (-4.258, 0.199)
Method (commercial)	1.629 (0.658, 2.673)	Method (commercial)	0.464 (-1.112, 1.953)
Method (electrofishing)	-8.773 (-15.973, -2.549)	Age-at-capture (commercial)	0.746 (-1.731, 3.069)
Age-at-capture (commercial)	1.318 (0.495, 2.162)		
Age-at-capture (electrofishing)	-5.854 (-10.79, -1.46)		
wet1 totalF	0.335 (0.076, 0.567)	Wet2 totalF	0.199 (-0.296, 0.653)
wet2 totalF	0.2 (0.045, 0.365)	Dry totalF	0.650 (0.209, 1.057)
wet2 totalF <sup>2</sup>	0.274 (0.133, 0.413)		
wet1 wetDur	0.407 (0.192, 0.604)	Wet1 wetDur	1.273 (0.879, 1.647)
		Wet1 wetDur <sup>2</sup>	1.211 (0.540, 1.8128)
		Wet2 wetDur	0.381 (-0.047, 0.808)



**Figure 2.9: Predicted Daly River Barramundi year class strength (YCS;  $\pm$  95% CI) as a function of A) wet1 total flow, B) wet2 total flow, C) wet1 duration and D) dry2 duration. Panels A-C are from the best fit model and D is from the third ranked model that was used for the water abstraction scenario testing (see text). YCS is shown as the number of three-year old fish predicted per sample. Adapted from Crook *et al.* (in review).**



**Figure 2.10: Predicted Roper River Barramundi year class strength (YCS; +/- 95% CI) as a function of A) wet 1 total flow, B) dry 2 total flow, C) wet 1 season duration and D) wet 2 season duration. YCS is shown as the number of three-year old fish predicted per sample.**

### ***Predicting the effects of water abstraction on YCS***

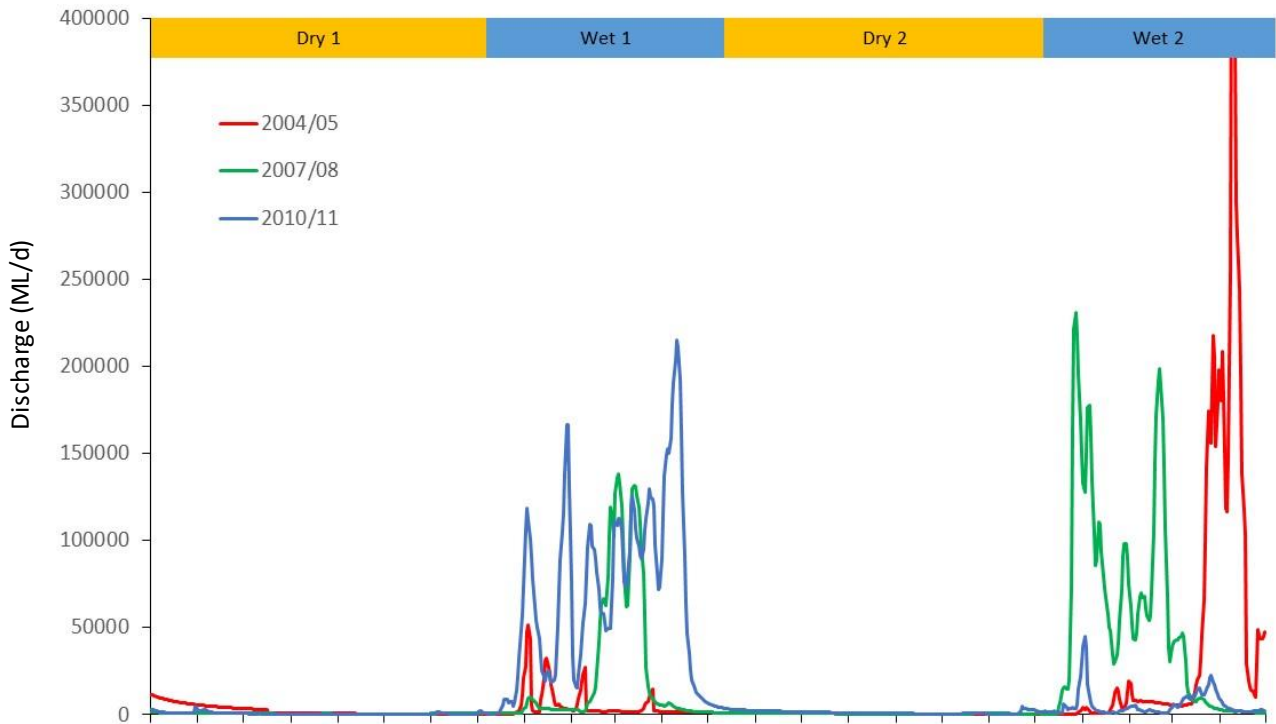
The river-specific YCS models were used to explore the potential impacts of flow abstraction on Barramundi recruitment in the Daly and Roper rivers. Flow abstraction scenarios were established during a meeting in August 2019 of the project team and water resource managers from the Water Resources Division of the NT DENR. The agreed scenarios for modelling included 10, 20, 30 and 40% abstraction of annual (whole year), dry season (May to October) and wet season (November to April) river discharge. These abstraction seasons used calendar dates rather than flow thresholds to facilitate direct integration of the results with current, date-based management. We also modelled a wet season abstraction scenario for both rivers in which water was only removed from the receding arm of the hydrograph according to environmental flow recommendations for the Daly River developed by Erskine *et al.* (2003).

Three hydrologically distinct years were selected for examination for each river, representing relatively low (2004/05), moderate (2007/08) and high (2020/11) annual discharge (Figure 2.11). We modified the daily hydrographs for each scenario by ‘abstracting’ the appropriate percentage (10, 20, 30, 40%) from the daily flow records during the relevant seasons. The modified hydrographs were then used to recalculate total flow and seasonal duration variables for each year which, in turn, were used to predict YCS and growth for each scenario and year.

To ensure that the modelled scenarios captured the effects of water abstraction across the time periods of interest, we used the model selection procedure to identify the best model that included variables from both dry and wet seasons. The best overall model for the Roper River included variables for both seasons and was used for the scenario modelling (Table 2.7). The two highest ranked models for the Daly River did not include dry season variables, so we used the third ranked model for the scenario modelling: the structure of the third ranked Daly River model is identical to the first ranked model except for the addition of a ‘dry2 dryDur’ term (Table 2.7, Figure 2.9).



(a) Daly River



(b) Roper River

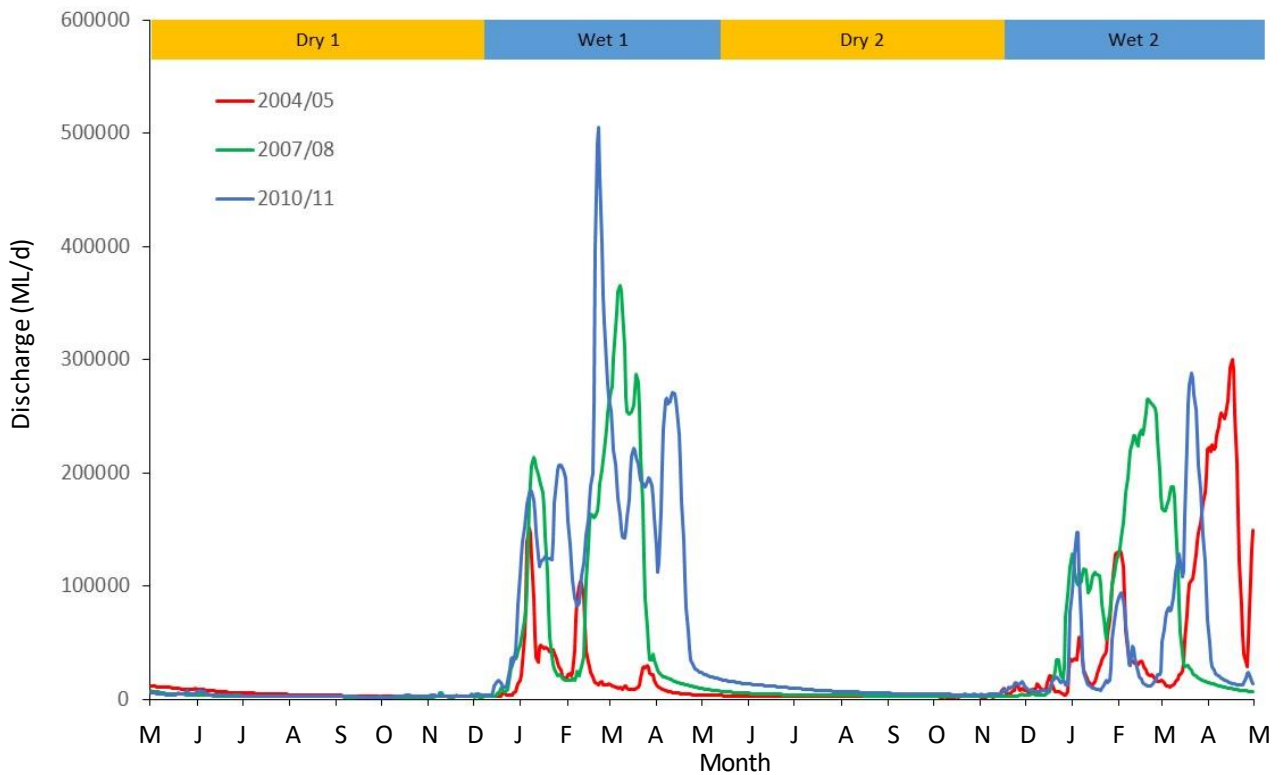
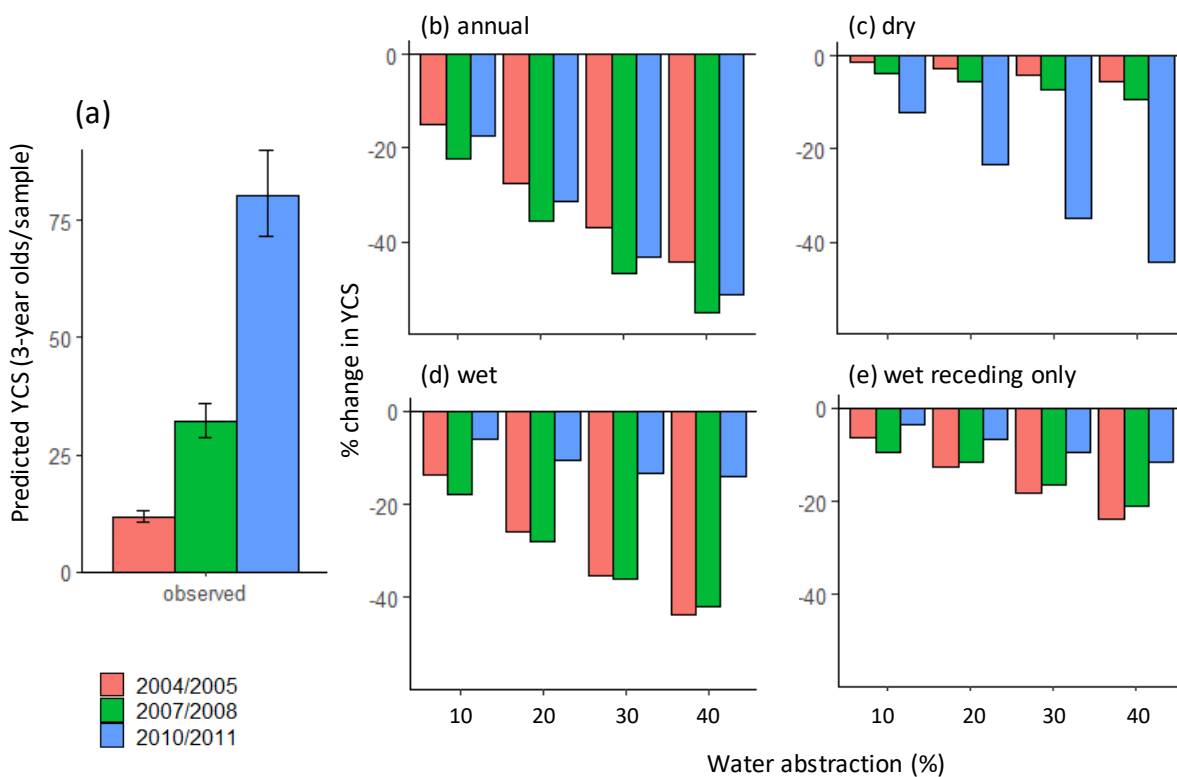


Figure 2.11: River discharge for the three years used in the flow abstraction scenario modelling for the (a) Daly River and (b) Roper River. Reproduced from Crook *et al.* (in review).

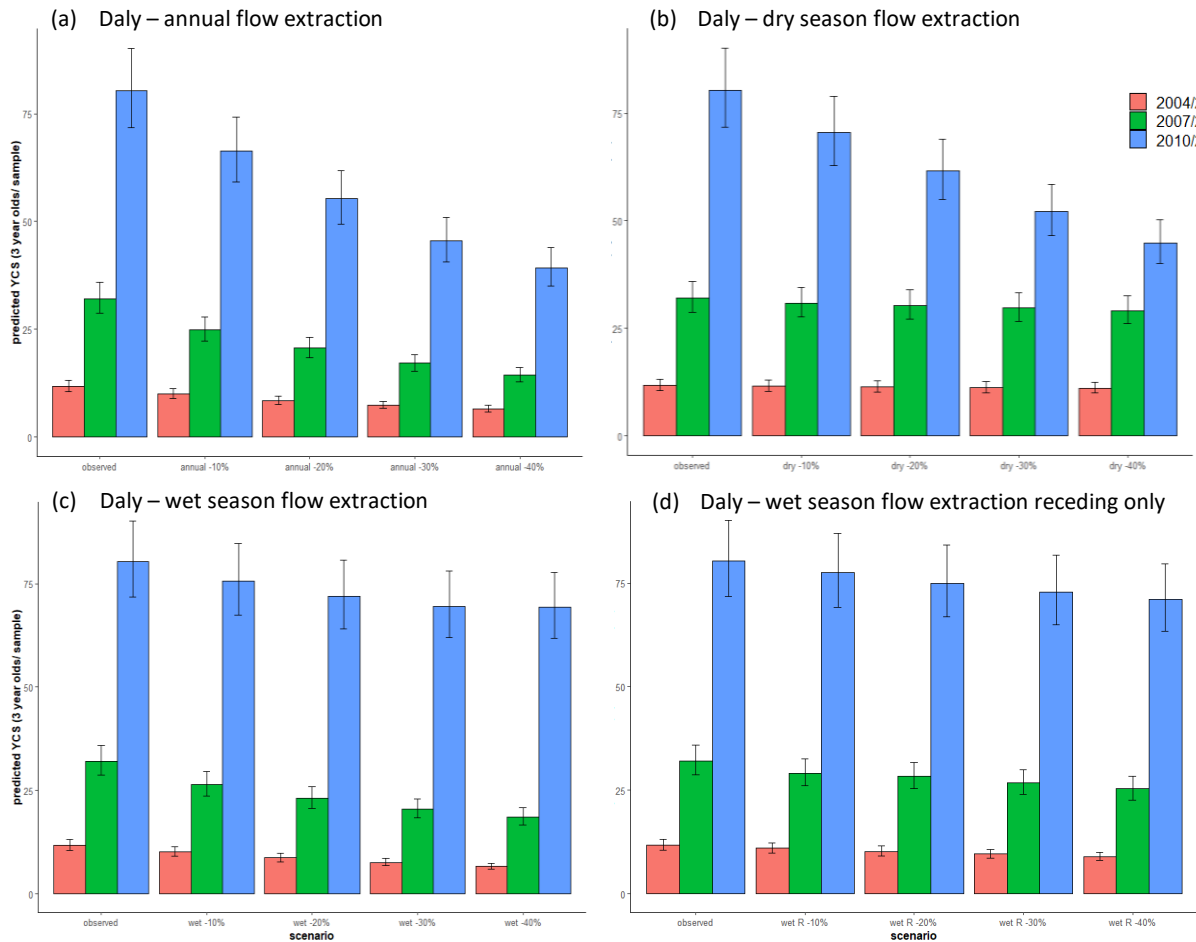
## Daly River

Predicted recruitment in the Daly River based on the observed river discharge varied greatly among years, with the number of recruits highest in the high flow year (2010-11) and lowest in the low flow year (2004/05) (Figure 2.12a). Water abstraction across the whole year resulted in strong percentage declines in recruitment across all years (Figure 2.12b). While the percentage declines were similar across years, the effect of annual abstraction on the number of recruits was highest in the high flow year due to the much higher predicted YCS in that year (Figure 2.13a).

Dry season extraction had a predicted negative impact on YCS across all years, but the effect was strongest in the high flow year both in terms of percentage decline (Figure 2.12c) and the number of recruits (Figure 2.13b). Wet season abstraction also had a negative impact on YCS across all years, however the effect was most pronounced in the low and moderate flow years (Figure 2.12d, 2.13c). Imposition of the receding arm abstraction flow rule during the wet season (as recommended by Erskine *et al.* 2003) reduced the predicted decline in percentage recruitment and number of recruits across all years in comparison to the unrestricted wet season abstraction scenarios (Figure 2.12e, 2.13d).



**Figure 2.12: Modelled % change of water abstraction on Barramundi recruitment in the Daly River.** (a) predicted YCS for Daly River for the three scenario years based on observed hydrology; (b) annual water extraction scenario results; (c) dry season water extraction scenario results; (d) wet season water extraction scenario results; (e) receding limb wet season extraction scenario results.

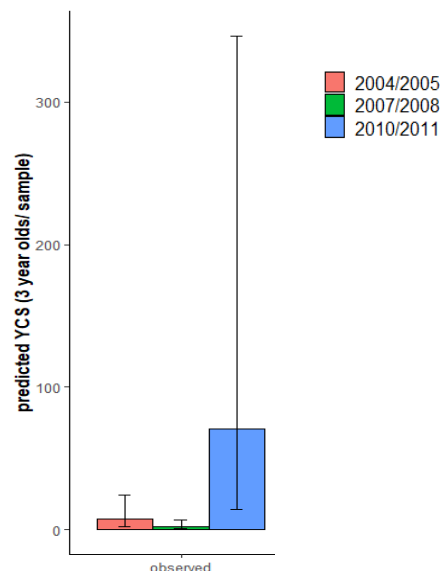


**Figure 2.13: Modelled effects of water abstraction on numbers of Barramundi recruits in the Daly River. (a) annual water extraction scenario results; (b) dry season water extraction scenario results; (c) wet season water extraction scenario results; (d) receding limb wet season extraction scenario results. Reproduced from Crook *et al.* (in review).**

### Roper River

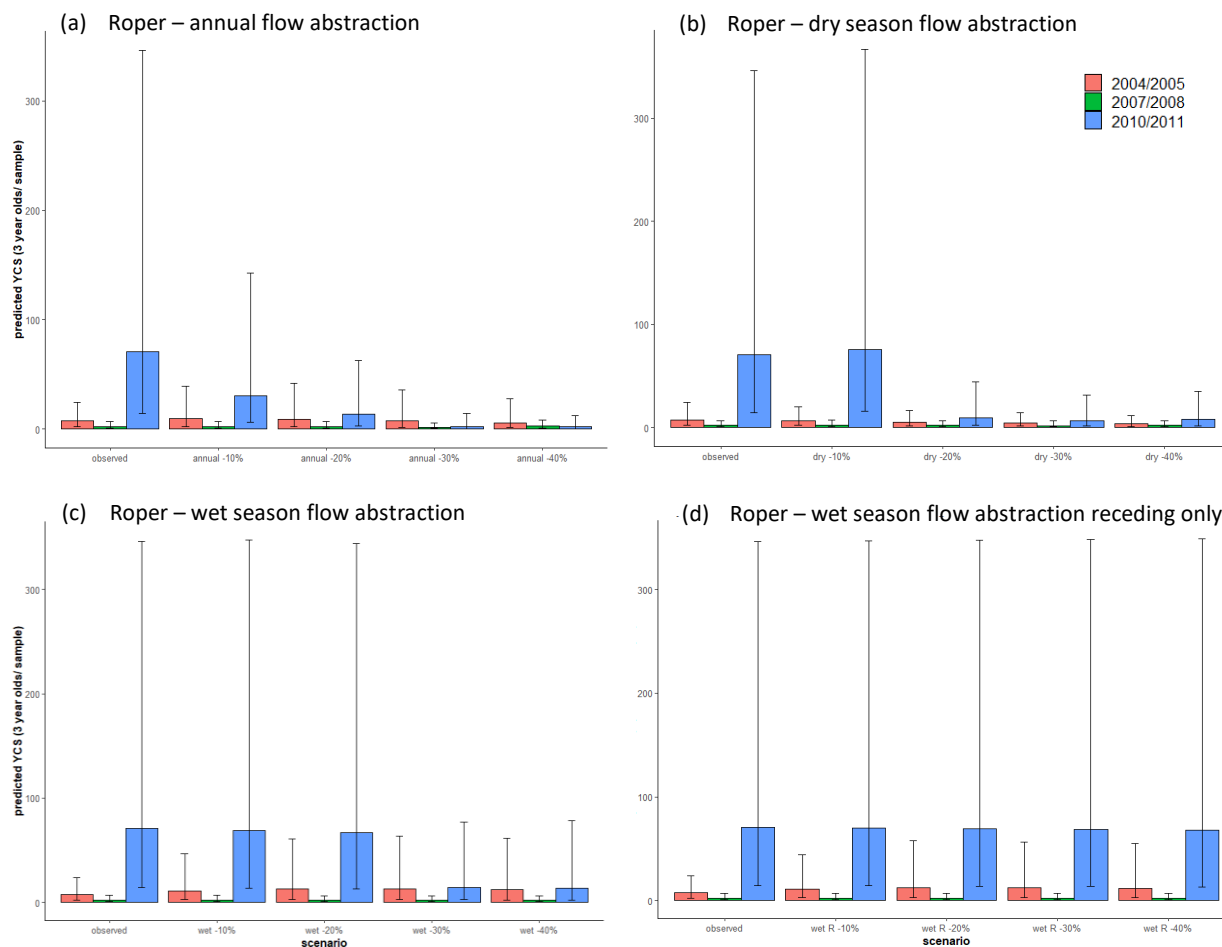
Estimates of predicted YCS for the Roper River had high levels of uncertainty (wide 95% CIs) in comparison to the Daly River analyses (Figure 2.14). Predicted recruitment was dominated by a large number of recruits in the high flow year (2010/11) and very low levels of recruitment in the moderate (2007/08) and low (2004/05) flow years.

The modelled effects of water abstraction in the Roper River in the moderate and low flow years were negligible relative to model error due to the low levels of recruitment in these years (Figure 2.15). Although the error around YCS estimates was also high in the high flow year, large decreases in predicted YCS were associated with annual flow abstraction in the high flow year (Figure 2.15a).



**Figure 2.14: Predicted YCS for Roper River for the three scenario years based on observed hydrology.**

Dry season flow abstraction of  $\geq 20\%$  had a strong negative predicted impact on YCS (Figure 2.15b) and wet season abstraction had a strong negative predicted effect at  $\geq 30\%$  in the high flow year (Figure 2.15c). Limiting wet season water abstraction to the receding arm of the hydrograph resulted in no effect of water abstraction on predicted YCS (Figure 2.15d).



**Figure 2.15: Modelled effects of water abstraction on numbers of Barramundi recruits in the Roper River. (a) annual water extraction scenario results; (b) dry season water extraction scenario results; (c) wet season water extraction scenario results; (d) receding limb wet season extraction scenario results. Reproduced from Crook *et al.* (in review).**

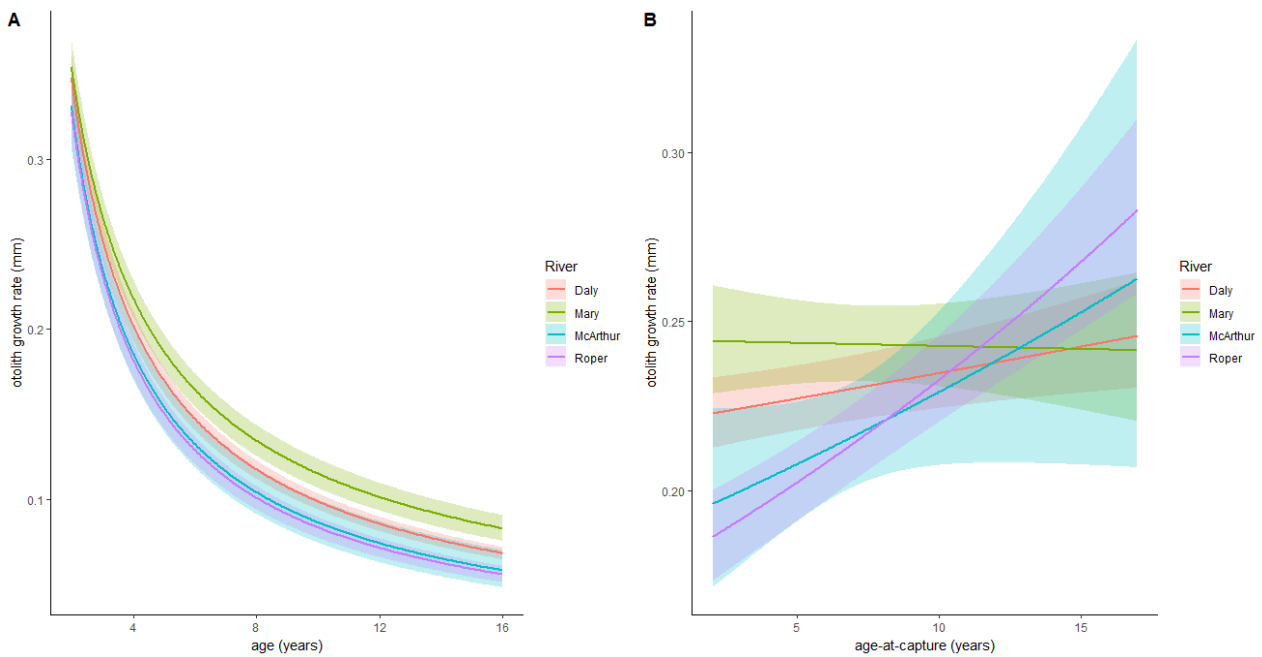
## 2.3.2. Growth rates

### *Regional patterns*

The best supported intrinsic growth model structure included interactions between age and river, and age-at-capture and river (Table 2.9). Overall, growth declined with age, but Mary River fish had higher age-specific growth than those in other rivers (Figure 2.16A; Table 2.10). Older fish at capture in the McArthur, Roper and Daly rivers all grew faster than those captured at younger ages (Figure 2.16B; Table 2.10). The greatest variation around average Barramundi growth (model intercept) was attributable to river-specific differences in annual growth, followed by fish-specific differences in annual growth (i.e. faster or slower growing individuals; Table 2.10). Fish growth rates across all rivers tended to increase through time from a low in 2002 (Figure 2.17). Daly and Roper annual growth peaked in 2009-2010, whereas Mary river growth peaked in 1995, 2011, and 2015. We found no strong evidence to suggest the presence of regional synchrony in growth across the four rivers, with all pairwise correlations between rivers for annual growth being non-significant ( $p > 0.10$ ; Figure 2.18). Nonetheless, there were weak positive and non-significant correlations between all rivers and the strength of these correlations tended to increase over time (Figure 2.18).

**Table 2.9: Results of intrinsic effects model selection exploring sources of annual growth variation across the four rivers. Shown are the degrees of freedom (df), log likelihood (logLik) and the  $\Delta AICc$  values for each fixed effect structure. The best model is highlighted in bold.**

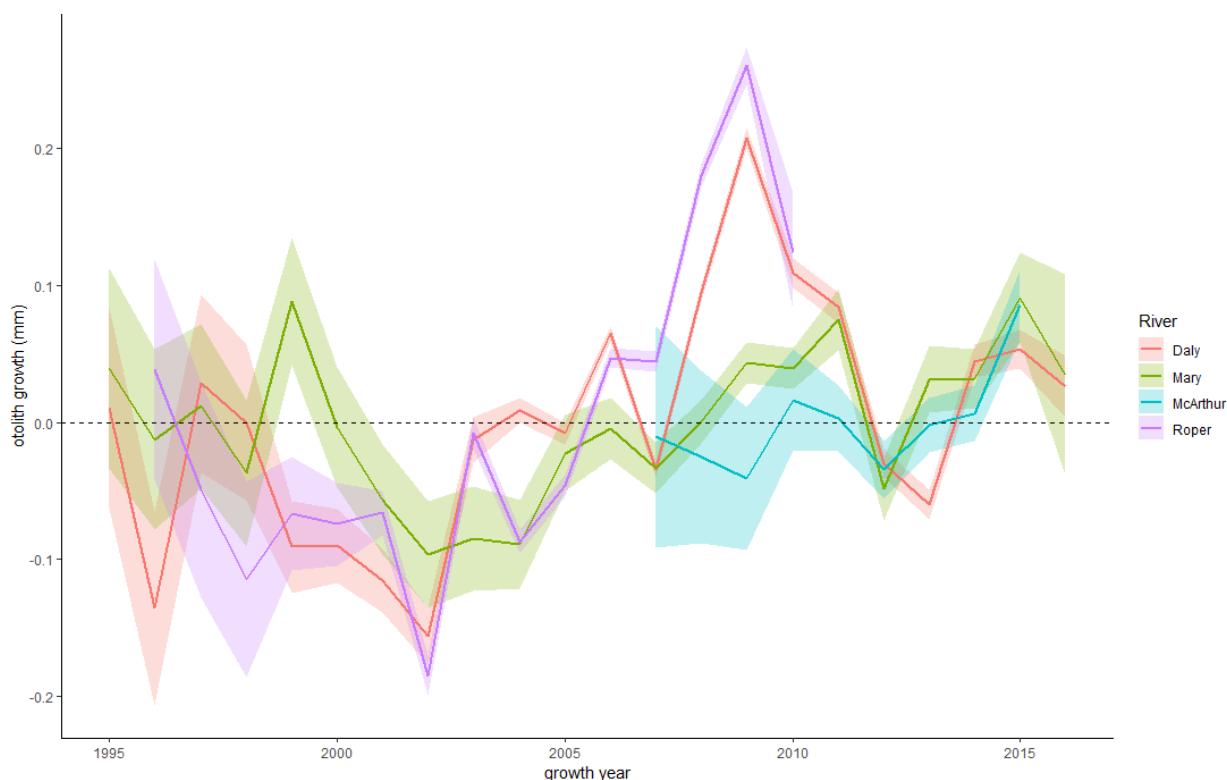
Model	df	logLik	$\Delta AICc$
<b>Age*River + Age-at-capture*River</b>	<b>17</b>	<b>3508</b>	<b>0</b>
Age*River + Age-at-capture	14	3495	19.9
Age + Age-at-capture*River	14	3492.5	25
Age + Age-at-capture + River	11	3489.3	25.4
Age + Age-at-capture	8	3483.9	30.1
Age*River	13	3484.2	39.4
Age + River	10	3477.6	46.7
Age	7	3470.9	54.1



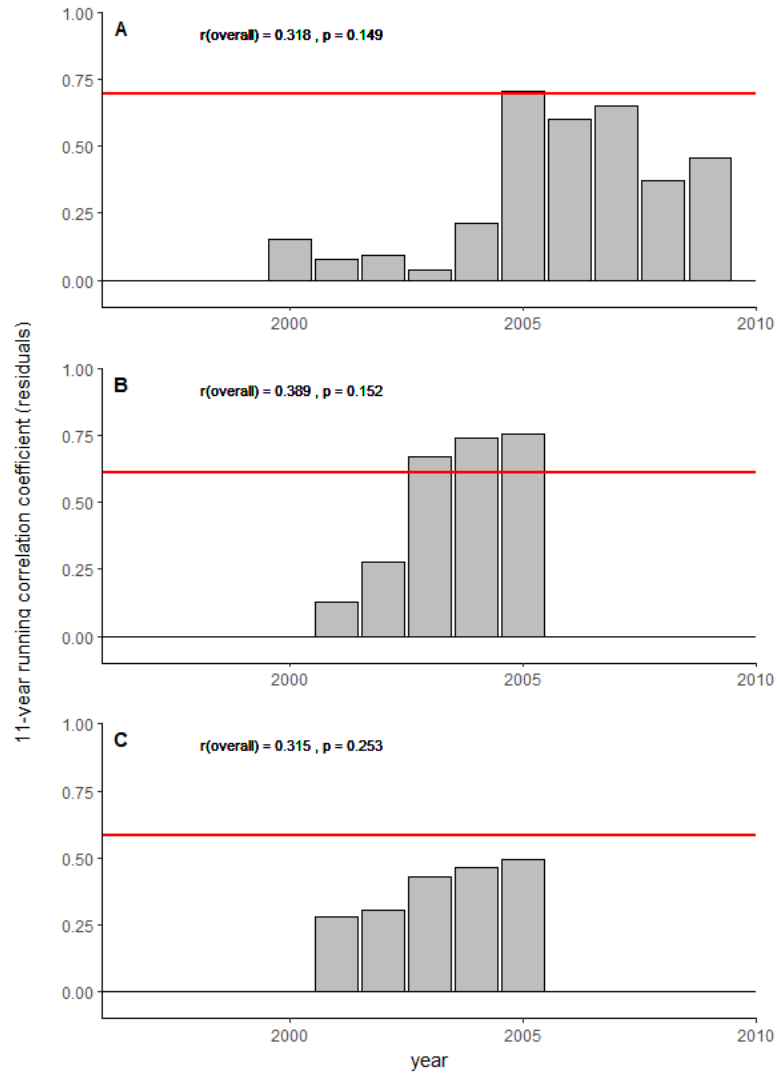
**Figure 2.16: Annual otolith growth variation (mm of otolith growth,  $\pm 95\%$  CI) in Barramundi from four rivers as a function of A) age and B) age at capture.**

**Table 2.10: Parameter estimates ( $\pm 95\%$  confidence intervals) for best intrinsic effects model explaining annual growth variation in Barramundi across four rivers.**

Intrinsic fixed effects	
Parameter	Estimate (95% CI)
Intercept (Daly)	-1.479 (-1.517, -1.435)
Age (Daly)	-0.344 (-0.351, -0.336)
Age-at-capture (Daly)	0.016 (0.004, 0.027)
River (Mary)	0.064 (0.004, 0.123)
River (McArthur)	-0.075 (-0.162, 0.016)
River (Roper)	-0.093 (-0.168, -0.024)
Age (Mary)	0.037 (0.016, 0.059)
Age (McArthur)	-0.022 (-0.069, 0.02)
Age (Roper)	-0.03 (-0.047, -0.014)
Age-at-capture (Mary)	-0.018 (-0.042, 0.004)
Age-at-capture (McArthur)	0.031 (-0.022, 0.088)
Age-at-capture (Roper)	0.051 (0.029, 0.074)
Random effects	
Variance component	Standard deviation
FishID	0.08774
Age FishID	0.05996
River:growth year	0.797
Residual	0.14455



**Figure 2.17: Temporal variation in Barramundi annual growth variation (mm of otolith growth,  $\pm$  standard error) across the four rivers. Dotted horizontal lines represent long-term mean growth (model fixed effect intercept).**

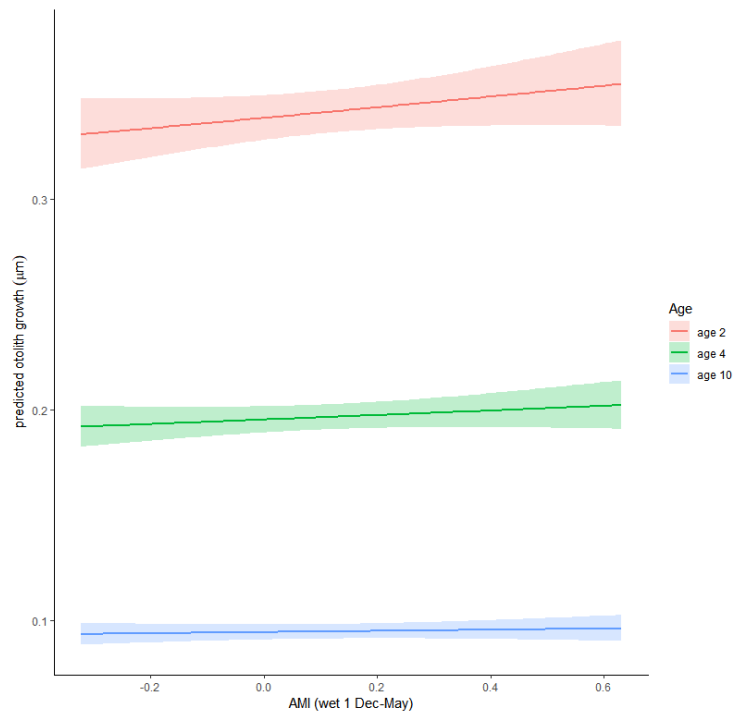


**Figure 2.18: 11-year running correlation between a) Daly and Mary river residual growth, b) Daly and Roper River residual growth and c) Mary and Roper River residual growth. Running correlations above the solid red line are significant at the 95% level.  $r(\text{overall})$  is the Pearson's correlation coefficient across the whole period. Note, 11-year running correlations could not be calculated with the nine years of McArthur River growth.**

The best regional growth model did not include wet season AMI (marginal  $r^2 = 0.757$ ). However, the second ranked growth model, which also received support from the data ( $\Delta\text{AICc} = 0.04$ ), included an interaction between wet1 AMI and age (Table 2.11). Thus, there was some evidence that younger Barramundi grew faster in years with stronger AMI, and that this effect declined with age (Figure 2.19).

**Table 2.11: Model selection exploring importance of wet season AMI in describing regional patterns in Barramundi growth. Shown are the top three models with degrees of freedom (df), AICc and  $\Delta\text{AICc}$  values for each model structure. The best model is highlighted in bold.**

Model	df	logLik	$\Delta\text{AICc}$
<b>Intrinsic</b>	<b>17</b>	<b>3507.97</b>	<b>0</b>
+ $\text{AMI}_{(\text{wet1})} * \text{Age}$	19	3509.95	0.04
+ $\text{AMI}_{(\text{wet1})}$	18	3508.87	0.19



**Figure 2.19: Predicted Barramundi growth (increment width +/- 95% CI) as a function of December-May AMI and fish age.**

### ***River-specific patterns***

The addition of local extrinsic variables improved the fit of all river-specific growth models, with the effect of these variables generally being highly age-dependent (Table 2.12). In comparison to the YCS analyses, the growth results were complex and difficult to interpret with regards to the effects of river hydrology on growth. Marginal  $r^2$  was 0.717 for the best Daly River growth model and 0.729 for the best Roper River model.

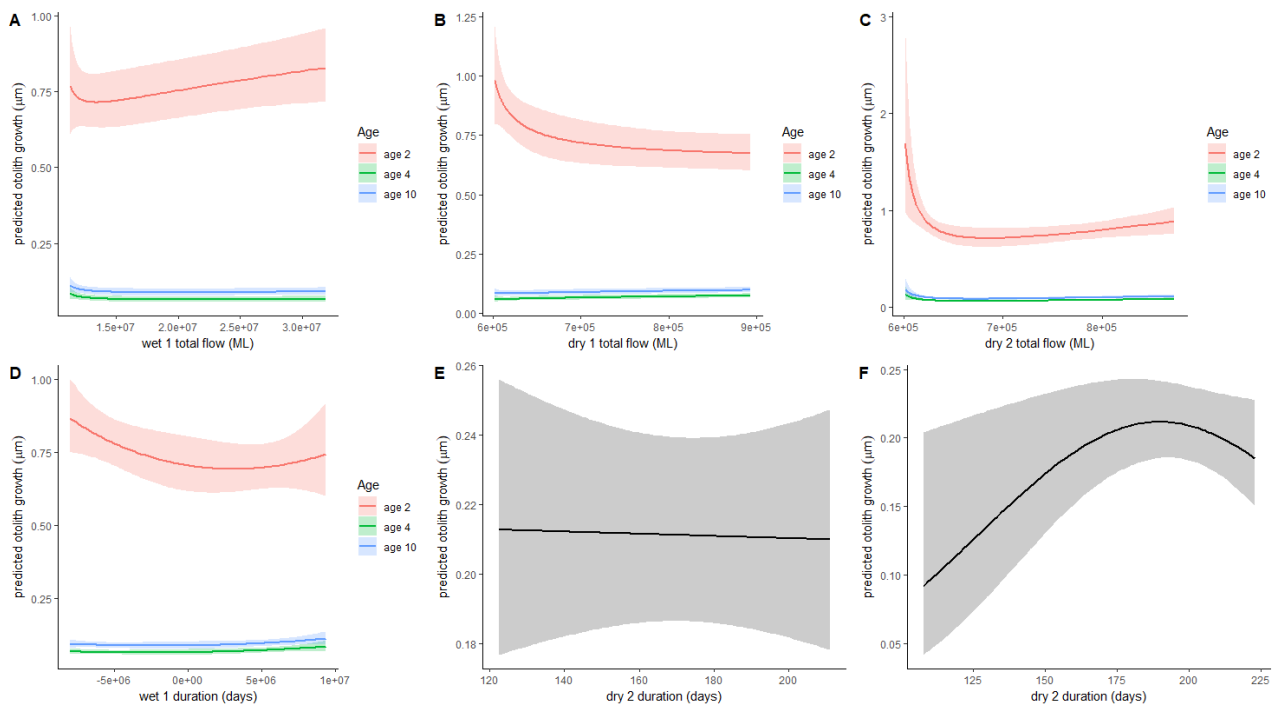
In the Daly River analyses, younger Barramundi tended to have faster growth rates than older fish in years of relatively high wet1 flows (Figure 2.20A), whereas younger Barramundi had slower growth rates when dry1 flows were high (Figure 2.20B). Younger Daly River Barramundi grew fastest when dry season flows were lower (Figure 2.20C). Older Barramundi had relatively fast growth rates in years with long wet seasons (Figure 2.20D) and all age classes grew faster in years where dry2 duration was long (Figure 2.20F).

Roper River Barramundi growth also displayed complex age-dependent relationships relative to hydrological and thermal conditions. The growth of all age classes was not influenced by wet season flow (Figure 2.21A). Younger fish grew faster when dry1 had high flows (Figure 2.21B) but marginally slower when dry2 had high flows (Figure 2.21C). The opposite pattern was observed for the growth of old fish during the dry season (Figure 2.21D-F). Young fish grew considerably faster when thermal conditions in the dry season were closely aligned with optimal temperatures identified in aquaculture (Figure 2.21G). However, these thermal conditions were associated with a decline in the growth of older fish (Figure 2.21G).

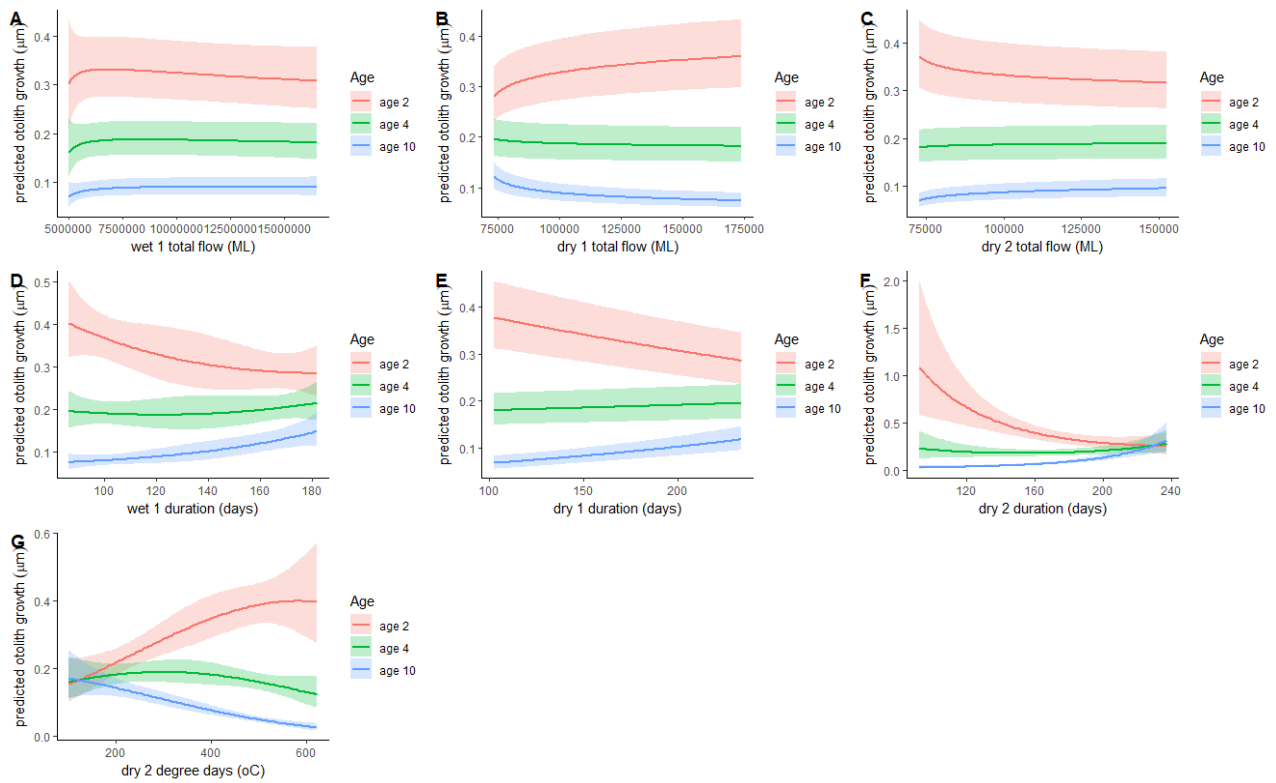


**Table 2.12: Comparison of the three best extrinsic factor growth models including hydrological variables from the wet and dry season, within each of three rivers. Shown are the degrees of freedom (df), and  $\Delta$ AICc values for each model structure. The best model for each river is highlighted in bold.**

Model	df	logLik	$\Delta$ AICc
<i>Daly</i>			
intrinsic	8	1594.51	46.4
<b>+ wet1 totalF * Age + wet1 totalF^2 + wet1 wetDur * Age + wet1 wetDur^2 + dry1 totalF * Age + dry1 totalF^2 + dry2 totalF * Age + dry2 totalF^2 + dry1 dryDur + dry2 dryDur^2</b>	<b>23</b>	<b>1632.8</b>	<b>0</b>
+ wet1 totalF * Age + wet totalF^2 + wet1 wetDur * Age + wet1 wetDur^2 + dry1 totalF * Age + dry2 totalF * Age + dry2 totalF^2 + dry1 dryDur + dry2 dryDur^2	22	1631.69	0.19
+ wet1 totalF * Age + wet totalF^2 + wet1 wetDur * Age + wet1 wetDur^2 + dry1 totalF * Age + dry2 totalF * Age + dry2 totalF^2 + dry1 dryDur^2 + dry2 dryDur^2	23	1632.68	0.23
<i>Roper</i>			
intrinsic	9	1644.44	261.09
<b>+ wet1 totalF * Age + wet1 totalF^2 + wet1 wetDur * Age + wet1 wetDur^2 + dry1 totalF * Age + dry2 totalF * Age + dry1 dryDur * Age + dry2 dryDur * Age + dry2 dryDur^2 + dry2 dryDD * Age + dry2 dryDD^2</b>	<b>27</b>	<b>1793.19</b>	<b>0</b>
+ wet1 totalF * Age + wet totalF^2 + wet1 wetDur * Age + dry1 totalF * Age + dry2 totalF * Age + dry1 dryDur * Age + dry2 dryDur * Age + dry2 dryDur^2 + dry2 dryDD * Age + dry2 dryDD^2	26	1792.6	1.17
+ wet1 totalF * Age + wet totalF^2 + wet1 wetDur * Age + wet1 wetDur^2 + dry1 totalF * Age + dry1 totalF^2 + dry2 totalF * Age + dry1 dryDur * Age + dry1 dryDur^2 + dry2 dryDur * Age + dry2 dryDur^2 + dry2 dryDD * Age	28	1793.39	1.63



**Figure 2.20: Predicted Daly river Barramundi growth. Age-dependent effects of A) wet1 total flow; B) dry1 total flow; C) dry2 total flow and D) wet1 duration. E) Effect of dry1 duration and F) effect of dry2 duration.**

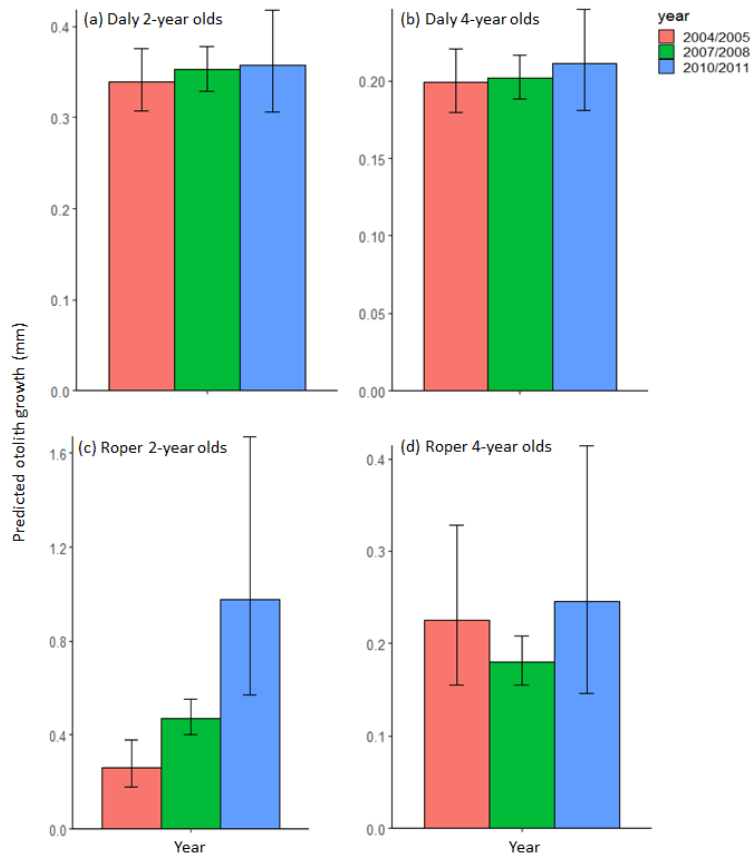


**Figure 2.21: Predicted Roper River Barramundi growth. Age-dependent effects of A) wet1 total flow; B) dry2 total flow; C) wet1 duration, D) dry1 duration, and E) dry2 duration.**

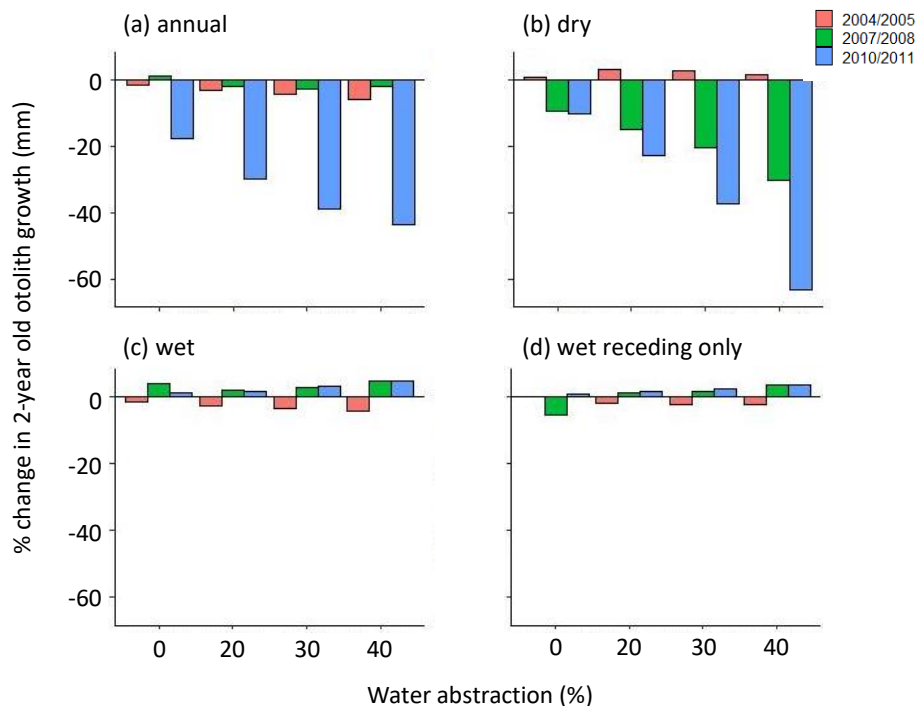
### ***Predicting the effects of water abstraction on growth***

The river-specific growth models were used to explore the potential impacts of flow abstraction on Barramundi growth in the Daly River and Roper River. Given the strong effect of fish age in the growth models, we examined growth rates in two age groups (age-2, age-4) for comparative purposes. The same flow abstraction scenarios and years used for the YCS analysis were examined in the growth scenario analysis. The best models for each river were used and dry season degree days in the Roper growth model was fixed at the long-term average for this exercise (Table 2.12).

The results of the growth scenario modelling showed no differences among the wet, average and dry years for 2-year old fish in the Daly river and 4-year old fish in both the Daly and Roper rivers (Figure 2.22). We therefore did not explore specific flow scenarios using these models. However, there were strong differences in the growth rates of 2-year olds in the Roper River, with growth highest in the wet year and lowest in the dry year (Figure 2.22c). These differences in growth were driven by variation in dry season flows. Water abstraction modelling predicted strong effects on Roper 2-year old growth of dry season flow abstraction in the high flow (2010/11) and moderate flow (2007/08) years but not in the low flow year, and negligible effects of wet season abstraction across all years (Figure 2.23).



**Figure 2.22: Predicted otolith growth for 2- and 4-year old fish in the Daly River (a, b) and Roper River (c, d) for the three scenario years based on observed hydrology.**



**Figure 2.23: Modelled % change of water abstraction on Barramundi otolith growth for Roper River 2-year old fish in dry (2004/05), average (2007/08) and wet (2010/11) years. (a) annual water abstraction scenario; (c) dry season water abstraction scenario; (d) wet season water abstraction scenario; (e) receding limb wet season abstraction scenario.**

## 2.4. Discussion

The results of the regional analyses revealed a strong positive relationship between Barramundi year class strength (YCS) and spawning season Australian Monsoon Index (wet1 AMI) across the four study rivers (Daly, Roper, Mary, McArthur). This finding appears to reflect a broad-scale influence of monsoonal activity for Barramundi recruitment, and aligns with the conclusions of several previous studies suggesting that large-scale climatic factors which drive regional rainfall strongly influence recruitment in Barramundi (e.g., Balston 2009a, b; Meynecke *et al.* 2006; Meynecke and Lee 2011). Mechanisms suggested to explain the positive relationship between rainfall and Barramundi recruitment include increased availability of supralittoral nursery habitat for settling larvae and juveniles, enhanced downstream migration opportunities for mature freshwater-resident fish, improved access to freshwater habitats for young-of-year fish and increased food web productivity during high river flows (Davis 1985; Russell and Garrett 1983; Staunton-Smith 2004; Robins *et al.* 2005).

The strength of the relationship between wet1 AMI and YCS suggests that AMI has the potential to be incorporated into population models that inform the management of Barramundi across much of northern Australia. For example, the model used by Tanimoto *et al.* (2012) to examine the effects of flow abstraction in the Fitzroy River in Queensland included predictions of annual recruitment derived from a Beverton-Holt stock-recruitment function, adjusted by thresholds in summer and spring river discharge. Discharge thresholds were based on the YCS analyses of Staunton-Smith *et al.* (2004) and Halliday *et al.* (2011) and the steepness parameter<sup>4</sup> was set at 0.5, 0.7 and 0.9 to assess the sensitivity of the population models to variations in the shape of the stock-recruitment curve. Incorporation of a climatic variable that can accurately predict annual recruitment at the regional scale has the potential to improve the generality, accuracy and precision of this type of model, and may be especially useful in situations where reliable gauged or modelled flow data is unavailable.

Similarly, AMI could be incorporated into population models focussed specifically on stock assessment for fishery management. A recent stock assessment for Barramundi fisheries in Queensland (Streipert *et al.* 2019) used a population model that did not incorporate rainfall or river flow data, but relied on a Beverton-Holt stock-recruitment function ( $h$  fixed at 0.7) to reflect the long-term dynamics of the population. Annual stochastic variability was added to the recruitment time-series by incorporating an adjustment factor ( $\exp[\epsilon(t) - 0.5\sigma^2]$ ) into the stock-recruitment function (see Schirripa *et al.* 2009; Streipert *et al.* 2019). Adjustment of the stock-recruitment function in this model using anomalies in AMI could potentially improve the ability to accurately estimate the status of Barramundi populations. Currently, DPIR does not conduct a stock assessment model for Barramundi. However, based on the results of the current study a model could be developed that adjusts the stock recruitment relationship using the AMI anomalies in a similar way to Streipert *et al.* (2019). An alternative approach could be to a simplified stock assessment model that removes the stock recruitment relationship as the results of this study indicate that stock size may not be important in influencing recruitment strength. It should be noted, however, that our regional YCS model was developed using only data from the NT. Evaluation of relationships between AMI and Barramundi recruitment in other geographic regions, as well as additional assessment of the predictive capacity of AMI models using independent data (see Myers 1998), would be required before this parameter can be confidently incorporated into population models outside of the NT.

The river-specific analyses conducted on the Daly and Roper rivers demonstrated strong relationships between YCS and a range of river hydrology variables. The YCS-hydrology relationships were complex and multifaceted, particularly in the Roper River, where a combination of ~~non-linear~~ wet season and dry season variables were included in the highest ranked model. YCS-hydrology relationships were simpler in the Daly River, with three wet season variables included in the best model. The third-ranked Daly River model that was used for the water abstraction scenarios had similar explanatory capacity to the first-ranked model but included a single dry season variable (dry2 duration). Relationships between annual growth and river

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<sup>4</sup> Steepness ( $h$ ) is the proportion of recruitment obtained when spawner biomass is reduced to 20% of its pristine (unexploited) level.

hydrology were highly complex and difficult to interpret, with the best models for both rivers composed of non-linear, age-specific relationships for both wet and dry season variables.

The existence of complex and multi-faceted relationships between Barramundi recruitment and river hydrology is to be expected considering the myriad of interacting processes that influence complex ecological systems like rivers and estuaries (see Boehlert and Mundy 1988). Identifying the best combination of predictor variables that can explain variation in Barramundi catch data is a model selection challenge shared across the sciences (Burnham and Anderson 2002, Hooten and Hobbs 2015). We adopted a systematic and comprehensive approach to generating biologically relevant predictor variables and time periods, and then comparing a suite of candidate models.

The type of hydrological variable included in our models was limited to those that could explain the total amount of water passing down the river at biologically relevant time periods, and those linked to seasonal duration. Other studies have looked at a wide array of potentially correlated predictor variables (rainfall, discharge, river and sea temperatures) calculated over different time periods and set at a range of lags of up to five years (e.g., Balston, 2009). Such an approach often identifies 'significant' relationships, but the underlying mechanism can be difficult to explain. We adopted an information-theoretic approach to model selection (Burnham and Anderson 2002) which enabled us to test plausible combinations of parameters and to use this to identify the best model within our candidate set. Other model selection approaches, such as stepwise model selection (e.g., Meynecke and Lee 2011), have well-known biases and short-comings and are not advocated in the general ecological literature (Whittingham *et al.* 2006).

Fisheries catch data includes signals from a range of sources, including those that reflect catchability and underlying recruitment dynamics (Halliday and Robins 2007; Meynecke *et al.* 2010). This can make it a challenging exercise to identify which factors are actually driving variation in underlying population dynamics. Modelling catch-at-age data allowed us to more closely explore actual recruitment (YCS) variation. Nonetheless, different gear selectivity and fishing effort can make it difficult to compare data collected from different rivers at different times. The statistical approach employed in our study (discussed in further detail in Morrongiello *et al.* 2014) explicitly addresses and controls for many potential biases in the underlying catch-age data by allowing for variable age-dependent mortality functions for different gears (e.g., recreational fishing vs commercial netting) and rivers (e.g., different mortality regimes in Daly vs Roper), and also conditions catches on the underlying effort (sample size). Morrongiello *et al.* (2014) also highlighted how traditional Gaussian catch curve analyses (Maceina 1997; Maceina 2003; Staunton-Smith *et al.* 2004) have limitations when data sets contain zeros (i.e., years of very poor recruitment), and they do not treat the underlying data as counts (non-negative) which, in fact, they are. The generalised linear model formulations of catch curve regression using Poisson and negative binomial distributions used here, coupled with an information theoretic model selection approach, therefore represents a methodological improvement on previous techniques to estimate the drivers of YCS variation in Barramundi.

In contrast to the YCS analysis, we did not identify a strong regional-scale relationship between Barramundi growth and AMI. Instead, we identified complex river-specific patterns associated primarily with dry and/or wet season river hydrology. Growth in fish is affected by a range of interacting physiological and environmental factors, including temperature, density-dependent competition, food web productivity and habitat quality (Crook and Gillanders 2013), so it is not surprising that our detailed analysis of growth responses to environmental conditions revealed high levels of complexity. Robins *et al.* (2006) also reported significant effects of river flow on Barramundi growth (expressed as  $K^5$ ) using seasonal von Bertalanffy growth models based on tag-recapture data from the Fitzroy River, Queensland. The use of tag-recapture data did not allow Robins *et al.* (2006) to explore relationships between growth and river hydrology at fine temporal time scales and, thus, their findings likely reflect the cumulative effects of the local hydrological regime over the whole period that tagged fish were at liberty. The analyses of Robins *et al.* (2006) are consistent with the current study, but do not capture the level of complexity that our results suggest underlies relationships between fish growth and river hydrology. Increased understanding of the intrinsic and extrinsic

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<sup>5</sup> In von Bertalanffy growth models,  $K$  (the growth rate coefficient) represents the exponential rate of approach to the asymptotic size.

factors that drive growth rates in Barramundi populations in different geographic and climatic regions is an important area for future research.

Scenario modelling based on the relationships identified in the GLMMs predicted that water abstraction during both the wet and dry seasons may have strong impacts on Barramundi recruitment in the Daly and Roper rivers and, to a lesser extent, annual growth for younger age cohorts in the Roper River. The predicted effects of water abstraction varied considerably between the rivers, were strongly seasonal, generally increased as the volume of water abstracted increased and were highly dependent on wet season strength in each year. An environmental flow rule recommended for the Daly River by Erskine *et al.* (2003) to limit wet season abstraction to periods when discharge is declining was predicted to have considerable benefits for YCS in the Daly River in the low and moderate flow years. This flow rule was also predicted to greatly reduce the impacts of 30% and 40% flow abstraction on YCS for the high flow year in the Roper River.

## **Conclusions**

The results of the current study provide strong evidence of the detrimental effects that water resource development may have on Barramundi fisheries in the future. Predicted effects of water abstraction on annual YCS exceeded 30% in many cases, which would certainly have major impacts on recreational, commercial and subsistence fisheries in these systems. Our projections are broadly consistent with recent qualitative and quantitative risk assessments undertaken as part of the Flinders and Gilbert Agricultural Resource Assessment (FGARA) examining the sustainability of agricultural development in the Flinders and Gilbert catchments in the Gulf of Carpentaria. These assessments rated the risk of altered flow regimes to Barramundi fisheries as “high” (Griffiths *et al.* 2014) and predicted strong impacts of flow abstraction on Barramundi catches and recruitment in the Flinders and Gilbert rivers (Bayliss *et al.* 2014). It is important to note that our predicted effects of water abstraction varied considerably among years, seasons and river systems, which likely reflects complex associations between river flows and fish recruitment and growth. This complexity introduces some uncertainty into the model predictions which should be considered when interpreting the information presented in this report. Nonetheless, our analyses provide statistically rigorous, system-specific predictions for the Daly and Roper rivers which can be used to directly inform water resource policy and management in the NT and, potentially, other regions of northern Australia.

The results of the current study also have significant implications for the assessment and management of Barramundi fisheries across Australia. For example, our finding that a high proportion of variance in recruitment can be explained without reference to spawning stock size raises important questions regarding the ways that stock-recruitment functions are used for modelling Barramundi populations. Our regional and river-specific YCS models were based on long-term time series (1991-2014) and explained ~86-94% of variance in catch-at-age across river systems with distinctive geographic, hydrologic and catchment properties. This finding suggests that broad-scale climate, expressed locally as variation in rainfall and river discharge, is a primary driver of recruitment across the study region. The broader applicability of this conclusion is supported by the many previous examples of positive correlations between Barramundi recruitment and rainfall/river discharge across a wide geographic range (e.g., Staunton-Smith *et al.* 2004; Robins *et al.* 2005; Halliday *et al.* 2010; 2012; Meynecke *et al.* 2014; Bayliss *et al.* 2014).

Barramundi population models used to inform water resource and fishery management (e.g., Tanimoto *et al.* 2012; Streipert *et al.* 2019) have assumed a static underlying relationship between spawning stock size and recruitment, which can be adjusted to account for annual recruitment variation by adding additional terms to the recruitment function. However, if annual recruitment is driven primarily by variation in environmental factors rather than population fecundity - for instance, the area of seasonally inundated nursery habitat (Davis 1985; Staunton-Smith *et al.* 2004) - then it is possible that the number of spawners has little influence on annual recruitment in Barramundi, particularly considering that this species is highly fecund and most populations in Australia are not severely depleted (Saunders *et al.* 2018). If this is the case, it may be necessary to re-evaluate the continued use of stock-recruitment functions and to explore alternative ways of incorporating environmental drivers of recruitment variability into Barramundi population models. Our results suggest that further research is required to develop our understanding of the importance of spawner-recruit relationships in driving Barramundi population dynamics.

A particularly significant finding of the study was that the explanatory power of the regional YCS model (intrinsic effects + wet1 AMI) was comparable to the river-specific models containing descriptors of local

river discharge. Intuitively, one might expect that local hydrological variables would much better characterise proximate drivers of recruitment and, thus, be far more effective as predictors of YCS than a broad-scale climatic variable. One possible explanation for our observations is that regional rainfall variation influences Barramundi recruitment via concurrent direct and indirect mechanisms. For instance, if the area of nursery habitat (seasonally inundated coastal floodplains and swamps) strongly influences the number of recruits (see Davis 1985; Robins *et al.* 2005), then increased rainfall might affect recruitment directly by filling nursery habitats with water and indirectly by increasing catchment runoff, which in turn increases river discharge and stage height, leading to the inundation of nursery habitat from the river via flood channels and overbank flow. The effects of direct filling of coastal nursery habitat by rainfall are not fully captured by gauged river discharge data, and this may explain why the regional climate (AMI) model had comparable explanatory power to the river-specific, hydrology-based models. Further research on relationships between YCS and the availability of nursery habitat and other environmental factors not directly associated with river hydrology would improve our mechanistic understanding of the drivers of Barramundi recruitment.

In summary, the current study's findings add to a large body of independent evidence suggesting causal linkages between the productivity of Barramundi fisheries and climate and river hydrology. Consideration of the full range of available evidence on these linkages provides a compelling argument for a cautious approach to water resource development in northern Australia - particularly in the context of a rapidly changing climate - and is critical to ensure that Government policy and management meets community expectations regarding the sustainable development of our shared water resources.

# 3. Evaluation of the use of gastric ossicles for age estimation in Giant mud crab<sup>6</sup>

## 3.1 Background

Until recently, direct ageing of crustaceans using calcified hard parts had not been considered possible because crustaceans grow by moulting their calcified tissues multiple times during life. However, several recent studies present evidence that some calcified structures (eye stalks and gastric ossicles) are conserved across the moulting process and contain growth marks with potential use for direct age estimation (Leland *et al.* 2011, 2015; Sarapuk 2014; Leland and Bucher 2017; Kilada *et al.* 2012; 2016; 2017; Krafft *et al.* 2016; Hasyima *et al.* 2017). While direct ageing of crustaceans may hold potential promise, some of the evidence suggesting that calcified structures are retained across moulting has recently been challenged (Vatcher *et al.* 2015; Sheridan *et al.* 2016; Becker *et al.* 2018) and the processes leading to mark formation are presently unclear (Leland and Bucher 2017; Kilada *et al.* 2017; Kilada and Driscoll 2017).

A recent study by Sarapuk (2014) examined the use of gastric ossicles for direct age estimation in Giant mud crab *Scylla serrata*. Based on a series of direct observations, calcein marking experiments and morphological measurements on 190 crabs, Sarapuk (2014) concluded that direct age determination of Giant mud crab via counts of growth bands in the gastric ossicles was a valid methodology. In a study published after commencement of the current project, Leland and Bucher (2017) reported on further research using gastric ossicles for direct age estimation of seven crustacean species, including Giant mud crab. Leland and Bucher (2017) undertook a series of trials and analyses, including calcein staining and ossicle chemistry analysis, and claimed to have validated annual increment formation in the gastric ossicles of Western rock lobster *Panulirus cygnus*, Eastern rock lobster *Sagmariasus verreauxi*, Ornate rock lobster *Panulirus ornatus* and Crystal crab *Chaceon albus*. Leland and Bucher (2017) conducted only limited analyses of Giant mud crab but concluded that the direct ageing method could also be readily applied to this species.

It was on the basis of these findings that the current study aimed to use growth increments in the gastric ossicles of Giant mud crab to undertake biochronological analyses in a similar manner to that described above for Barramundi. However, as the analysis of Giant mud crab ossicles commenced during the current project, significant issues were identified that raised concerns regarding the reliability of using gastric ossicles for age estimation in this species and, potentially, other species of crustacean. In particular, measurement of the putative annual increments in sectioned ossicles suggested that ossicle growth is not cumulative across ontogeny (a key premise of the use of calcified hard parts for direct age estimation and biochronological analysis) and that calcified tissues in the ossicles do not appear to be retained across the moult cycle. In light of these observations, the planned biochronological analyses were not considered achievable and a decision was made to change the emphasis of this project component towards a comprehensive assessment of the direct ageing technique for Giant mud crab. By changing the emphasis of this project component, we address future research recommendation 6 (Periodicity evaluations for Southern Rock Lobster and Giant mud crab) from Leland and Bucher (2017), and provide a broader assessment of the issues associated with direct ageing of crustaceans to help guide future directions in this area.

The following section describes the analyses conducted to evaluate the utility of sectioned gastric ossicles for direct ageing of Giant mud crab. The specific aims were to determine:

- 1) whether putative annual age estimates can be reliably reproduced;
- 2) if age estimates are compatible with previous studies; and

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<sup>6</sup> The results of this chapter are published as: Crook, D.A., Adair, B., Morrongiello, J.R., Grubert, M.A. Saunders, T.M., Douglas, M.M. and King (2018). Muddy waters: an assessment of the suitability of zygocardiac ossicles for direct age estimation in the Giant mud crab *Scylla serrata*. *Limnology and Oceanography: Methods* 16, 895-905.

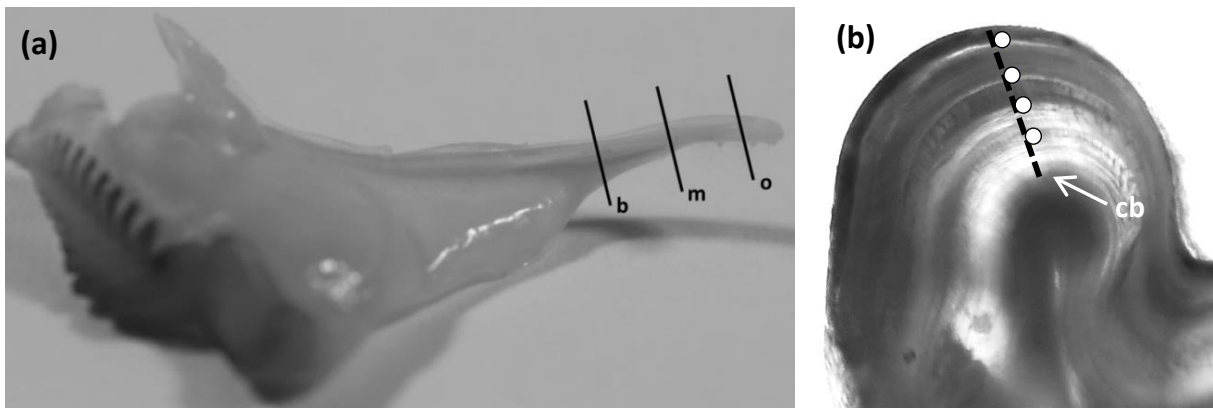


3) if ossicle growth is cumulative across ontogeny.

The results of the study are discussed with regards to the utility of gastric ossicles for direct ageing of Giant mud crab and issues associated with the use of this type of structure for ageing crustaceans more generally.

## 3.2 Methods

A total of 210 Giant mud crab (mean 166 mm, range 126-200 mm carapace width [CW]; 48% females, 52% males) were sourced from commercial fishers between 2015 and 2017 from three estuaries in the NT: the Adelaide River (n=51), the Roper River (n=69) and the McArthur River (n=90). The dorsal carapace was separated from the body, exposing the internal anatomy and the gastric mill was removed. The membranous material surrounding the gastric mill was dissected and removed to reveal the ossicles (mesocardiac, pterocardiac and zygocardiac), and then preserved in an ethanol, glycerol and distilled water medium (60:30:10) (Leland *et al.* 2015). Preserved ossicles were rinsed in fresh water, air dried and embedded in two-part clear epoxy resin. A low-speed saw was used to cut a 200 $\mu$ m thick transverse section through the mid-point of the thin portion (hereafter referred to as the 'protuberance') of the zygocardiac ossicle following Leland *et al.* (2015) (Figure 3.1a). Sections were then polished using a graded series of lapping film, rinsed and inspected until the view of the internal structure of the ossicle was optimised using a stereo microscope at x50 magnification.



**Figure 3.1:** (a) Photograph of whole zygocardiac ossicle showing the location of the sectioning plane used for direct age estimation in Giant mud crab (m = mid). Additional locations sectioned to examine the consistency of increment counts along the length of the zygocardiac ossicle are also shown (b=base, o=outer). (b) cross-section of a zygocardiac ossicle showing the location of cuticular boundary (cb), locations of increments (numbered 1-3) and location of ossicle cross-section width measurements (grey line). Reproduced from Crook *et al.* (2018).

Instruction and training in ossicle preparation and interpretation was undertaken by members of the project team prior to the study at specialised workshops held in Darwin, Australia (July 2014) and Lismore, Australia (March 2016), followed by additional consultations with one of the workshop presenters. The 210 sectioned ossicles were then examined by the trained reader, with age estimated by counting the number of pairs of opaque and translucent bands using the cuticular boundary as a starting point (Leland and Bucher 2017). Ageing was blind-repeated six times for all ossicle sections. The first read was considered preliminary and was excluded from further analyses. Variation within readings was calculated using age bias plots and average percent error (APE) (Campana 2001).

Ten randomly selected Giant mud crab samples were analysed to examine the consistency of increment counts along the length of the ossicle protuberance. These ossicles were sectioned at three locations (base, mid, tip) and increment counts conducted for each section. To explore ossicle morphology, 60 whole ossicles were randomly selected and their weight, length (proximal to distal tip) and width (widest point) were recorded. All 210 sections were also assessed for cross-section diameter (cuticle boundary to ossicle section edge).

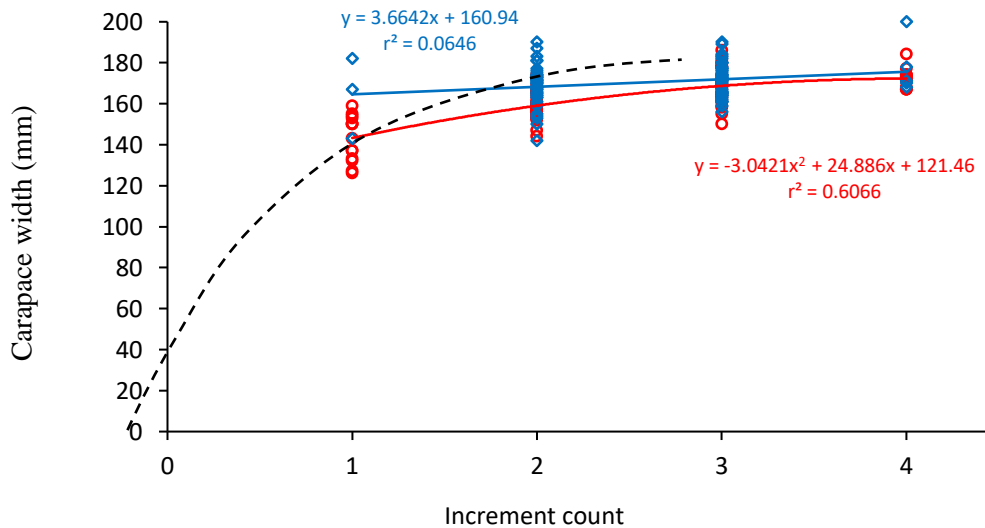
### 3.3 Results and Discussion

Similar to previous studies (Leland *et al.* 2011; Sarapuk 2014; Leland and Butcher 2017), we found that sectioned Giant Mud Crab ossicles had identifiable marks that potentially represent growth increments. However, these sections were very difficult to interpret consistently by comparison to fish otoliths. Issues encountered included indistinct increments, incomplete and discontinuous increments, non-translucent sections and numerous apparently secondary growth checks. Of the 210 sections examined, only 15% were rated as having good (category 4) or very good (category 5) readability. Readability scores increased significantly with higher increment counts (two-way analysis of variance [ANOVA];  $F_{3,202} = 17.430$ ,  $P < 0.001$ ), raising concerns that low increment counts may be due to poor readability rather than the actual number of increments present in the ossicle. Crab sex had no effect on readability scores ( $F_{1,202} = 0.021$ ,  $P = 0.886$ ) and the interaction between age and sex was not significant ( $F_{3,202} = 2.595$ ,  $P = 0.054$ ).

Crabs with low increment counts (1-3) were aged reasonably consistently over the six repeated readings, but higher age estimates (up to 5 years) occurred more frequently in the early reads. The 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> reads showed greater convergence of estimated ages than earlier reads and the maximum age estimate across the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> reads was 4 years. Average percentage error (APE) was 19% calculated for the 2<sup>nd</sup> versus 3<sup>rd</sup> reads and improved to 13% by the 5<sup>th</sup> versus 6<sup>th</sup> reads. The age estimates from the 6<sup>th</sup> (last) read were considered the most reliable based on these findings and were used for subsequent analyses. It should be noted that APE scores would likely be higher than 13% in situations where multiple readers are required to undertake the estimates due to inter-reader error. A best-case APE of 13% is high compared to fish otolith studies, which often report APE scores of <5% including inter-reader error. APE scores for Barramundi otolith ageing by the two readers in the previous section were ~5%. Leland and Bucher (2017) reported similar problems in readability of ossicle sections: for example, ~60% of sections were rated as poor to intermediate and ~10 and 20% of samples were rejected as unreadable for Western rock lobster and Eastern rock lobster respectively.

Increment counts from the sub-set of 10 crabs whose ossicles were sectioned at three locations exhibited considerable variation in estimated ages among the locations (i.e., base, mid, tip; Figure 15a). In only one out of the 10 cases did the increment count from the base of the ossicle match the count at the mid-point (the location used in previous studies) and estimated ages varied by up to 2 years between the base and the mid-point. Age estimates from sections taken from the tip of the ossicle matched the mid-point in four out of ten cases, with estimates differing by up to 3 years between the tip and mid-point in the remaining cases. Our results of variable age estimates with section location are consistent with Leland *et al.* (2015), who found that primary increment counts varied from 5 to 17 in the same ossicle depending on where the section was taken. The above results show that there is considerable potential for error in age estimates both from increment interpretation and section preparation, particularly considering that the maximum putative age of Giant mud crab based on ossicles increment counts is only 4 years (this study; Sarapuk 2014).

Despite these methodological issues, however, age-at-length relationships based on our age estimates were broadly similar to a von Bertalanffy growth function generated from a previous tag-recapture study of Giant mud crab from the same regions as the current study (Knuckey 1999, Figure 3.2). The similarity between our estimates and those of Knuckey (1999) may - at least superficially - suggest that the increments observed in the sectioned ossicles represent annuli that can be used for direct age estimation. However, we identified several issues associated with the growth characteristics of Giant mud crab gastric ossicles that warranted further investigation before we could be confident in their suitability for direct age estimation.

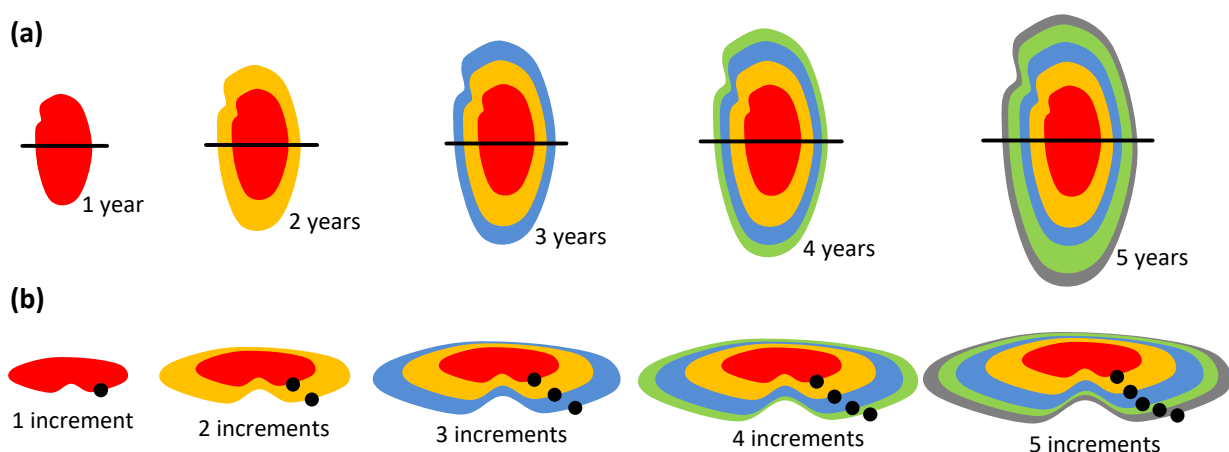


**Figure 3.2:** Carapace width versus zygo-cardiac ossicle increment counts for the 109 male (unbroken red line and circles) and 101 female Giant mud crab sampled during the study (unbroken blue line and diamonds). The broken line shows age-at-size estimates derived by Knuckey (1999) using the von Bertalanffy growth function applied to tag recapture data from males and females combined. Redrawn from Crook *et al.* (2018).

### **Issues associated with gastric ossicles as direct ageing structures**

As mentioned previously, direct age estimation from calcified structures relies on the cumulative deposition of new material as the structure grows. For a hard structure to be useful for direct ageing, the deposited material must be preserved across ontogeny in such a way that all previous growth increments can be reliably observed and counted. The concept of cumulative growth over the life of the animal is fundamental to biochronological analysis of calcified hard parts.

In the case of fish otoliths, for example, calcium carbonate is deposited on the outer surface of the otolith, building from a central primordium (Figure 3.3). Annual increments occur due to seasonal variation in calcium carbonate and protein deposition. Importantly, there is no physiological reworking of the calcium carbonate material and, hence, otoliths represent a stable chronological record of growth. In this context, we identified two major issues regarding the growth characteristics of Giant mud crab ossicles that raise questions regarding their suitability for age estimation. These issues are summarised briefly below and further details are available in Crook *et al.* (2018).



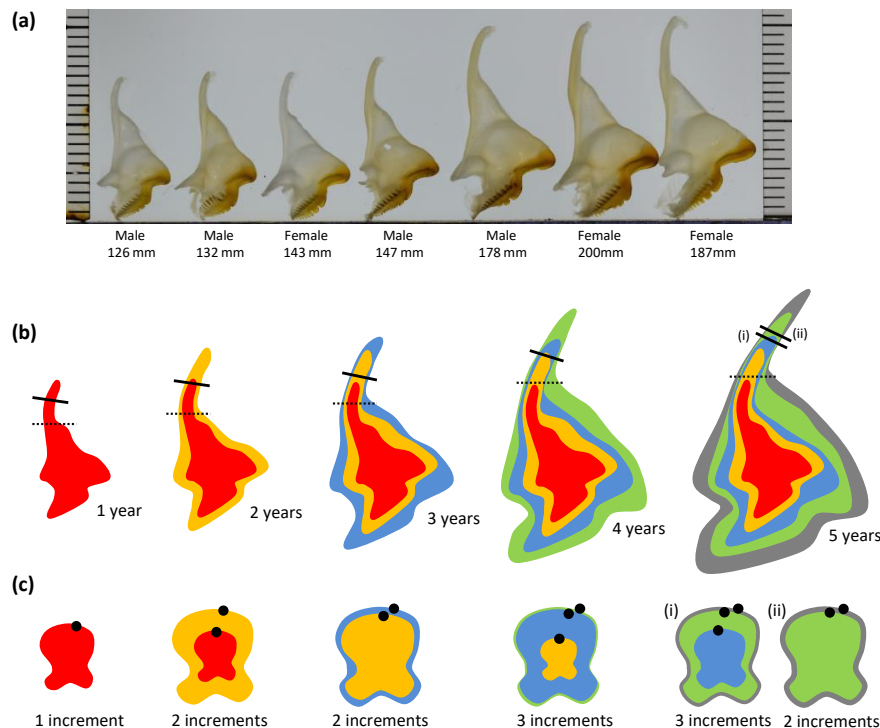
**Figure 3.3:** Schematic showing the influence of growth morphology on age estimation in fish otoliths. (a) sagittal sections of an otolith showing its size at each year of age. The calcified material laid down during each year of growth shown is shown as a different colour. Black dots represent annual increments. (b) transverse sections of the same otolith at the locations shown by the solid lines in (a). Redrawn from Crook *et al.* (2018).

## Effects of growth morphology on increment counts

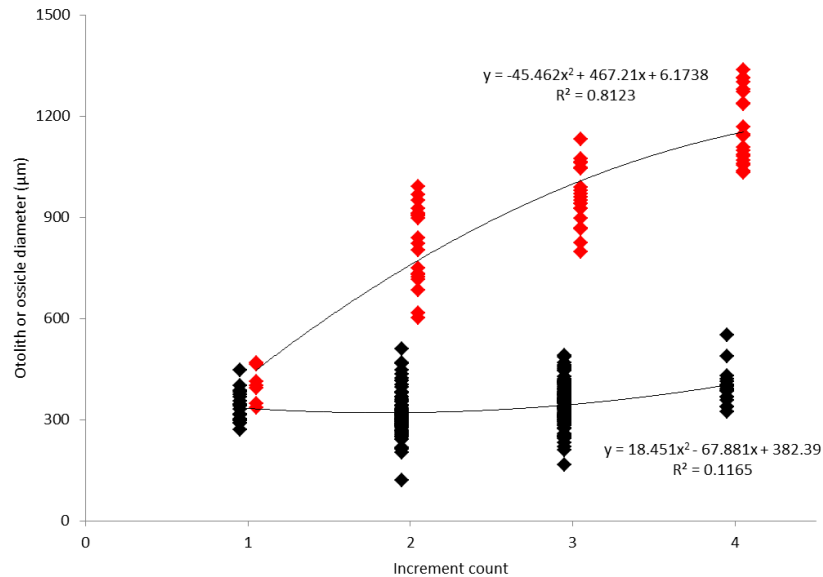
Previous studies of Giant mud crab used the mid-point of the protuberance of the zygo-cardiac ossicle (see Figures 3.1 and 3.4) for ageing because this region has the clearest putative increments (Leland *et al.* 2011; Sarapuk 2014; Leland and Bucher 2017). However, examination of the growth morphology of the zygo-cardiac ossicles of Giant mud crab across ontogeny suggests that sections taken from this location will not contain all previous growth increments, even if we assume that growth is cumulative throughout life. As shown in Figure 3.4, the mid-point position of the ossicle changes relative to the overall shape of the ossicle as it increases in size and, as a consequence, sections taken from the mid-point in older crabs will not contain all growth increments from the early life history. This feature of the growth morphology of zygo-cardiac ossicles is likely to result in systematic underestimation of increments in larger crabs and inconsistencies in increment counts due to minor variations in the location of the sampled section.

### Do ossicles exhibit cumulative growth?

If gastric ossicles exhibit cumulative growth, it follows that the diameter of the ossicle section should increase with successive increments, as occurs in otoliths (see Figure 3.5). However, we found only a very weak relationship between ossicle section diameter and increment count (quadratic regression model:  $F_{2,204} = 13.453$ ,  $P < 0.001$ ,  $r^2 = 0.1165$ , Figure 20). Furthermore, ossicle diameter was predicted to be higher in putative 1-year olds than 2-year olds based on the regression equation and there was an overall increase in ossicle diameter of only ~20% between putative age-1 and age-4 crabs (notwithstanding that increment count explained little of the variance in ossicle diameter;  $r^2 = 0.1165$ ). This contrasts with an equivalent analysis using Barramundi otolith increment data from the previous section (ages 1-4 years,  $n = 68$ ) where: 1) there was a strong relationship ( $r^2 = 0.8123$ ) between age and otolith diameter, 2) all successive cohorts were predicted to have larger otolith diameters than previous cohorts, and 3) otolith diameter increased by ~200% between ages 1 and 4 based on the regression equation (Figure 3.5).

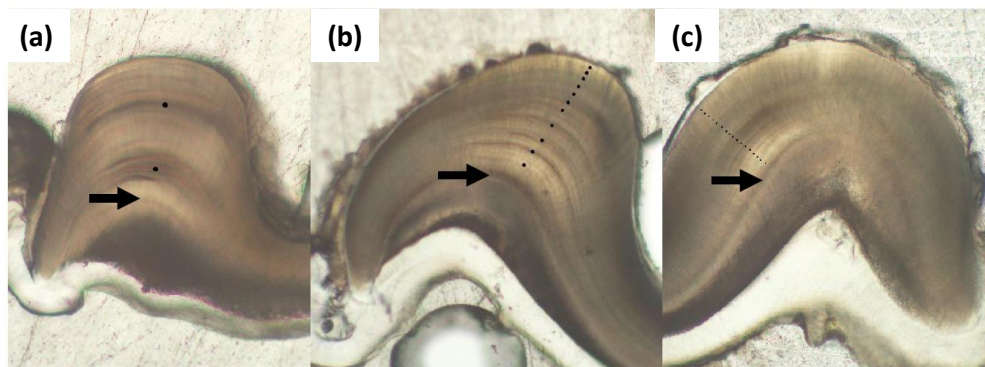


**Figure 3.4:** Schematic showing the influence of growth morphology on age estimation in Giant mud crab zygo-cardiac ossicles. For the purposes of this illustration, cumulative growth of the ossicles is assumed. (a) Photographs of a progression of zygo-cardiac ossicle morphologies across the size ranges of crabs examined in the study, (b) lateral sections of a zygo-cardiac ossicle showing its size at each year of age. (c) cross-sections at the mid-point of the thin protuberance of the zygo-cardiac ossicle at the locations shown by the solid lines in (b). The broken line shows the portion of the ossicle below which incremental structure is not discernible (authors pers. obs.). Increment counts in (c) show how counts at the mid-point location potentially lead to systematic underestimation of age in older crabs and inconsistency in counts due to small changes in the position of the section plane (i versus ii). Redrawn from Crook *et al.* (2018).



**Figure 3.5: Comparison of relationships between ossicle or otolith section diameter and increment count (putative age) in Giant mud crab (black diamonds) and Barramundi (red diamonds). This figure demonstrates the difference between cumulative growth in fish otoliths versus the non-cumulative growth of Giant mud crab ossicles. Note the strong, increasing relationship between Barramundi otolith diameter and age versus the very weak relationship between Giant mud crab ossicle diameter and putative age. Redrawn from Crook *et al.* (2018).**

This analysis strongly suggests that, unlike otoliths, ossicle growth in Giant mud crabs is not cumulative across ontogeny, and that ossicles are either entirely shed during moulting or are re-worked over time. This supports previous studies on the moult process for other species of crustacean (Brösing 2014; Vatcher *et al.* 2015; Sheridan *et al.* 2016; Becker *et al.* 2018). Further to this, Leland and Bucher (2017) presented ossicle sections from three individual southern rock lobsters (*Jasus edwardsii*) and estimated their ages as 1.6 to 16.6 years (Figure 21). If the ossicles in this figure were exhibiting cumulative growth, we would expect the spacing between the early increments to be broadly similar in both size and appearance between young and old crabs. In contrast to this expectation, the annual increments identified by Leland and Bucher (2017) are much closer together in older crabs than young crabs. It is possible that the lobsters with lower estimated ages in Leland and Bucher (2017) grew much faster than older individuals and that this explains the difference in increment spacing and appearance between old and young individuals. However, such a process was not mentioned by Leland and Bucher (2017) and it appears more likely that the gastric ossicles of these lobsters are either shed during the moult cycle or are extensively re-worked over ontogeny. Leland and Bucher (2017) hypothesised that the discrepancy in increment widths between young and old *J. edwardsii* is due to “compression” of retained increments over time, but they did not elaborate on a causal mechanism that might explain such a process.



**Figure 3.6: Photographs of pterocardiac ossicles showing primary growth increments (black dots) used to assign putative ages to Southern rock lobster *Jasus edwardsii*. The black arrow indicates the cuticular boundary. (a) 59 mm CL female with 2 marks (putative age = 1.6 years), (b) 152 mm CL male with 11 marks (putative age = 10.6 years), (c) 170 mm CL male with 17 marks (putative age = 16.6 years). Scale not provided in original figure. Reproduced from Leland and Bucher (2017) (<https://creativecommons.org/licenses/by/3.0/au/legalcode>).**

Our conclusions of non-cumulative growth in Giant mud crab zygo-cardiac ossicles contrast with those of Sarapuk (2014) and Leland and Bucher (2017), who suggested that zygo-cardiac ossicles are retained across the moult cycle and have utility for direct age estimation in Giant mud crab. One of the key points of evidence used by Sarapuk (2014) to suggest that ossicle material is retained across ontogeny was a calcein staining experiment which found that calcein stains were retained in Giant mud crab ossicles across moults. However, whilst ossicles exhibited calcein fluorescence post-moult, Sarapuk (2014) was unable to distinguish a definitive calcein mark indicating the time of staining and, therefore, could not validate the annual periodicity of growth bands based on the trials.

Although the retention of calcein stains in Giant mud crab ossicles across moults may appear incompatible with our findings, a recent study by Sheridan *et al.* (2016) provides a potential mechanism to explain how calcein stains may occur in calcified structures that are completely shed during the moult. Sheridan *et al.* (2016) examined gastric ossicles of Norway lobster *Nephrops norvegicus* and found that all of the ossicles of the gastric mill were shed during moulting and that new ossicles were calcified post-moult. Although the old ossicles were moulted, they found that the new ossicles of lobsters stained with calcein prior to moulting exhibited calcein fluorescence post-moult. Sheridan *et al.* (2016) suggested that this occurs because the old ossicles and gastroliths are digested, and their calcium (to which the calcein is bound) is resorbed from the gastroliths and the consumed exuviae. Whilst these findings provide a plausible explanation for calcein fluorescence in newly formed ossicles, detailed research on the moulting cycle is required to determine whether such a process occurs in Giant mud crab and other crustacean species.

In a study of Redclaw crayfish *Cherax quadricarinatus*, Leland *et al.* (2016) described the sequential addition of urocardiac ridges on the mesocardiac tooth plate as crayfish size increased, despite the fact that the mesocardiac ossicles of this species are known to be completely shed during moulting. Based on this observation, Leland *et al.* (2016) suggested that a “memory-like” mechanism may explain the existence of chronological information in moulted structures. Whilst this hypothesis may be plausible, such a mechanism to explain annulus formation in gastric ossicles would represent a new paradigm in the use of calcified hard parts for ageing aquatic organisms, which to date has relied upon the assumption of cumulative growth and preservation of accreted material across ontogeny. We believe that such an explanation requires extensive investigation to establish the underlying processes and their practical implications for age determination.

Finally, our study demonstrates the potential problems of using correspondence between putative age estimates and other sources of data that are themselves un-validated or have high uncertainty, as a means of corroborating direct age estimates. While correspondence among different data sources may provide some assurance that age estimates align with expectations, this does not amount to independent validation and can lead to a circularity of logic regarding age estimate validity. The validation of growth increments requires detailed information on the timing and frequency of increment formation and at least some understanding of the underlying processes. While there is evidence that calcified structures may have potential utility for direct age estimation in some crustacean species, our study suggests that substantial further research is needed before a sufficient level of understanding is achieved for crustaceans more generally.

# 4. Conclusions

## 4.1. Barramundi

The results of the biochronological analyses showed that Barramundi year class strength (YCS) was strongly correlated with a regional climatic parameter - the Australian Monsoon Index (AMI) - across all four of the study rivers (Daly, Roper, Mary, McArthur). This finding appears to reflect a broad-scale, positive influence of monsoonal activity and associated rainfall for Barramundi recruitment. This finding aligns with the conclusions of several previous studies which have suggested that increased rainfall is associated with increased recruitment in Barramundi populations. We did not find a regional-scale relationship between Barramundi growth and regional climate, most likely due to localised variations in factors such as density-dependent competition, food web productivity and habitat quality.

River-specific analyses conducted on the Daly and Roper rivers demonstrated strong relationships between YCS/growth and hydrology. These relationships were complex and variable among the rivers, but relationships between YCS/growth and the volume and duration of river discharge tended to be positive or neutral. Scenario modelling based on these relationships predicted that water abstraction during both the wet and dry seasons is likely to have a strong impact on YCS in the Daly and Roper rivers and, to a lesser extent, growth in the Roper River. The predicted effects of water abstraction on barramundi YCS generally increased as the volume of water abstracted increased, with the strength of response varying with wet season strength in each year. Our modelling also supported a previously proposed flow rule (Erskine *et al.* 2003), suggesting that water abstraction during receding wet season flows would lower the impacts on YCS, particularly in low and moderate flow years.

## 4.2. Giant mud crab

The Giant mud crab component of this study examined 210 sectioned Giant mud crab gastric ossicles and identified marks that may represent growth increments that could be used for ageing, as proposed by previous studies (Leland *et al.* 2011, 2015; Leland and Bucher 2017; Kilada *et al.* 2012; 2016; 2015; 2017; Krafft *et al.* 2016; Hasyima *et al.* 2017). Age-estimates were also broadly similar to von Bertalanffy growth model estimates from previous work using tag-recapture data (Knuckey 1999). However, our study identified and explored important issues regarding the suitability of this method for providing reliable age estimates for Giant mud crab. These issues included the following:

- (i) ossicle readability is generally poor and sections are difficult to interpret consistently in comparison to fish otoliths;
- (ii) the morphological growth characteristics of gastric ossicles make it unlikely that growth bands from the early life history will be present in sections taken from older crabs, potentially leading to systematic underestimation of age;
- (iii) sections taken from different locations along the ossicle resulted in highly variable age estimates, suggesting that minor variations in sample preparation may affect age estimates;
- (iv) strong evidence of non-cumulative growth in ossicles suggests that ossicles are either completely shed during the moult cycle or extensively reworked across ontogeny;
- (v) a recent study by Sheridan *et al.* (2016) shows that calcein dye can be resorbed from the gastroliths and exuviae, suggesting that calcein fluorescence in ossicles post-moult does not necessarily demonstrate ossicle retention across the moult cycle.

Based on our analyses, we caution against the use of ossicle sections for direct ageing of crustaceans until the ontogenetic growth morphology of these structures has been comprehensively evaluated, the periodicity of increment formation validated using increment counts from independent readers, and the underlying processes for increment formation and retention identified.

# 5. Implications

## 5.1 Barramundi

A key finding of the project for fisheries management was the strong correlation between YCS and wet season Australian Monsoon Index across all four rivers. Identification of a single climatic index that can predict Barramundi recruitment at the regional scale has the potential to facilitate forecasting of harvestable biomass over a 3-year horizon (i.e., the time taken for recruits to enter the fishery). Incorporation of this index into fishery modelling and planning could improve the efficacy and timeliness of management interventions for NT Barramundi fisheries and may also have applications for other fisheries and jurisdictions in tropical Australia.

The model outputs showed a strong effect of water abstraction in wet and dry season on Barramundi YCS. These findings have important implications for water resource development in NT and northern Australia, and suggest that careful consideration must be given to the likely detrimental impacts of water abstraction on Barramundi fisheries. These results have been communicated directly to NT water resource managers via a series of meetings and presentations. The project team aims to maintain ongoing dialogue with water managers to facilitate further incorporation of the project outputs into water resource planning in the future.

## 5.2 Giant mud crab

The results of the current study raise important doubts about the suitability of using gastric ossicles for the direct ageing of Giant mud crab and, potentially, other crustacean species. Since commencement of our work, two additional studies have questioned the validity of using cuticle bands for direct ageing of crustaceans. Becker *et al.* (2018) conducted detailed investigations of crustacean cuticle structure and growth using dissection, light microscopy, micro-computed tomography and cryo-scanning electron microscopy. Based on their analyses, they concluded that the gastric mill is moulted and that ossicles are replaced during ecdysis. Similarly, Sheridan *et al.* (2020) examined the cuticle structure of crustacean eyestalks and concluded that the presence of bands is due to the lamellate appearance of the crustacean cuticle and not the deposition of annual bands. Despite these findings, however, several studies have continued to use cuticle band counts to directly estimate age in crustaceans (e.g., Gnanalingam *et al.* 2019; Bosley *et al.* 2019).

Given the fundamental importance of age information for fisheries assessment - and considering our reliance on accurate fisheries assessments to ensure that fishery harvests are sustainable - it is imperative that methods for ageing are accurate, precise and validated by strong scientific evidence. In contrast to the conclusions of Leland and Bucher (2017), our study suggests that considerable further research on method validation is required before direct ageing of crustaceans is routinely integrated into fishery research and management in Australia. In the meantime, we caution against the use of this age estimation technique.

# 6. Recommendations

## 6.1. Barramundi

1. Our results provide empirical evidence of the detrimental effects that future water resource development may have on Barramundi fisheries. Consideration of this information is necessary to ensure that community expectations are met with regards to the sustainable development of northern Australia's water resources.
2. The models developed in this project have the potential to enhance the accuracy of stock assessment models for Barramundi by accounting for environmental recruitment drivers;
3. The potential utility of the Australian Monsoon Index as a forecasting tool for tropical fisheries should be explored for other fisheries in northern Australia.



4. Further research is required to develop our understanding of stock-recruitment relationships in Barramundi populations in order to refine population models.

## 6.2. Mud crab

1. The morphological growth characteristics and non-cumulative growth of gastric ossicles in Giant mud crab (and potentially other crustacean species) suggests that caution should be applied to direct age estimates based on gastric ossicle increment counts unless further detailed research validates the method.
2. If direct ageing using gastric ossicles is pursued as a method to support crustacean fisheries management in future, then further research is needed to understand the physiological processes that produce banding in these structures.
3. Any future validation experiments to examine retention of calcified structures across the moult cycle should account for the potential reabsorption of calcein or other dyes from moulted gastroliths and exuviae.

# 7. Further development

## 7.1. Barramundi

1. Development of strategies for effectively integrating outputs of YCS scenario models into water resource policy and management.
2. Regular evaluation of the accuracy of modelled estimates of the effects of climate and hydrologic change on Barramundi recruitment and growth.
3. Increased understanding of the mechanistic processes (e.g., density-dependence, nursery habitat availability) that drive temporal and spatial variation in recruitment and growth in Barramundi populations.
4. Increased understanding of the role of intra-specific variation in physiology, behaviour and life history on recruitment and growth in Barramundi.
5. Application of the modelling approach to other coastal species that may be influenced by river hydrology and large-scale climatic drivers.
6. Consider how the results can be implemented in a harvest strategy framework; i.e. the need for stock assessment models for this species given that environmental drivers appear more important in determining recruitment strength than stock size.

## 7.2. Mud crab

1. Detailed assessment of the physiological processes that produce banding (i.e. putative increments) in crustacean ossicles, including electron microscopy of ossicle ultra-structure across the moult cycle.
2. Quantitative assessment of the timing of band formation in crustacean ossicles (for example, marginal increment analysis).
3. Greater understanding of the moult process to resolve the issue of gastric ossicle retention across the moult cycle for key crustacean species.

## 8. Extension and Adoption

Date	Communication Activity	Stakeholders
Mar 2017	Oral presentation on preliminary results and study justification	NT FRAB, FRDC
Apr 2017	Oral presentation on preliminary results and study justification. AFANT general meeting, Darwin	Peak recreational fishing body
May 2017	Oral presentation of preliminary results at Palmerston Game Fishing Club, Palmerston.	Recreational fishing club
Aug 2017	Onsite discussions about project with commercial crab fishers, Port Roper	Commercial crab fishers
Nov 2017	Oral presentation of project concept, preliminary results and discussion of flow scenarios	NT Department of Environment and Natural Resources water resource managers
Nov 2017	Onsite discussions about project with commercial crab fishers, Port Roper	Commercial crab fishers
Mar 2018	Oral presentation: project update and preliminary results	NT FRAB, FRDC
Apr 2018	Oral presentation of preliminary results and study justification. AFANT general meeting, Darwin	Peak recreational fishing body
Nov 2018	Onsite discussions about project with commercial crab fishers, Port Roper	Commercial crab fishers
Dec 2018	Presentation of preliminary project findings to water and fisheries managers	NT Department of Environment and Natural Resources, NT Department of Primary Industries and Resources
May 2019	Presentation of project objectives and preliminary results at Barra Nationals fishing competition, Daly River	Recreational fishers
May 2019	Onsite discussions about project with commercial crab fishers, Port Roper	Commercial crab fishers
August 2019	Meeting to decide on flow scenarios for modelling	NT Department of Environment and Natural Resources (Water Resources Division)
August 2019	Oral presentation “Predicting the ecological impacts of water extraction on Top End rivers”. Top End Public Water Forum, Katherine.	General public

October 2019	Oral presentation: project update and latest results	NT RAC, FRDC
October 2019	Presentation of project findings to fisheries researchers and managers.	NT Department of Primary Industries and Resources
October 2019	Oral presentation on Giant mud crab ageing study. Australian Society for Fish Biology annual conference, Canberra	Fisheries scientists and managers
January 2020	Phone meeting to discuss preliminary scenario modelling results	NT Department of Environment and Natural Resources (Water Resources Division)
February 2020	Presentation and discussion of final scenario modelling results and implications for water management	NT Department of Environment and Natural Resources (Water Resources Division)
February 2020	Presentation of results at Research Institute for the Environment and Livelihoods 2020 stakeholder outreach event, Darwin	Wide range of NT natural resource management stakeholders
January 2020	Zoom meeting to discuss final scenario modelling results	NT Department of Environment and Natural Resources (Water Resources Division)

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## 9. Project coverage

Radio interview with ABC Darwin, by Alison King, 11<sup>th</sup> March 2019.

ABC Interview and online story, ABC radio, by Alison King, 11<sup>th</sup> September 2019.

Radio interview with ABC morning show Darwin by David Crook, 29 January 2020.

## 10. Project materials developed

This project has developed:

- Final report
- Two peer-reviewed scientific papers, one of which is published and another in preparation at the time of writing.
- Quantitative models and water abstraction scenario results provided to NT Department of Environment and Natural Resources (Water Resources Division) and NT Department of Primary Industries and Resources (Fisheries) for use in future water and fisheries policy development, management and modelling.
- A final project fact sheet (Appendix 5).

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# Appendix 1. Project staff

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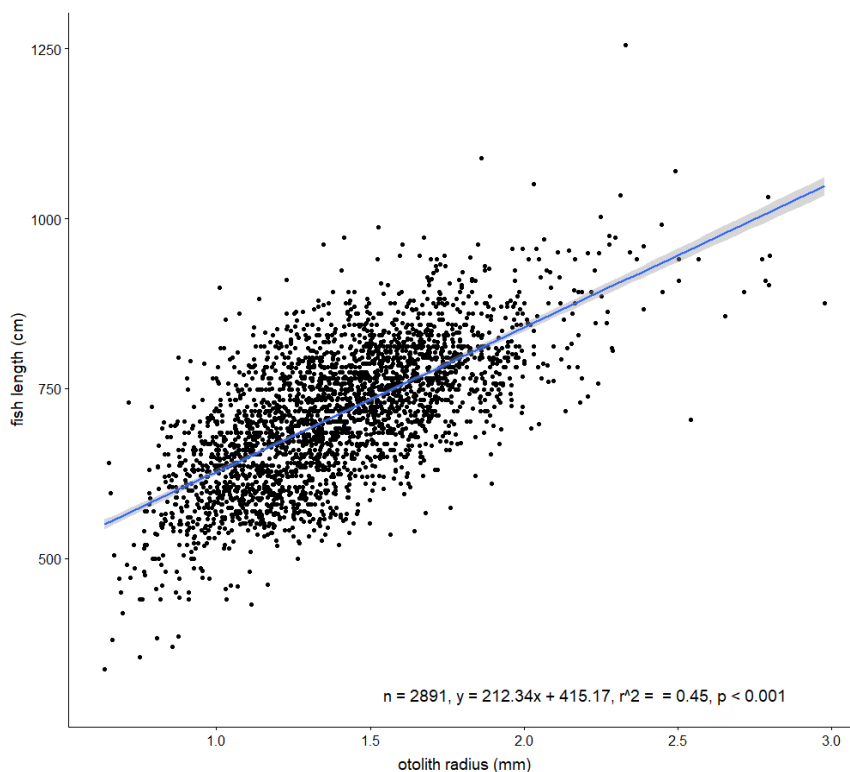
Dr Mark Grubert, NT Department of Primary Industry and Resources (Fisheries Division)

Dr Michael Douglas, University of Western Australia.

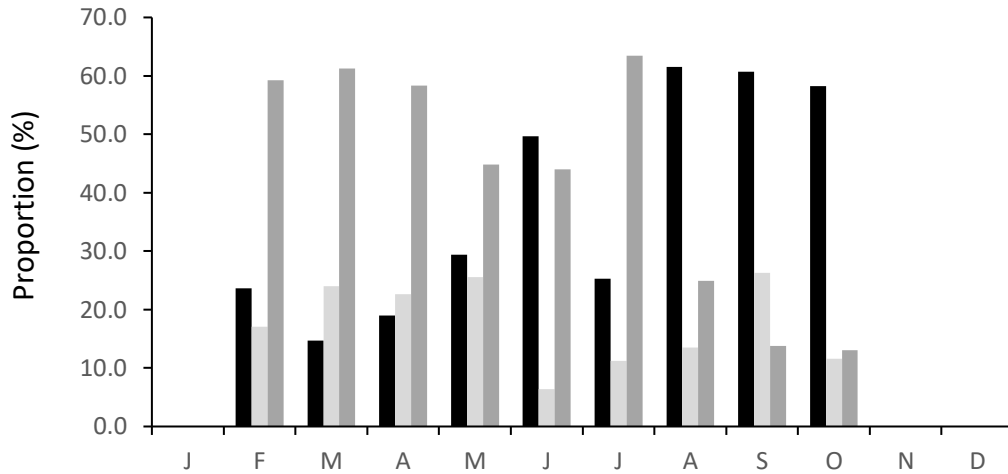
# Appendix 2. Intellectual Property

Intellectual Property: All intellectual property arising from this research project has been made publicly available.

# Appendix 3. Supplementary figures



**Figure S1: Relationship ( $\pm$  95% CI) between fish length and otolith radius in Barramundi.**



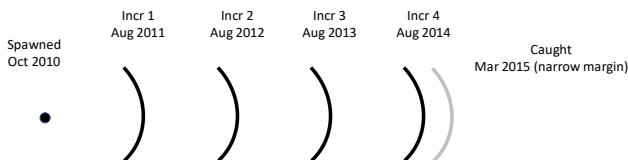
**Figure S2: Proportion of monthly samples of Barramundi otoliths with marginal increment categorised as narrow (black), intermediate (light grey) and wide (dark grey).**

## Appendix 4. Age adjustment

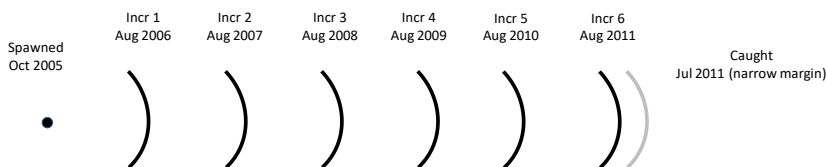
This Appendix presents examples demonstrating the Barramundi age adjustment scheme used in the study for each marginal increment type and different sample dates. These adjustments are based on an October 1<sup>st</sup> birthday and annual increment formation on August 1<sup>st</sup> (see Section 2.2.2 above), with a 4-month adjustment window either side of August where appropriate.

### Narrow margins

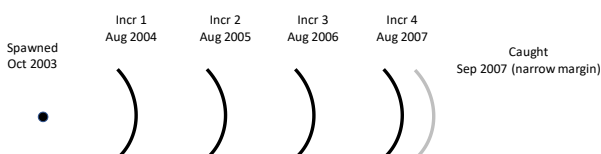
**Example 1:** Fish caught in March 2015 with 4 increments. This fish is allocated to the 2010 year-class (i.e., 2010/11 wet season). Interpretation of a narrow margin in March is that the fish has not yet formed its 2015 increment. A 1-year age adjustment is made for this fish (2015 - [4+1] = 2010).



**Example 2:** Fish caught in July 2011 with 6 increments. This fish is allocated to the 2005 year-class (i.e., 2005/06 wet season). Interpretation of a narrow margin in July is that the fish formed its 2011 increment earlier than 1<sup>st</sup> August. No age adjustment is made for this fish (2011 - 6 = 2005).

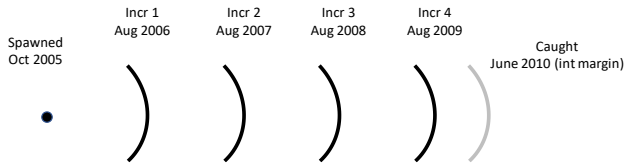


**Example 3:** Fish caught in September 2007 with 4 increments. This fish is allocated to the 2003 year-class (i.e., 2003/04 wet season). Interpretation of a narrow margin in September is that the fish recently formed its 2007 increment. No age adjustment is made for this fish (2007 - 4 = 2003).

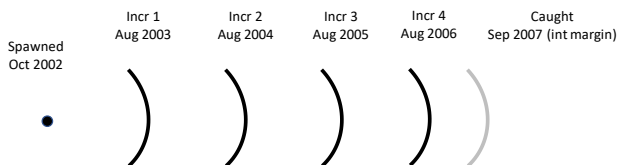


## Intermediate margins

**Example 1:** Fish caught in June 2010 with 4 increments. This fish is allocated to the 2005 year-class (i.e., 2005/06 wet season). Interpretation of an intermediate margin in June is that the fish is yet to form its 2010 increment. A 1-year age adjustment is made for this fish ( $2010 - [4+1] = 2005$ ).

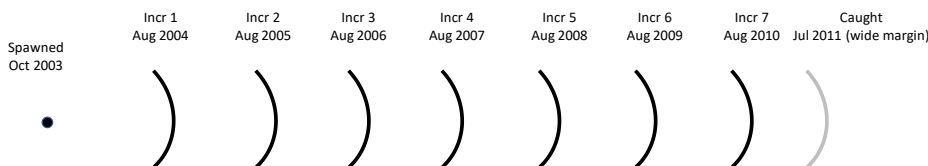


**Example 2:** Fish caught in September 2007 with 4 increments. This fish is allocated to the 2002 year-class (i.e., 2002/03 wet season). Interpretation of an intermediate margin in September is that the fish is yet to form its 2007 increment. A 1-year age adjustment is made for this fish ( $2007 - [4+1] = 2002$ ).

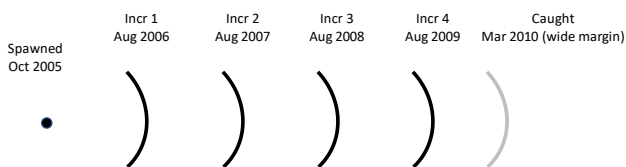


## Wide margins

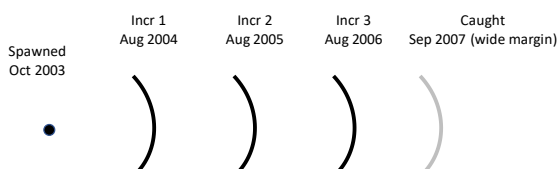
**Example 1:** Fish caught in July 2011 with 7 increments. This fish is allocated to the 2003 year-class (i.e., 2003/04 wet season). Interpretation of a wide margin in July is that the fish is yet to form its 2011 increment. A 1-year age adjustment is made for this fish ( $2011 - [7+1] = 2003$ ).



**Example 2:** Fish caught in March 2010 with 4 increments. This fish is allocated to the 2005 year-class (i.e., 2005/06 wet season). Interpretation of a wide margin in March is that the fish is yet to form its 2010 increment. A 1-year age adjustment is made for this fish ( $2010 - [4+1] = 2005$ ).



**Example 3:** Fish caught in September 2007 with 3 increments. This fish is allocated to the 2003 year-class (i.e., 2003/04 wet season). Interpretation of a wide margin in September is that the fish is yet to form its 2007 increment. A 1-year age adjustment is made for this fish ( $2007 - [3+1] = 2003$ ).



# Appendix 5. Project fact sheet



## Effects of freshwater flows on recruitment and growth of Barramundi

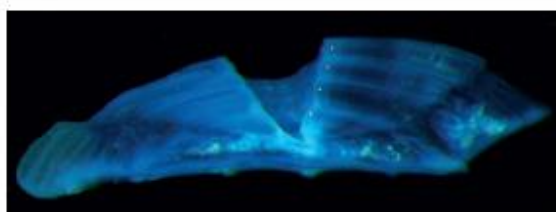


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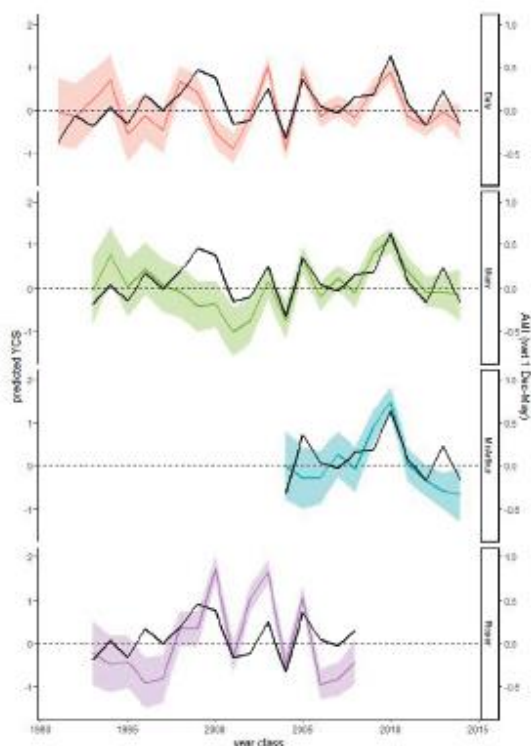
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### Climate and river flow drive the productivity of our barramundi fisheries

- > There is much current interest in expanding agriculture and mining across Northern Australia, which will require additional use of water from rivers.
- > Whilst such developments provide important economic opportunities, increased water use will alter river flows and may impact aquatic ecosystems and the fisheries they support.
- > This project analysed Barramundi otoliths (see below) to improve our understanding of relationships between fishery productivity and river flows, and to predict the likely effects of increased water use on Barramundi fisheries in the Northern Territory.
- > Results showed that the number of recruits each year (year class strength, YCS) was strongly correlated with a measure of regional wet season strength (the Australian Monsoon Index, AMI) across the Daly, Mary, Macarthur and Roper rivers.
- > River-specific analyses demonstrated strong relationships between Barramundi YCS and growth and various aspects of river flow. These relationships were complex and variable among rivers.



Sectioned Barramundi otolith (earstone) viewed under a microscope. Four annual growth checks are marked (4-year old fish)

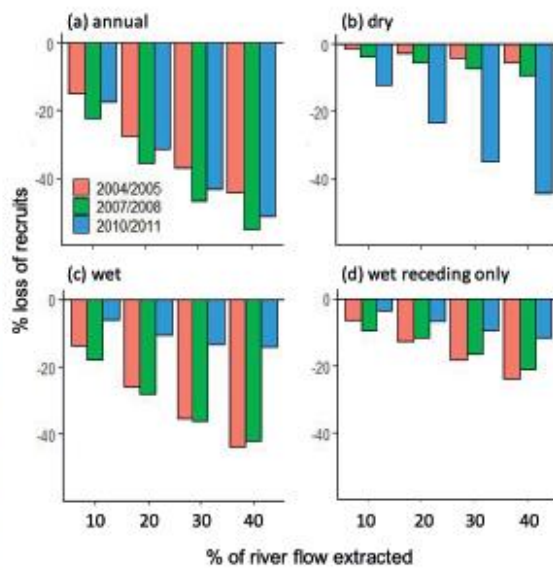


Predicted Barramundi year class strength (+/- 95% confidence intervals) for Daly, Mary, Macarthur and Roper rivers versus wet season Australian Monsoon Index (black line).

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- > Scenario modelling for the Daly and Roper rivers predicted that increased consumptive water use during both the wet and dry seasons would have strong impacts on Barramundi recruitment.
- > Results from the study have been provided to water managers to support decisions relating to future water resource development in the NT and other regions of northern Australia.



Modelled effects of consumptive water use on Barramundi recruitment in the Daly River in dry (2004/05), average (2007/08) and wet (2010/11) years. (a) all year; (b) dry season; (c) wet season; (d) wet season on receding flows only.



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 Produced August 2020 | 66530 Unprint 08/20



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