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Fish stocking programs

Assessing the benefits against potential long term genetic and ecological impacts

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FRDC Project Number 2009/040

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FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Fish stocking programs: assessing the benefits against potential long-term genetic and ecological impacts

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

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2009/040 Fish stocking programs: assessing the benefits against potential long-term genetic and ecological impacts

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OBJECTIVES:

1. Assess movements and ecological impacts of stocked barramundi in a model river and impoundment
2. Determine if barramundi stocking has any discernable adverse genetic impacts on wild populations in a previously stocked river system

Outcomes achieved

The principal outcome of this project was to provide fisheries managers and other relevant stakeholders with the quantitative data needed to assess some of the likely ecological and genetic impacts of barramundi stocking. Access to these data will enable them to address sustainability concerns by refining stocking permit conditions and protocols to ensure that they are aligned with world's best practice. This, in turn, will help to ensure that the substantial benefits of fish stocking are continued to be enjoyed by industry and the community. The results of this project were well received when presented to a dedicated workshop of industry, community stocking groups, fisheries and natural resource managers in Townsville in May 2012. The results will also be available to be used in the development of freshwater fisheries policy by Fisheries Queensland.

Native fish stocking activities in Australia are very well established and their benefits well known, but there is a body of evidence from mostly international studies that demonstrates that this type of activity can have negative impacts. There are concerns in some sections of the community that some of these negative impacts are already occurring in Australia.

An earlier survey (2008) of key stakeholders including fisheries and conservation managers, community groups and industry representatives identified a number of key management questions related to fish stocking in Australia, including:

- Is there leakage of stocked fish into sensitive environmental habitats?
- Do stocked fish threaten vulnerable species?
- Have historic stocking programs caused changes in the genetic population structure of wild stocks?
- Are there impacts on survival and growth and displacement of wild fish populations?
- Is stocking density of barramundi in rivers and impoundments at sustainable and/or optimal levels?

The first four of these questions formed the basis for the objectives of this current project and were addressed with a particular emphasis on barramundi stocking in the north Queensland Wet Tropics Bioregion.

To address Objective 1, barramundi were strategically stocked into sites in the Johnstone River and Tinaroo Falls Dam at densities similar to what were commonly used in historic stocking programs. Their movements, diet, growth and condition were monitored. Wild stocks of barramundi exist in the Johnstone River but Tinaroo Falls Dam supports only a 'put and take' fishery. Stocked barramundi in both the Johnstone River and Tinaroo Falls Dam showed no inclination to move upstream into smaller tributary streams where they would be more likely to encounter and/or impact on prey species that are of conservation significance, for example, some species of amphibians. In the Johnstone River, stocked barramundi generally occupied the same locations and habitat types as wild fish, while in Tinaroo Falls Dam recently stocked juvenile barramundi mostly moved away from tributary streams and into the main body of the impoundment.

Dietary studies of both stocked and wild (Johnstone River) barramundi suggest a low predation rate on prey species that may be considered to be of conservation significance. Rather than being selective in their dietary preferences, barramundi in the study areas are opportunistic predators, consuming a range of different prey items. The diet of barramundi varied depending on the abundance of prey species in the habitats where they were captured. At the current stocking densities, no evidence was found to suggest widespread cannibalism of stocked and/or wild barramundi in the Johnstone River or of stocked fish in Tinaroo Falls Dam.

Growth rates of stocked and wild fish in the Johnstone River were similar, but barramundi in Tinaroo Falls Dam grew at a faster rate, probably because of an abundance of suitable prey and refuge habitat. The condition factor of the stocked barramundi in Tinaroo Falls Dam was similar to that observed in both wild and stocked fish in the Johnstone River.

The recapture rates of stocked fish underline the importance of habitat in determining the relative abundance of barramundi. Juvenile barramundi were found in higher densities in slower, deeper sections of the Johnstone River with plenty of cover rather than in shallow, fast-flowing reaches or narrow, shallow, swift-flowing tributaries. Indeed, very few barramundi stocked into the latter habitat types were ever recaptured, suggesting that they either perished or moved to other more suitable locations. Great caution should be exercised in considering stocking a top level predator like barramundi directly into areas, for example, above natural barriers, where they are likely to survive and become a novel predator on known environmentally-sensitive species (that may not have sufficiently developed anti-predator mechanisms).

Despite barramundi stocking in the Johnstone River being undertaken continuously from 1993 until 2005 and again in 2009, no evidence was found of either a loss of genetic diversity in the wild population or increased inbreeding levels. Furthermore, there was no evidence of movement of genes from the original broodstock back into the wild population of barramundi in the Johnstone River (introgression). Individual family contributions in pre-stocking cohorts and in subsequent recaptures were highly skewed. Tracking the proportions of the various families of stocked barramundi both prior to release and in the recaptured population, while suggesting differential survival between family groups, may simply be due to sampling error.

These results suggest that, for the management issues investigated, barramundi stocking at the current rate in the Johnstone River and Tinaroo Falls Dam have had minimal impact. This is likely to hold true for other catchments in the Wet Tropics Bioregion with similar hydrology patterns, catchment morphologies and land uses. However, increased stocking densities and stocking in other regions will undoubtedly produce different results and there is a need for further research to determine optimal/sustainable stocking levels. There is also a need to develop and implement appropriate hatchery protocols to ensure the future genetic viability of wild barramundi fisheries in Queensland.

Keywords: Freshwater fish stocking, sustainability, environmental impacts, barramundi, Wet Tropics Bioregion.

Acknowledgements

This project would not have been possible without the assistance of the many commercial and recreational fishers who provided biological samples. In particular we wish to thank Steve Howe and Mario Corica.

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Department of Agriculture, Fisheries and Forestry , Queensland staff members including Wayne Hagadoorn, Sam Hedge, Stuart Hyland, Mark Leith, Malcolm Pearce, Joe Sariman and others who assisted either in field operations or by collecting samples while engaged in other duties. Giana Gomes also helped in the processing of some of the genetic samples. We would also like to thank members of the Tablelands Fish Stocking Society, and in particular Jenny and John Mondora, who provided the fish for release into Tinaroo Falls Dam for this project and then assisted in tagging and releasing them into the impoundment. We would also like to thank Richard Saunders who assisted in writing and editing this document. The picture of the front cover was supplied courtesy of John and Jenny Mondora.

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Background

Fish stocking in Australia

In Australia, fish stocking activities have resulted in the creation of valuable new 'put and take' fisheries as well as enhancing existing wild fisheries (e.g. Cadwallader and Kerby, 1995; Rowland, 1995; Holloway and Hamlyn, 1998). As a result, stocking activities have been of considerable economic benefit to industry and the community, particularly rural and regional communities. Recently, questions have been raised about the environmental sustainability of fish stocking activities, and these need to be addressed if industry and the community are to continue to enjoy the benefits of fish augmentation programs (see Phillips, 2003).

Fish stocking is not a new practice in Australia, with several species of northern hemisphere trout released regularly for over a century. Furthermore, the ready availability of Australian native species, due to advances in fish breeding technologies, has resulted in a huge increase in the magnitude of stocking activities since the late 1970s.

In Queensland alone, there are 73 community fish stocking groups active that are permitted to release fingerlings into approximately 150 locations. In New South Wales in 2010/11, around three million fingerlings were released, up from around 1.24 million in 2001/02. Annual production of Murray cod (*Maccullochella peelii peelii*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*) by commercial hatcheries totalled 5–8 million fingerlings (Rowland and Tully, 2004). In Victoria between 730,000 and a million native fish are released annually with golden perch and Murray cod the dominant species (Department of Primary Industries, 2005).

Benefits of stocking

There have been many positive outcomes from fish stocking activities in Australia. For example, advances in breeding technology for barramundi (*Lates calcarifer*) in the 1980s resulted in the creation of successful "put and take" recreational fisheries for that species in many Queensland impoundments including Tinaroo Falls Dam. The increase in visitor numbers that resulted from the creation of the Tinaroo Falls Dam fishery has been of significant economic benefit to the local rural community. A cost-benefit analysis of the barramundi stocking program in Tinaroo Falls Dam concluded that each dollar spent on fish stocking returned a potential $31 of economic benefit to the Queensland economy (Rutledge et al. 1991). Hogan (pers. comm.) estimates the value of the Tinaroo Falls Dam fishery alone to be in excess of $10 million. There are also many other examples of highly successful stockings creating large recreational fisheries in impoundments and rivers in New South Wales (Rowland, 1995) and other parts of Australia.

As well as impoundment stocking, a number of fish species have been released into many inland and coastal rivers around Australia. Some of these stockings are designed to enhance or promote the recovery of existing fisheries. Barramundi stocking in Queensland is one example where hatchery-produced fish have been released to enhance existing wild fisheries.

A long-running study by Russell and colleagues in the Johnstone River in north Queensland examined the efficacy and cost-benefits of barramundi stock enhancement (Russell and Rimmer, 1997, 1999, 2000; Russell *et al.*, 2002). The data obtained from this study suggest that, after moderate stocking activity, stocked fish can contribute between about 10 and 15% of the commercial and recreational catch respectively (Rimmer and Russell, 1998). The stocking of Murray cod, golden perch and silver perch has re-established these species in some rivers on the Northern Tablelands of New South Wales (Rowland, 1995). In addition,

stocking has been used in some places as a tool for the conservation of the endangered eastern freshwater cod (*Maccullochella ikei*), trout cod (*M. macquariensis*) and Mary River cod (*M. mariensis*) (www.environment.act.gov.au/__data/assets/pdf_file/0004/156820/Fish_stockplan_2009-2014_final.pdf). In Victoria, trout cod are stocked in small numbers to establish self-sustaining populations to ensure the survival of this species in the wild (Department of Primary Industries, 2005).

Issues with stocking

Adding large numbers of fish, particularly high level predators, to an ecosystem will have environmental consequences. In Australia, potential issues associated with the stocking of native fishes were reviewed by Harris (2003) and discussed at a workshop "Managing Fish Translocation and Stocking in the Murray-Darling Basin" (Phillips, 2003) held in Canberra in September 2002. Gillanders et al.(2006) reviewed the impacts of native fish stocking on fish within the Murray-Darling Basin and recommended that, given the continued increase in stocking of hatchery-reared fish and the potential for such interactions with wild fish, it was essential to take a "responsible approach" and to "monitor and experimentally evaluate any stocking program".

In the Wet Tropics of north Queensland, a review of stocking activities and consideration of the potential impact of fish stocking was done by Burrows (2002). An Environmental Impact Statement (EIS) on freshwater fish stocking in New South Wales (New South Wales Fisheries, 2003) noted numerous considerations were likely to pose a risk to the environment. The EIS also highlighted the lack of specific research into the impacts of stocking on the receiving environment.

These reviews and assessments have identified several common threats from stocking including the loss of population genetic diversity, impacts on indigenous aquatic communities (including threats to listed species), and the spread of diseases. These threats have been recognised globally and have provided some of the impetus for calls for "responsible fish stocking" (Blankenship and Leber, 1995b, a, 1997; Lorenzen *et al.*, 2010) and these calls have been reinforced in Australia (Taylor *et al.*, 2005).

Inherent in the recommendations for responsible fish stocking is the ability to distinguish wild from stocked fish. Numerous marking techniques have been developed and evaluated including chemical marking (Munro *et al.*, 2008; Woodcock *et al.*, 2011), genetic identification (Robbins *et al.*, 2008) as well as dart, anchor and coded wire tags (Ingram, 1993; Booth and Weyl, 2008). The ability to distinguish wild from stocked fish is essential to evaluate the effectiveness of a stocking program and to facilitate study of the biological impacts of fish stocking. Internationally, such evaluations have been produced, particularly for salmonid stocking in Europe and North America (see Pearsons, 2008). In Australia, while technologies to determine the origin of fish have been the subject of research effort (see Woodcock *et al.*, 2011), little empirical work has been done on the biological impacts of stocking and it remains poorly understood (Taylor *et al.*, 2005).

Biological impacts of stocking

The stocking of non-native species or native species translocated to areas they do not naturally occur, have impacts akin to any invasive animal. Specifically, they compete for resources with indigenous fauna and may prey upon them. Furthermore, when stocking occurs to supplement a pre-existing wild population, there is potential for intra-specific genetic competition. Thus, there are concerns about the biological effects of stocked fish on endemic fish populations, and effects on other fish species and aquatic fauna and flora. The

biological impacts of fish stocking can be broadly categorised into three types (1) ecological, (2) disease and (3) genetic.

Ecological impacts of stocking

It is well established that fish have a strong influence on ecosystem processes and the mechanisms by which this occurs are complex and diverse. Competition for resources such as food and space is a primary mechanism (Pearsons, 2008). Such density-dependent mechanisms of impact have been demonstrated (Achord *et al.*, 2003) and it follows that successful stocking can limit the available resources for wild fish populations.

Direct predation by stocked fish is a simple, clear mechanism of ecosystem alteration. Increased mortality of wild fish due to predation by stocked fish has been demonstrated for salmonids (Pearsons and Fritts, 1999) and is likely for other piscivorous fishes such as barramundi. Such direct predation can have flow-on effects beyond the aquatic environment. For example, in North America the dietary changes of a seabird have been at least partly attributed to a stocking program that altered the trophic dynamics in the Laurentian Great Lakes (Hebert *et al.*, 2008). Beyond piscivory, stocked fish consume other animals.

There has been speculation that deliberate stocking or accidental introductions of novel fish predators can place at risk significant amphibian and crustacean assemblages, particularly in areas like the high mountain streams of the Queensland Wet Tropics (Burrows, 2002). Burrows (2002) cited European and North American examples of significant reductions, even localised extinctions, of frog populations resulting from the introduction of novel predators (e.g. Bradford, 1989). Impacts on frog populations in Australia have also been documented with introduced trout taking significant numbers of tadpoles of the spotted tree frog (Gillespie, 2001). Other studies have suggested that novel predators can influence the distribution, size structure and behaviour of prey species even though the affected prey species may already have endemic predators (Concepcion and Nelson, 1999; Leberer and Nelson, 2001).

The complexity of ecosystems means that the impact of fish stocking can be far reaching and direct predation is one mechanism by which ecosystems can be impacted. Pearsons (2008), while focussing on salmonids, provides an excellent overview of mechanisms of ecological impacts of stocking and, amongst others, lists direct and indirect predation, behavioural anomalies and changed nutrient dynamics as key impacts on recipient ecosystems and species.

In Australia and New Zealand, declines of native galaxias species have been associated with the stocking of non-native salmonids, primarily brown (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*). These species of trout prey directly on native galaxias (Tilzey, 1976; Ault and White, 1994). Evidence of the impact of such predation was provided by the removal of rainbow trout from a stream in the Australian Capital Territory after which galaxias species were able to recolonise a nine kilometre section of that stream from which they had previously been excluded (Lintermans, 2000). Indirect impacts of stocking trout have also been documented. Specifically, the diet of galaxias overlaps considerably with the introduced salmonids (Glova *et al.*, 1992; Glova and Sagar, 1993). In New Zealand, introduced salmonids have been demonstrated to consume the majority of all benthic invertebrate production (Huryn, 1996, 1998).

Even without considering direct predation, such predation pressure, combined with a broad dietary overlap, could leave no food resources for native galaxias (McDowall, 2003). In spite of these and other studies, the evidence for the impact of salmonid stocking on galaxias has been criticised as being circumstantial, but the evidence is nonetheless compelling (McDowall, 2006). Beyond the impact of trout on native galaxias, their heavy predation on

invertebrates can also have far reaching consequences for the tropho-dynamics of ecosystems (McIntosh and Townsend, 1996; Biggs *et al.*, 2000).

A further Australian example of ecological impacts was the translocation, for fishery purposes, of the large piscivorous gudgeon the sleepy cod (*Oxyeleotris lineolatus*) into the upper reaches of the Burdekin River, Queensland in the 1980s. This area of the river is isolated from its lower catchment by the Burdekin Falls. Sleepy cod does not naturally occur in the Burdekin River Basin and the population initially remained small and restricted to the site of introduction for the course of a decade. However, after the occurrence of a large flood and subsequent entry into a prolonged period of drought, it expanded its population size and distribution to a point where it was present in every available tributary of the Burdekin basin (Pusey *et al.*, 2006). This, in addition to the translocation of other species into the upper reaches of the Burdekin River (e.g. barramundi, *L. calcarifer*), has had a substantial effect on the fish fauna of the river. Post-introduction fish surveys suggest that the spread of sleepy cod was concomitant with a significant decline in abundance of the purple-spotted gudgeon (*Morgunda adspersa*) in the Burdekin River (Pusey *et al.*, 2006). Further, it would appear that the purple-spotted gudgeon has been driven to near extinction in parts of the Burdekin River when the sleepy cod has reached high abundance (Pusey *et al.*, 2006).

While the above examples are of species (both exotic and native) that were translocated to regions where they did not naturally occur, there is currently limited information on the ecological impacts of enhancement of natural stocks on wild conspecifics or the receiving ecosystems in Australia. While not directly addressing the impacts of fish stocking, some work has been carried out on ecosystem carrying capacity by considering habitat limitations (Taylor and Suthers, 2006; Smith *et al.*, 2011) and dietary requirements (Taylor and Suthers, 2008). This highlights the unresolved question as to whether enhancement programs increase biomass of the target species or displace wild fishes. Indeed, even for highly "successful" stock enhancements of salmonoids in Alaska this question remains valid (Neff *et al.*, 2011).

Disease

Disease outbreaks can have devastating impacts on fish populations (e.g. Rahimian and Thulin, 1996; Gaughan, 2002), and while disease transfers from hatchery fish to wild populations can occur, it has rarely been well documented (but see McVicar, 1997). There are, however, numerous examples of disease transfer from farmed fish to wild fish (see Amos and Thomas, 2002; Olivier, 2002). Inter-species transfer of pathogens is also of concern, for example of fish carrying viruses that can impact amphibians (e.g. Ranavirus: Daszak *et al.*, 1999). These examples serve to demonstrate that there are disease risks associated with introducing captive animals into the natural environment. Management of these risks is an essential component of good stocking practices (Lorenzen *et al.*, 2010).

Genetic impacts of fish stocking

Fish that have been reared in captivity often have reduced and skewed allelic genetic diversity compared to progenitor populations (Taylor, 1991; Palm and Ryman, 1999; Frost *et al.*, 2006). This is because hatchery progeny are usually derived from few parental broodstock that do not possess the wide accompaniment of alleles represented in the species as a whole. Small effective population sizes within hatcheries also result in the production of large numbers of highly related progeny (Frost *et al.*, 2006). Furthermore, captive broodstock are subject to different selective pressures (e.g. domestication selection) to wild fish and their progeny may be poorly adapted to survive in the wild (Lynch and O'Hely, 2001; Ford, 2002).

Thus, when the progeny of these fish are stocked into a wild population, and they survive and breed, there is the potential for these fish to impact the genetic diversity of the wild population through several mechanisms. Firstly, if the ratio of the receiving population to the

stocking population is too high, this can lead to swamping of the indigenous gene pool with highly related genotypes resulting in increased inbreeding and reduced genetic diversity in the receiving population (Neff *et al.*, 2011). Further, if the stocking population has been subject to domestication selection, intentionally or otherwise, and the genotypes they carry are mal-adapted for wild conditions, it may reduce the genetic fitness of the wild population, which can have long-term implications for the resilience of that population (Christie *et al.*, 2012). Finally, there is the risk of introgression hybridisation where the hybrids between wild and stocked fish inherit a gene (or genes) whose frequency has been artificially increased in the hatchery, but which are detrimental to survival in the wild (Philipp *et al.*, 2002; Marzano *et al.*, 2003). This can result in a reduction in the effective population size and loss of genetic variability (Ward, 2006). However, it is difficult to assess the detrimental impacts of these genetic effects without an understanding of the adaptive significance of the lost or altered traits in the local environment (Houde *et al.*, 2011).

Natural selection in the wild will remove individuals with low fitness, but when these individuals have swamped the natural population and reduced natural genetic diversity, the net effect can be a decline in the overall population size. These effects have been documented for some species. In one example from Spain, the genetic effects of stocking hatchery-reared brown trout into wild populations included (1) stocked fish failing to reproduce, (2) wild brown trout populations experiencing substantial introgression from hatchery stocks and (3) virtual extinction of local endemic populations (Garcia-Marin *et al.*, 1991; Garcia-Marin *et al.*, 1999). Inbreeding depression and loss of genetic variation from bottlenecks (very small effective population size, N_e) have been documented in Atlantic salmon and several trout species (Waples and Drake, 2004).

The need for genetic management during stock enhancement programs is recognised worldwide. Genetic diversity is positively correlated with fitness (Reed and Frankham, 2001), and interfering with natural genetic diversity can result in populations with less resilience to disease and a reduced ability to meet environmental challenges. Lowering of genetic diversity of progeny coming out of hatcheries is inevitable without the guaranteed contribution of a large number of brood-stock and appropriate breeding programs for the production of offspring. An understanding of the level of interbreeding between stocked and wild fish is essential to determine appropriate stocking rates. In Australia, there is no current understanding of the genetic interaction between wild and stocked fishes.

Responsible fish stocking

Calls from scientists and the community for "responsible stocking" that takes into account the biological impacts have been made repeatedly over the past fifteen years at national and international levels (Blankenship and Leber, 1995a; Lorenzen, 2005; Taylor *et al.*, 2005; Lorenzen *et al.*, 2010). The call for responsible fish stocking requires research into the impacts of stocking on genetic diversity, ecology of aquatic ecosystems and disease.

Most of the information on sustainability of fish stocking in Australia is from desktop studies using information gleaned from overseas work, particularly for salmonids, the relevance of which is uncertain. To date, there have been no published Australian studies that have sought to directly assess the ecological impacts of stocking programs. Therefore, it would be unwise to excessively extrapolate the outcomes of cold water and temperate fish stocking studies in the northern hemisphere to temperate, tropical and sub-tropical ecosystems in Australia where species assemblages and ecological characteristics differ significantly.

If the benefits of fish stocking to the community and industry are to continue, then mechanisms need to be put in place to ensure that it is carried out in an ecologically-sustainable manner. Therefore, this project aimed to (1) assess movements and ecological

impacts of stocked barramundi in a model river and impoundment, and (2) determine if barramundi stocking has had any discernible adverse genetic impacts on wild populations in a previously stocked river system.

Consultation

Prior to the commencement of this project, delegates at a national expert workshop held as part of the FRDC sponsored project "Towards responsible native freshwater fish stocking" acknowledged the need for further work on sustainability issues related to barramundi stocking programs and assisted in the development of a project outline. The peak recreational fishing organisation in Queensland (Sunfish) reviewed the proposal for the current project and gave it a high priority. Other groups that were consulted include Sunwater, the Wet Tropics Management Authority, the Freshwater Fishing and Stocking Association of Queensland, the Recfishing Research Steering Committee and resource managers in the Department of Agriculture, Fisheries and Forestry (DAFF) Queensland. This project also addressed priority issues for, and was supported by, the Queensland Freshwater Management Advisory Committee. More recently, a workshop was held in Townsville in May 2012 where a range of stakeholders including fisheries and natural resource managers, industry representatives, stocking groups and community bodies were briefed on the results of the project.

Need

Recreational fish stocking is widely practised throughout Australia and has delivered considerable benefits to anglers, and substantial economic flow-on effects have been previously documented in some fisheries (Rutledge *et al.*, 1990; Rutledge, 1990). The long-term "success" of freshwater fish stocking in Queensland and other states is, however, contingent on demonstrating that it is an ecologically sustainable practice, having no or minimal detrimental effects on wild populations and ecosystems. Some environmental groups and government agencies (e.g. Wet Tropics Management Authority) are now questioning if freshwater fish stocking is a sustainable activity. The Threatened Species Scientific Committee considered a nomination to list the introduction of live native or non-native fish into Australian watercourses that are outside their natural geographic distribution as a key threatening process.

One of the criteria that fisheries managers now use for assessing freshwater fish stocking applications is the risk they pose to local aquatic communities, although there is very little real information on the likely ecological and genetic impacts of native freshwater fish stocking activities to support this decision making process. This type of information is therefore urgently needed if our stocking industry is to adopt "world's best practice" to ensure future sustainability.

These same concerns were expressed at a 2008 FRDC national expert workshop "Towards responsible native freshwater fish stocking", where the potential ecological and genetic impacts of fish stocking, particularly for barramundi and Murray cod, were nominated as the most pressing research and management issues. This proposed project is part of a coordinated national response to these critical issues.

Objectives

1. Assess movements and ecological impacts of stocked barramundi in a model river and impoundment.

2. Determine if barramundi stocking has any discernible adverse genetic impacts on wild populations in a previously stocked river system.

General methods

The overall experimental plan for this study involved releasing batches of hatchery reared juvenile *L. calcarifer* into a Queensland wet tropics river and impoundment (Figure 1), and then closely monitoring their immediate post-stocking movements, diet, growth and condition. The river selected for this study was the lower freshwater reach of the Johnstone River and the impoundment was Lake Tinaroo (Figure 2). Both these study locations had previously been stocked with *L. calcarifer* and the Johnstone River has an existing wild barramundi fishery.

Study locations

Lake Tinaroo – is the largest artificial water body in the Wet Tropics bioregion of north Queensland and is an in-channel impoundment on the Barron River (17°10′S, 145°35′E) on the Atherton Tablelands. It has a capacity of 407 000 ML, a surface area of 33.7 km^2, a shoreline of 39 km and a surface elevation of 670 m.

Johnstone River – is a large catchment of the Wet Tropics bioregion that has its source on the Atherton Tablelands at an elevation of 1385 m ASL. The system flows into the Coral Sea near the township of Innisfail (17°32′S, 146°02′E). On the coastal plain, the river bifurcates into two major arms—the North and South Johnstone Rivers with catchment areas of 994 km^2 and 640 km^2 respectively. Both of these rivers have significant upland and lowland components to their catchments that are separated by steep gorge sections with numerous waterfalls and cascades. These act as migratory barriers to most native catadromous species such as *L. calcarifer*. The vegetation in the lowland sections is heavily cleared for agriculture, however, remnant riparian rainforest consisting of mostly mesophyll vine forest with dominant palms (MFPVF, Type 3) (Tracey, 1982) remains.

Stocking

Fish stocked into the Johnstone Rivers were sourced from two commercial hatcheries using broodstock of local genetic provenance (Keenan, 1994). These hatcheries supplied 5615 and 3808 fish respectively 50–70 mm Total Length (TL) and these were stocked into the Johnstone River during November–December 2009. During February 2010, a separate batch of fish at ~200 mm TL were released into Lake Tinaroo. These fish were spawned earlier in 2009 and were grown out to a larger size than the Johnstone River fish to meet the requirements of the local community fish stocking association. Full stocking numbers by site are given in

Table 1. Numbers of barramundi stocked by site.

Site	November 2009	December 2009	February 2010
North Johnstone (NJU)	1625	1724	
North Johnstone (NJL)	2183	1854	
South Johnstone (SJU)		2037	
Lake Tinaroo (Severin Creek)			998
Lake Tinaroo (Kauri Creek)			999
Lake Tinaroo (Robson Creek)			999
Total Stocked	3808	5615	2996

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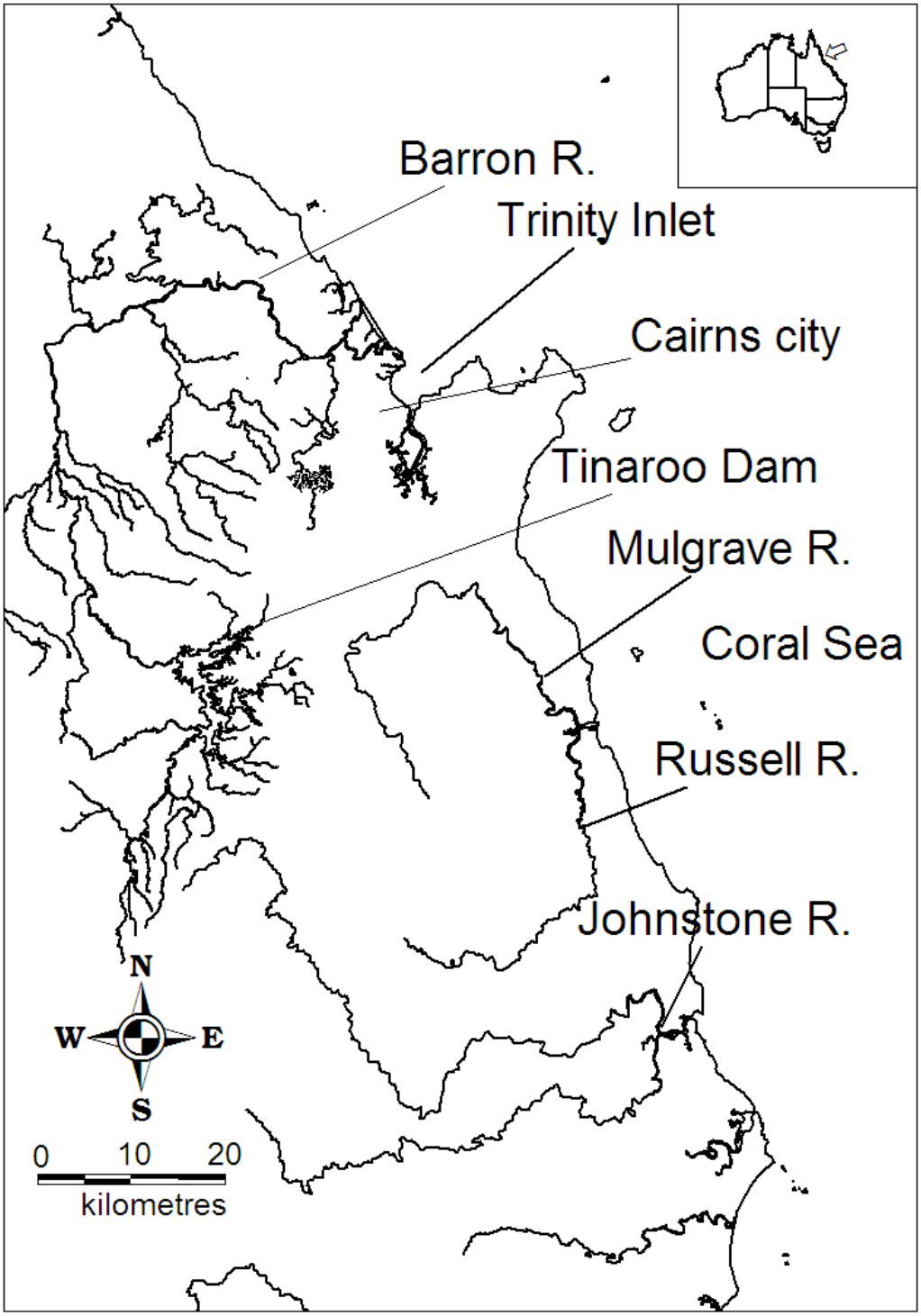


Figure 1. Study areas in north Queensland

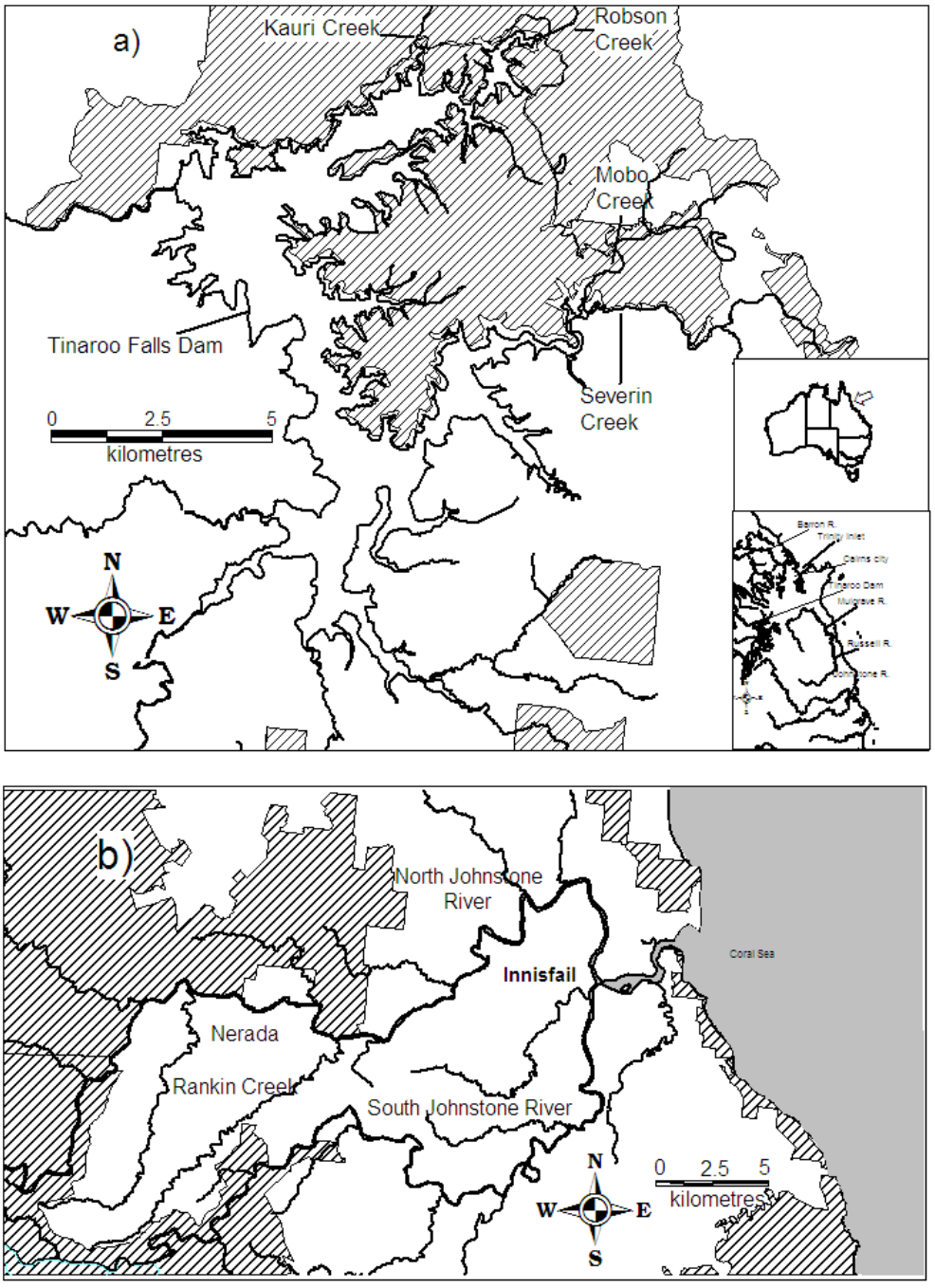


Figure 2 Tinaroo Falls Dam (a) and Johnstone River (b) study areas. Crosshatched areas are the Wet Tropics World Heritage area.

Table 1. Numbers of barramundi stocked by site.

Site	November 2009	December 2009	February 2010
North Johnstone (NJU)	1625	1724	
North Johnstone (NJL)	2183	1854	
South Johnstone (SJU)		2037	
Lake Tinaroo (Severin Creek)			998
Lake Tinaroo (Kauri Creek)			999
Lake Tinaroo (Robson Creek)			999
Total Stocked	3808	5615	2996

Release sites

Johnstone River

In the North Johnstone, stockings for this current study occurred in the tidally influenced lower freshwater reaches of the main channel and further upstream (~ 25 km) at Nerada in Rankin Creek and the adjacent main river channel (Figure 3). In the South Johnstone River, barramundi stockings only took place in Utchee Creek and in the adjacent main river channel. Both the South and North Johnstone Rivers have numerous feeder creeks (stream order ≤ 3) that potentially provide habitat for species of conservation concern (e.g. regionally endemic fish and amphibians). The upper catchments of many of these tributaries drain the Wet Tropics World Heritage Area (WTWHA), a large conservation reserve (894,420 ha) that extends along the north–east Queensland coast from Cooktown in the north to just north of Townsville in the south (http://rainforest-australia.com/wet_tropics_world_heritage.htm).

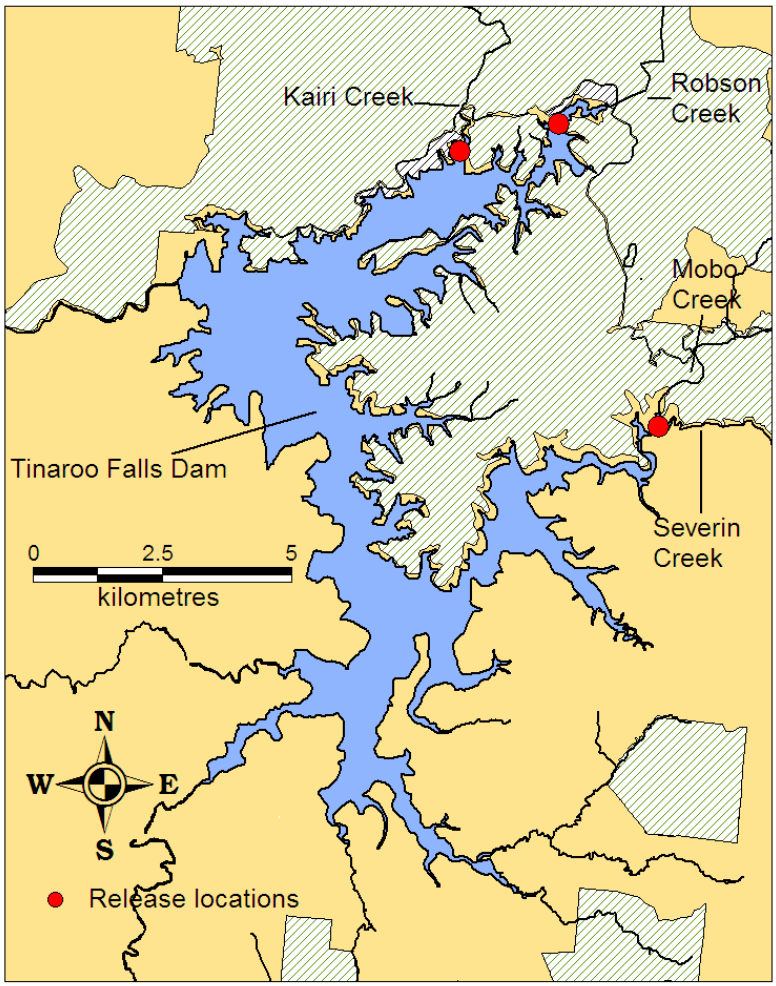
Stockings sites were selected to 1) encompass the natural range of *L. calcarifer* in the freshwater reaches of the Johnstone River, and 2) ensure stocked barramundi had the opportunity to migrate into potential areas of conservation concern (e.g. small feeder streams) by releasing them into locations adjacent to these areas. At all sites, fish were released into areas that provided refuge from predators (i.e. macrophyte beds, rock piles and woody snags) to maximise survival.

Lake Tinaroo

These fish were released into three large arms of the dam: the Severin Creek arm, Robson Creek arm and the Kairi Creek arm (Figure 3). These sites were adjacent to significant feeder creek networks in the Danbulla National Park (also part of the WTMA). The terrestrial vegetation in this area consists mostly of intact rainforest (Complex Notophyll Vine Forest, Type 1c) (Tracey, 1982), and is considered an area of high conservation value with regionally endemic species (i.e. crustaceans, fishes and amphibians).

Tagging program

To facilitate later identification of the stocked fish during the monitoring program, all fish, depending on size, were marked with either a coded wire tag (CWT) or a dart or anchor tag.



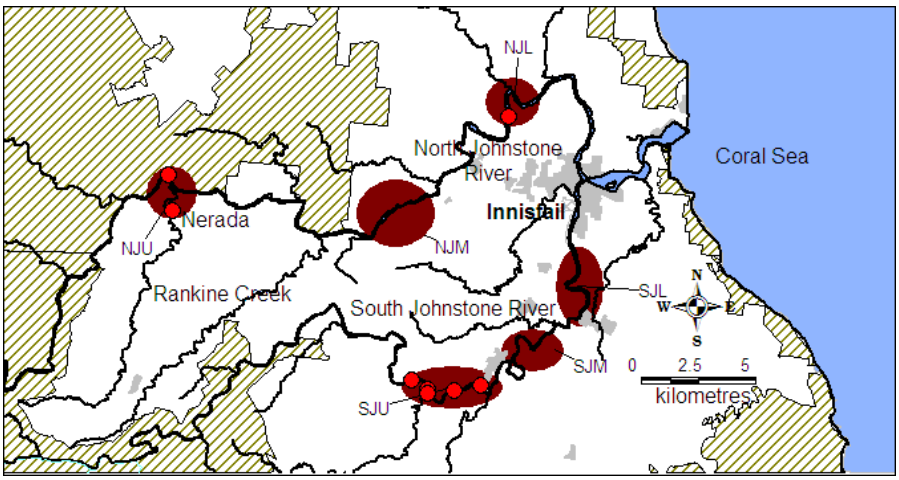


Figure 3. Stocking and sampling locations in Lake Tinaroo (top) and the north and south Johnstone Rivers (bottom).

Oblique-hatched areas denote the Wet Tropics World Heritage Area, red dots are stocking locations and shaded oval areas are sampling zones in the Johnstone River.

Johnstone River

Prior to their release into the Johnstone River, all *L. calarifer*, because of their relatively small size (50-70 mm TL), were marked with a CWT (Northwest Marine Technologies Inc., Shaw Island, WA, USA, www.nmt.us). Tags, which measured 1.00 x 0.25 mm diameter, were laser etched with a unique batch number. CWTs were inserted under the skin and into the muscle layer of the cheek (gill cover) using an automatic tag injector (model MKIV). Following tagging, fish were passed through a Quality Control Device (model QCD) to confirm successful tag insertion. Barramundi were then returned to holding tanks for a period of at least two days, and again passed through the QCD to ensure the CWTs had been retained. Fish stocked into the South Johnstone River were double tagged (left and right cheeks), while those released into the North Johnstone River were single tagged (left or right cheek only). Prior to their release, fin clips were taken from samples of *L. calcarifer* purchased from each of the hatcheries to be used for later genetics analyses (see 'Genetic tissue sampling' section below for details).

A handheld magnetic wand detector was used to determine if fish recaptured during field electrofishing surveys were wild or stocked. Upon recapture in the river, if a CWT marked fish had grown larger than ~200 mm, it was externally tagged with a Hallprint type TBF-2 (45 mm) fine anchor T-bar tag (Hallprint Pty. Ltd, Hindmarsh Valley South Australia, www.hallprint.com). Details of the tagging procedure are outlined in Russell *et al.* (2003). These tags are marked with a unique number in the flag end to allow non–destructive identification of individual fish. Fish greater than 350 mm were tagged with a Hallprint 85 mm plastic tipped dart tag, which was inserted between the posterior ptyregiophores of the second dorsal fin rays using a hollow tagging needle. Any wild fish caught were also externally tagged using these methods.

Lake Tinaroo

At Lake Tinaroo, because of their larger size (~ 200mm TL), all hatchery reared fish were able to be externally tagged using a Hallprint type TBF-2 (45 mm) fine anchor T-bar tag before being measured (nearest mm TL) and then released in February 2009. These fish were slightly larger and older (spawned mid 2009) than the fish released into the Johnstone River (spawned late 2009 and ~60 mm TL). Lake Tinaroo is an artificial *L. calcarifer* fishery with no wild stocks, therefore there was no need to distinguish between wild and stocked fish, but there are other age cohorts of unmarked stocked barramundi in the impoundment. The local community fish stocking association assisted in marking the barramundi before their release. If any previously stocked *L. calcarifer* were captured during subsequent electrofishing surveys, these were also tagged.

Field sampling

Fish sampling techniques

All research sampling during this project was undertaken using electrofishing. In small and shallow streams, this was undertaken using a Smith-Root Model LR24 backpack (Smith Root Inc., Vancouver, Washington, www.smith-root.com) or a smaller 3.5 m aluminium boat fitted with a Smith-Root Model 2.5 GPP electrofisher unit and one netter. A Smith-Root 7.5 GPP boat-mounted electrofisher was used for larger and deeper streams (stream order ≥ 4) or Lake Tinaroo. These boat surveys were conducted with two netters aboard a 4.5 m aluminium boat.

Electrofishing was undertaken by creating an electric field in the water between the anode(s) and the boat (cathode). The boat was then manoeuvred so the anode(s) were close to the bank and/or structures such as snags, overhangs, macrophyte beds or rock bars. Between October 2009 and July 2011, routine electrofishing surveys were undertaken every six weeks in both

the Johnstone River and Lake Tinaroo. *Lates calcarifer* caught during these surveys were first anesthetised using Aqui-S (Aqui-S Ltd., Lower Hutt, New Zealand, www.aqui-s.com), with dosages ranging between 20–40 mg L^{-1}. Following application of the anesthetic, all fish were weighed, measured (TL), gut–flushed and, if unmarked, tagged with an anchor or dart tag before being released. All Johnstone River barramundi (both stocked and wild) that had not previously been caught in this study were also fin–clipped for a DNA sample. The capture locations of each fish were recorded using a Garmin Model 60CSx GPS (Garmin International Inc., Kansas City, Kansas, www.garmin.com). At the conclusion of each survey, electrofishing power on time (seconds) was recorded as a measure of fishing effort.

The aim of these surveys was to assess the site fidelity of stocked fish, and to determine the movements of individuals into areas of conservation concern—particularly small, upstream tributaries that drain the WTWHA (Figure 3). Sites were chosen to represent a range of habitats that *L. calcarifer* could potentially disperse between (i.e. not above migratory barriers such as waterfalls). These included main channel sites in the lower and upper freshwater sections of the Johnstone River catchment, and associated tributaries and headwater streams. In Lake Tinaroo, monitoring took place in the main arms of the impoundment that fish were released into, and associated feeder streams (Figure 3).

Water quality

Surface water quality parameters (dissolved oxygen, turbidity, temperature and salinity) were routinely recorded at each sampling event in both the Johnstone River and Lake Tinaroo using a Yeokal YK615 water quality analyser. Temperature and light *in situ* dataloggers (Hobo ©) were deployed at multiple locations in both systems.

Growth and condition

The TL of all fish captured was measured to the nearest ± 1 mm. A sub–sample of both wild and stocked fish approximately < 500 mm were also weighed (± 1 g) using Arlec digital scales (Melbourne).

Diet

Stomach contents of captured *L. calcarifer* were removed by stomach flushing (gastric lavage) (Kamler and Pope, 2001). This method was elected as the most appropriate as it is non–destructive, and was considered an effective technique for fish > 140 mm (Hartleb and Moring, 1995). *Lates calcarifer* (both stocked and wild) were first anesthetised as described above, and the stomach flushing was then undertaken by inserting soft polypropylene tubing (tube diameter varied depending on fish size) into the fore–gut. A 12 volt mechanical pump was then used to pump freshwater into the stomach of the fish. Once the stomach had visibly expanded with water, gentle pressure was applied and prey items were regurgitated into a sieve. These food items were subsequently stored in vials containing 70% ethanol. A small subsample (n =10) of stomach–flushed *L. calcarifer* was collected opportunistically (i.e. if a fish was injured/diseased) and the euthanased fish was subsequently dissected in the laboratory to determine the efficacy of this technique.

Genetic tissue sampling

Broodstock and pre–release fingerlings

Archival quality genomic DNA extracts of high purity were obtained from the former Queensland Department of Primary Industries broodstock tissue samples (80% ethanol preserved fin tissue), which was previously collected in 2004 as part of a genetic audit of Queensland hatcheries. Some of these fish were used to produce fingerlings to be stocked into the Johnstone River.

DNA samples (pectoral or caudal fin clips) and TL measurements were also taken in 2009 from samples of the fingerlings purchased from two commercial hatcheries (Hatchery 1, n = 92 and Hatchery 2, n= 184) for release into the Johnstone River as part of this current project. These samples were taken to a) establish the number of families represented within each hatchery batch and b) to determine the respective contribution to pre–stocking of each of the families.

Pre–stocking genetic profile

To obtain a genetic profile of the Johnstone River *L. calcarifer* population prior to the commencement of stocking activities, DNA was extracted from a number of archived otolith samples held at the Queensland Government's Northern Fisheries Centre. These otolith samples were collected from barramundi captured in the Johnstone River system prior to 2000 and were stored dry at room temperature in individual sample vials. The otoliths that were eventually used were selected on the assumption that the very earliest that stocked *L. calcarifer* could contribute to the spawning population was in 1996, when as three-year-old fish they would be ~ 300–550 mm TL (Russell and Rimmer, 1997). Otoliths from barramundi that were at least three years old in 1996 were judged as wild fish and included in the analyses. There is no evidence of any unauthorised barramundi stocking in the Johnstone River.

Genetics field sampling

During field sampling of stocked areas in the Johnstone River, caudal or pectoral fin clips were taken from all *L. calcarifer* (i.e. both stocked and wild) upon their first recapture. Stocked fish were identified via the presence / absence of an internal wire–coded microtag in the flesh of the cheek. This was determined using a handheld wand detector. After capture, all fish large enough (> 170 mm) were marked externally with a Hallmark fine anchor T-bar tag or dart tag to allow ready identification if recaptured at a later date. This also aided in eliminating the number of duplicate genetic samples collected and processed in the laboratory.

Analyses

Diet

In the laboratory, prey items in the gut samples were first identified to the lowest possible taxonomic level given their state of digestion, using a stereoscopic binocular microscope. Items were then blotted dry and weighed to the nearest 0.1 g before being volumetrically measured by calculating the displacement of each food group in a graduated measuring cylinder (Hyslop, 1980). The frequency of occurrence of individual prey items in each stomach was also recorded (Hyslop, 1980). Initially prey items were identified to the lowest taxonomic level possible, but later grouped (where possible) into families for analyses. An index of preponderance was calculated (Natarajan and Jhingran, 1961) to determine the prevalence of different prey species in the diet. The formula for calculating this index is:

$$I_p = V_i O_i \, (\sum V_i O_i)^{-1}$$

where I_p is the index of preponderance, and V_i and O_i represent the percentage volume and occurrence of a particular prey item respectively.

Detrended Correspondence Analysis (DCA; (McGarigal *et al.*, 2000) was used to assess for differences in diet (I_p) of *L. calcarifer* between areas using the software package PC-ORD version 4.0 (McCune and Mefford, 1999). In these analyses, five broad zones, North Johnstone lower (NJL), South Johnstone lower (SJL), North Johnstone middle (NJM), North Johnstone upper (NJU) and Tinaroo Falls Dam (TIN) (Figure 3) were considered. Data were first log (x + 1) transformed and the down-weighting procedure within PC-ORD was applied to reduce the influence of rare species. Food items consumed by less than five fish were removed completely from the analysis. This was because very rare species are likely to appear randomly and their inclusion in the analyses may disproportionately obscure patterns of more commonly consumed prey items (Gauch, 1982). The interaction between diet and site was plotted in a two-dimensional ordination diagram.

Growth and condition

Condition factor of stocked *L. calcarifer* in Lake Tinaroo and both stocked and wild Barramundi in the Johnstone River was calculated using Fulton's condition factor (K). The formula for this is:

$$K = \frac{100 \times w}{l^3}$$

where w is the fish weight in grams and l is the length in centimetres (Bagenal and Tesch, 1978). In analysing these data, stocked and wild fish in the 2009-2010 cohort were separated into four distinct zones; North Johnstone lower (NJL), South Johnstone lower (SJL), North Johnstone middle (NJM) and Tinaroo Falls Dam (TIN) (Figure 3). Tinaroo Falls Dam was omitted from the analyses because it only contained stocked fish and Zones NJU, SJM and SJU in Figure 3 were not included because of small sample sizes. ANOVAs using total length as a covariate were used to compare condition factors of stocked and wild *L. calcarifer* within zones and between zones. Growth data were expressed as average daily growth ($mm.day^{-1}$).

Movements and relative abundance

The recorded locations of recaptured fish were used to determine local movements of both stocked and wild *L. calcarifer* in the Johnstone River and stocked fish in Lake Tinaroo. To assist with collating and analysing abundance and movement data, each fish was also assigned to a 1 km^2 reference grid identical to that used in the Suntag recreational fishing database (http://database.info-fish.net/suntag/recaptures/inputform.asp). This allowed for the ready inclusion of recapture data from the Suntag recreational fishing database. Relative abundance or Catch Per Unit Effort (CPUE) in the form of number of fish caught in each grid per hour was calculated for both stocked and wild fish. A Kruskal-Wallis test was used to compare the median CPUE values of stocked and wild *L. calcarifer* caught in the Johnstone River and in stocked fish from Tinaroo Falls Dam. CPUEs were also used to give an indication of site fidelity and also the relative importance of geographic locations within both the Johnstone River catchment and Lake Tinaroo. See 'Movements' section for further details.

Movements

Introduction

There have been numerous studies on the movements of wild barramundi (*Lates calcarifer*) both within Australia and also overseas (e.g. Dunstan, 1959; Dunstan, 1962; Davis, 1986; Milton *et al.*, 2000; Sawynok and Platten, 2009). Barramundi are a facultative catadromous species (e.g. Jones and Sujansingani, 1954) that utilise a range of riverine habitats, including freshwaters, but must return to high salinity environments to spawn and complete their life cycle (e.g. Dunstan, 1959; Moore, 1982; Davis, 1986, 1987; Russell and Garrett, 1988). Many of the published movement studies on this species therefore relate only to seasonal spawning migrations of *L. calcarifer* where there is a general downstream migration towards coastal spawning grounds (Davis, 1986; Griffin, 1987).

Early tagging studies of *L. calcarifer* to determine movement were first undertaken in Papua New Guinea and Australia (Dunstan, 1959; Dunstan, 1962). Although very few tags were ever reported as being recaptured in Australia, none were ever recovered in Papua New Guinea. A much more intensive tagging study was later undertaken by Moore and Reynolds (1982) in Papua New Guinea where over 15% of the nearly 6400 fish released were subsequently recaptured. They found that adult fish released into inland waters migrated to coastal spawning grounds, while at least some of those that were released directly onto spawning grounds moved to inland waters—generally to the same areas from which they originally migrated. Moore and Reynolds (1982) also documented a progressive movement of juveniles from spawning grounds to coastal nursery areas and then upstream into inland waters.

In Australia, Davis (1986) found in the Northern Territory a general seaward movement of mature fish from freshwater, which he associated with spawning. Contrary to the findings of Moore and Reynolds (1982) in Papua New Guinea, he found no evidence of any subsequent return of *L. calcarifer* to freshwater after spawning. Davis (1986) also found some limited evidence of exchange of fish between river systems. Also in the Northern Territory, Griffin (1987) concluded that *L. calcarifer* were catadromous, moving upstream as 0+ year old fish where they generally remained until maturity.

Russell and Garrett (1988) conducted an intensive study of the movements of juvenile fish resident in small coastal streams in north–eastern Queensland. In this study, they found that juvenile *L. calcarifer* remained resident in small tidal creeks until they were about a year old, after which they dispersed into adjacent streams and coastal habitats. The streams investigated by Russell and Garrett (1988) were much smaller systems than either those in the Northern Territory where the work was done by Davis (1986) and Griffin (1987) or in Papua New Guinea (Moore and Reynolds, 1982). Since these initial studies, the most comprehensive tagging study on wild barramundi in Australia (and probably worldwide) has been conducted by recreational fishers under the auspices of the Australian National Sportsfishing Association. Details of the tagging and recapture of barramundi through this study are recorded in the Suntag recreational fishing database (see www.info-fish.net). Reports on movements of mostly wild *L. calcarifer* (and other species) are available as a series of reports (e.g. Sawynok, 1992, 1996, 1998, 2004, 2005, 2007, 2009; Sawynok and Platten, 2009; Sawynok, 2010), but there are also some reports specifically on the movements of stocked *L. calcarifer* (e.g. Sawynok and Pearce, 2006, 2007; Sawynok and Platten, 2007, 2009).

While almost all of the movement studies conducted on *L. calcarifer* to date have been done using traditional tagging techniques, such as with anchor or dart tags, more recent work has been undertaken using otolith microchemistry (Milton *et al.*, 2000) and by stocking hatchery

reared fish marked prior to their release with coded wire tags (CWT) (Russell and Rimmer, 2004; Russell *et al.*, 2004). The major disadvantage of using CWT is that, in the absence of a second, visually apparent external tag or mark, these fish cannot be readily identified without the use of a special detector (Nielsen, 1992).

The most extensive, continuous tagging study on stocked *L. calcarifer* was undertaken by the now Queensland Department of Agriculture, Fisheries and Forestry (DAFF) from 1993 to 2005 when some 287 000 stocked barramundi marked with CWT were released into the Johnstone River in North Queensland (see Appendix 3). In addition, a further 9523 CWT *L. calcarifer* were released into the Johnstone River in 2009 as part of this current study (see General Methods section). Release locations for the 2009 stocking are shown in Figure 3. In February 2010, another 2996 *L. calcarifer* were also released into Lake Tinaroo and stocking locations in this impoundment are shown in Figure 3. Stocking fish implanted with CWT provides a means of tracing both localised and wider movements. Rimmer and Russell (1998) found that most stocked fish (62%) in the Johnstone River were recaptured within 3 km of their release site, but 38% undertook intra-riverine movements of up to 37 km. A small number (1%) of stocked *L. calcarifer* undertook inter-riverine and coastal movements both to the north and south of the Johnstone River (Russell *et al.*, 2004). This current study aimed to evaluate the movements of stocked *L. calcarifer* in both the Johnstone River and Lake Tinaroo to determine if stocked fish were moving into potentially environmentally sensitive areas, including the adjacent Wet Tropics World Heritage Area.

Methods

Techniques used to mark the fish prior to stocking and those used to recapture stocked and wild fish, are outlined in the 'General methods' section. In this chapter, fish released into Lake Tinaroo as part of this study will be referred to as the 2009 age class, and those put into the Johnstone River will be identified as 2009-2010 age class. The fish released into the Johnstone River were marked with CWTs only. To assist with determining which river the fish were released into (without recovering the CWT by killing the fish), those stocked into the North Johnstone River were tagged with a single CWT in either the left or right cheek, while those released into the South Johnstone River were tagged with two CWTs, one in each cheek. When these fish were recaptured as part of the electrofishing monitoring program (see 'General methods' section) they were marked with an anchor or dart tag prior to being re-released.

Most of the recapture data from this current study and from previous stockings in both the Johnstone River and Lake Tinaroo were recorded in the Suntag database. This database was used, in part, to obtain information on movements of wild *L. calcarifer* and barramundi stocked into the Johnstone River prior to 2006 as part of previous programs. The Tableland and District Fish Stocking Association provided the barramundi for this current stocking and assisted in the tagging and release of the fish into Lake Tinaroo. Where possible, all recaptured fish were assigned to a year class either as a result of size or a combination of size, and the presence and location of a CWT. It was not possible to confidently assign all fish, particularly larger barramundi (e.g. > ~500 mm TL) to a year class.

The stocking strategy adopted for this current program involved the release of multiple batches of hatchery reared *L. calcarifer* at freshwater sites in the lower Johnstone River catchment (Figure 3). These sites were chosen because of their proximity to smaller tributary streams that drained potentially environmentally sensitive areas of the catchment. Details of stocking numbers and times are given in the 'General methods' section.

CPUE, which was used as an index of relative abundance for both stocked and wild *L. calcarifer,* was calculated as the number of fish electrofished hr^{-1}. The statistical package Genstat® Release 11.1 (VSN International; www.vsni.co.uk) was used for statistical analyses. The GIS software package Mapinfo® Version 6 (Pitney Bowes Software Inc., www.pbinsight.com/MapInfo-Pro) was used to create maps of the Johnstone River and Lake Tinaroo and CPUE data were square root transformed to create thematic maps.

Results

Movements

Inter-riverine movements

Since the commencement of *L. calcarifer* stocking in the Johnstone River in 1993, there have been only 22 records of both stocked and wild fish making inter-riverine movements to or from this system. Of these, two were stocked fish marked with a CWT that had been released into the Johnstone River in 1999 and 2003. One of these fish had moved south into the adjacent Mourilyan Harbour and the other north into the Mulgrave River. The fish that moved into the Mulgrave River was at liberty for 862 days while the Mourilyan Harbour fish was at liberty for 545 days before recapture (Figure 1). From the original 22, seven wild (non-CWT) fish that were tagged with plastic dart or anchor tags in the Johnstone River as part of past research programs (or by recreational fishers) moved into adjacent river systems. Of these, six moved north into the Russell/Mulgrave River system and the other further north into Trinity Inlet. These fish were at liberty between 429 and 1483 days.

Other barramundi, including at least eight stocked fish, had moved from the Russell/Mulgrave Rivers and were subsequently recaptured in the Johnstone River. These fish were stocked at a larger size (~200-300 mm TL) by a local community stocking group and marked with plastic dart or anchor tags prior to release to facilitate identification. No *L. calcarifer* stocked as part of this current program have as yet been found to have undertaken inter-riverine movements.

Intra-riverine movements between the North and South Johnstone Rivers

Since the commencement of regular sampling using predominantly research electrofishing in the early 1990s, both stocked and wild fish have been recorded as moving in both directions between the North and South Johnstone River systems or into the estuary (Table 2) or adjacent coastal foreshores (see Appendix 3). A comprehensive summary of the movements of stocked *L. calcarifer* in Queensland waterways from previous studies is given in Appendix 3 (Sawynok and Platten, 2009).

Table 2. Number of recaptures of stocked and wild barramundi that have made large intra-riverine movements since the commencement of monitoring in the early 1990s.

Some fish were recaptured on more than one occasion. Stocked fish numbers are in parentheses.

Capture site	*North Johnstone R.*	*South Johnstone R.*	*Ninds Creek*
North Johnstone R.		88 (65)	14 (7)
South Johnstone R.	71 (20)		9 (8)
Ninds Creek (estuary)	14 (5)	15 (2)	
Coastal foreshore		3 (1)	0
Total	85 (25)	106 (68)	23 (15)

None of the *L. calcarifer* stocked in 2009 as part of the current program has so far been found to have made extensive intra-riverine movements. However, 14 fish with single CWTs were subsequently recaptured in the lower South Johnstone River. Given that only double–tagged fish (both left and right cheeks) were released into the South Johnstone River, it is likely that there has been some movement of stocked fish from the North to the South Johnstone River. No double tagged Barramundi were recaptured in the North Johnstone River, suggesting no or minimal movement of stocked fish out of the South Johnstone River.

Wild *L. calcarifer* tagged during this current study have made some large intra-riverine movements. Seven barramundi with an average recapture size (± S.E.) of 517 ± 21.3 mm TL were found to have moved from the North Johnstone River into the South Johnstone River. These fish were at liberty for an average of 940 ± 190 days. In the North Johnstone River, three wild *L. calcarifer* between 413 and 519 mm TL were tracked as moving 37 km downstream from the Nerada site to the lower North Johnstone River.

Linear movements between riverine zones

There were nine wild 2009-2010 year class *L. calcarifer* that had been marked with plastic tags and then subsequently recaptured. All were caught moving either upstream (n = 4) or downstream (n = 5) in the North Johnstone River. The largest movement was a single fish that moved upstream from the middle zone (NJM) to the upper (Nerada) zone (NJU) (see Figure 3 for zonal definitions). When all year classes (including 2009-2010) of both stocked and wild *L. calcarifer* are considered, there were 81 inter-zonal movements. One of these was by a stocked fish that was 775 mm TL when it was recaptured at Nerada (NJU) after being released in the middle zone (NJM) of the North Johnstone River. In the South Johnstone River, three fish from the 2009-2010 stocking cohort were found to have moved downstream. In the North Johnstone River, CWT fish had moved into the NJM zone and up to 6 km upstream of the lower freshwater reach release site. It is not known if these fish moved either upstream or downstream to reach their new recapture locations.

Site fidelity

Of all the 2009-2010 age class fish that were captured, tagged with plastic tags and then subsequently recaptured, 244 were caught in the same zone where they were originally tagged and released. Of these, 135 were stocked fish and 109 were wild fish. The average time at liberty for wild fish was 117 days with a maximum of 457 days and a minimum of 8 days. Stocked fish had a minimum average time at liberty of 163.5 days with a maximum of 616 days and a minimum of 8 days. If the initial release date when fish were stocked into the river was considered, not when they were first caught and tagged with a dart or anchor tag, times at liberty were considerably longer than those reported above.

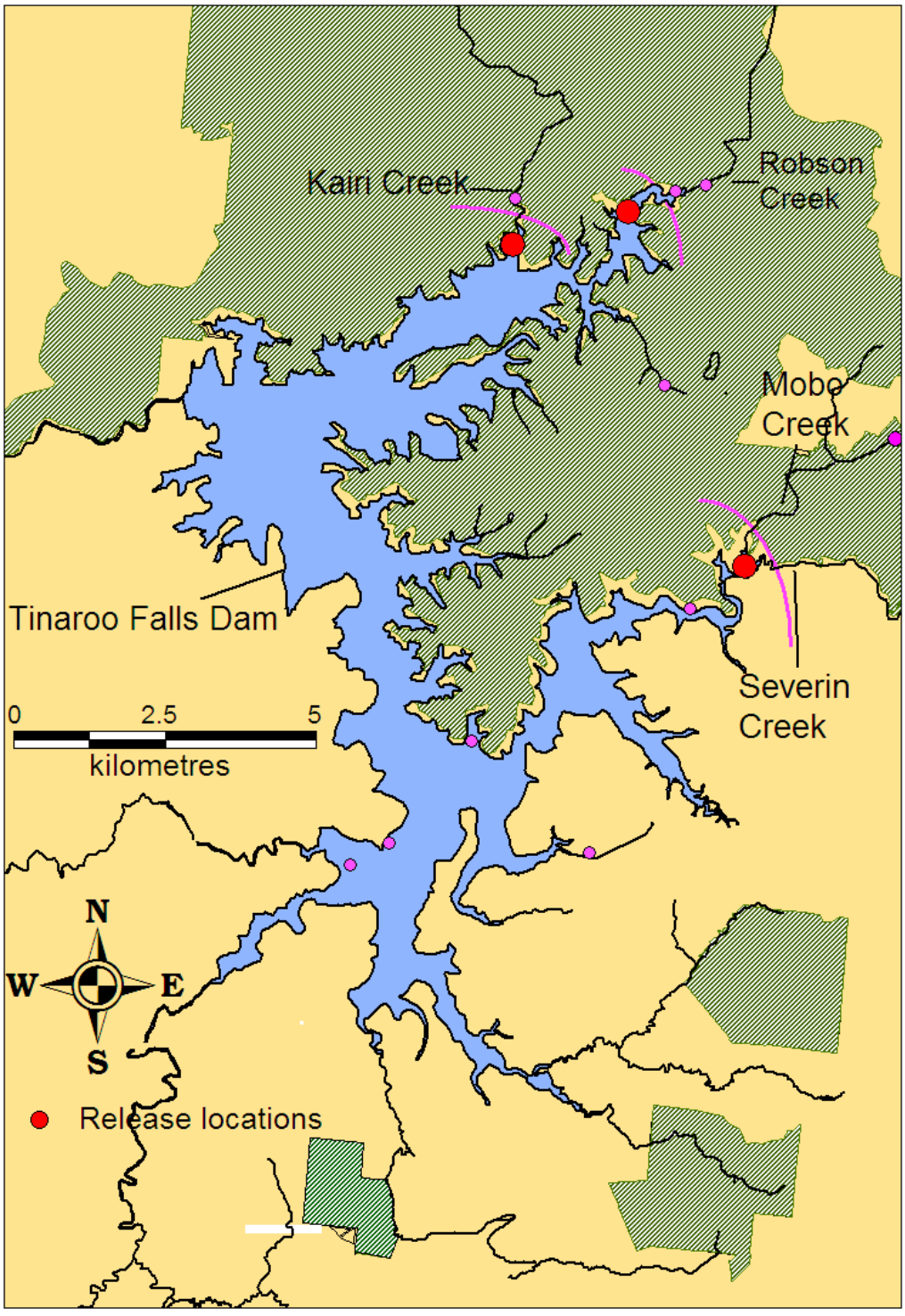


Figure 4. Sites (pink dots) in Lake Tinaroo where no stocked 2009 barramundi were caught. Pink arcs show the limit of upstream movement of barramundi into tributary streams.

Of the 2996 *L. calcarifer* that were released into Lake Tinaroo in early 2010, 188 were subsequently recaptured at least once. In addition, a further 119 *L. calcarifer* that were not part of this current program, but were stocked into Lake Tinaroo on previous occasions, were recaptured. Only five stocked 2009 age class *L. calcarifer* were not recaptured in the same general locale (i.e. Robson Creek arm or Severin Creek arm) as they were originally released.

However, on a finer scale, there were 31 *L. calcarifer* caught in the same 1 km^2 grid where they were originally released with 23 of these caught within 31 days of release. This indicates a gradual dispersal away from the specific release locations into other parts of the impoundment. However, a *L. calcarifer* 1180 mm TL from a previous stocking was electrofished at one of the release locations suggesting that larger fish do, from time to time, forage in areas of the dam near the mouths of feeder streams. No barramundi, either from these current or previous stockings, were caught in the upper reaches of Lake Tinaroo feeder streams (Figure 4).

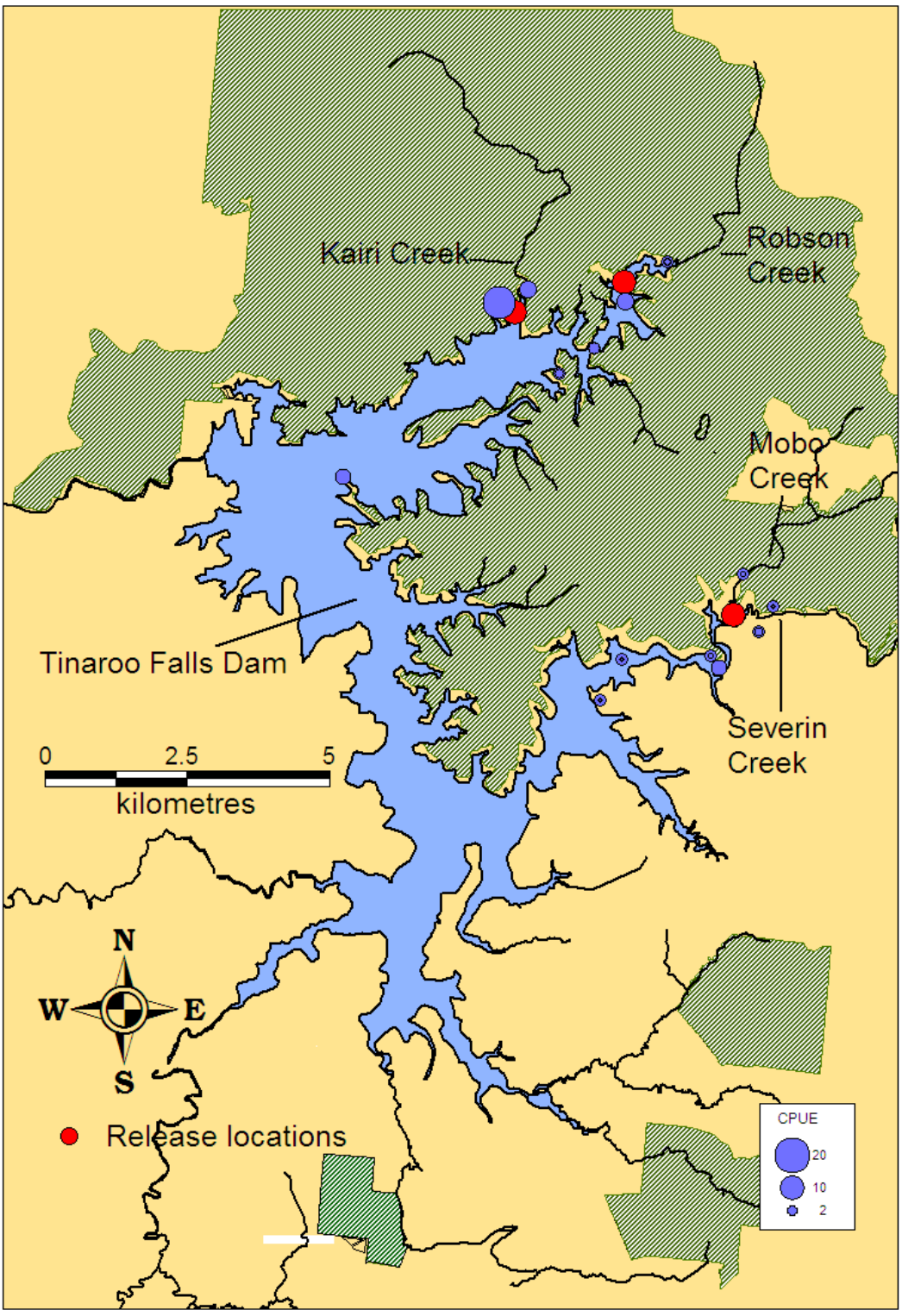


Figure 5. CPUE of stocked 2009 age class barramundi in Lake Tinaroo. The size of mauve markers indicated the relative CPUE at individual sites.

Dispersal of 2009-2010 wild and stocked L. calcarifer

Lake Tinaroo

Areas where stocked fish were not sampled include upstream tributaries of Lake Tinaroo that drain the Wet Tropics World Heritage area (Figure 4). The pink arcs on the tributary creeks in Figure 4 show the approximate upstream limits of the distribution of stocked *L. calcarifer*. Sites on the western and southern sides of the impoundment showed no evidence of the presence of 2009 age class barramundi and it is likely that, at the time of sampling, large numbers of these fish had not dispersed to these areas. Most of the recaptured *L. calcarifer* from the February 2010 stocking were in the arms of Lake Tinaroo close to where the fish were originally stocked. There was some suggestion, however, of a gradual westerly dispersal into the main body of the impoundment. The high relative abundance of 2009 *L. calcarifer* adjacent to the Kauri Creek release site (Figure 5) was evidence of short–term site fidelity in that area of the impoundment. Similarly, the CPUEs of 2009 *L. calcarifer* adjacent to the Severin Creek and Robson Creek arms also suggests some stocked fish, initially at least, remain close to where they were originally released.

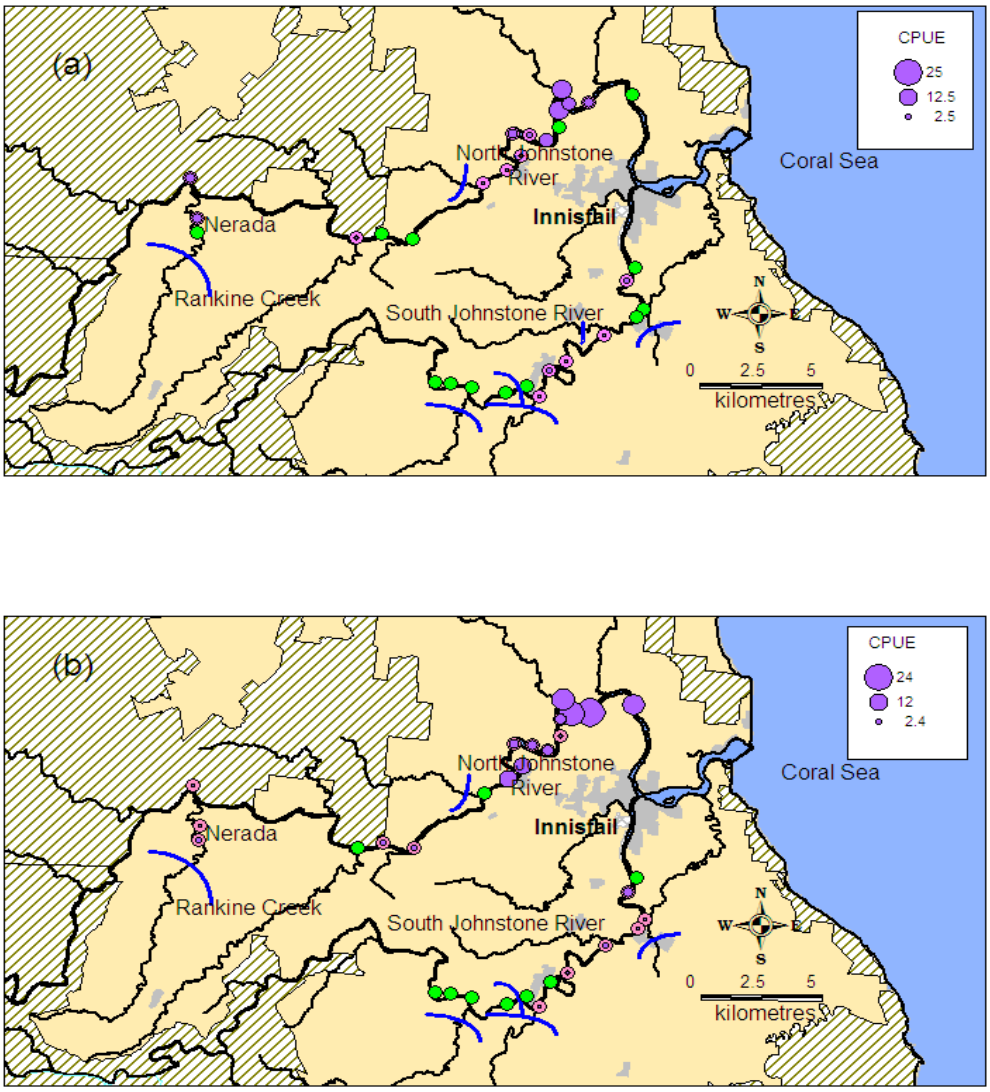


Figure 6. CPUE (mauve markers) for (a) stocked and (b) wild 2009-2010 age class barramundi.

Green markers show sites on the main streams where no fish were caught and blue arcs indicate limits of penetration of *L. calcarifer* up major tributaries. The size of mauve markers indicated the relative CPUE at individual sites.

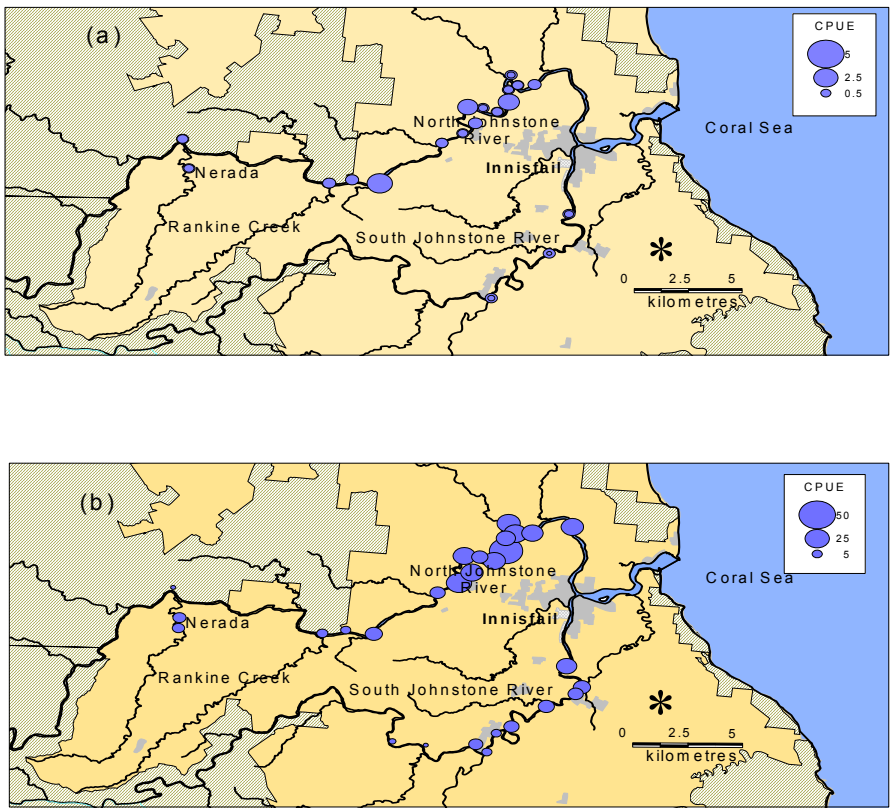


Figure 7. CPUE of (a) stocked and (b) wild Barramundi from all age classes except 2009-2010 in the Johnstone River

Johnstone River

Figure 6 shows the CPUE of 2009-2010 year class stocked *L. calcarifer* in the lower Johnstone River. The highest relative abundances of these stocked fish were in the lower North Johnstone River close to or at the lower freshwater release location. The CPUE of stocked (and wild) *L. calcarifer* decreased with increasing distance upstream from this release location up until the middle reaches of the river. The CPUE at the Nerada and South Johnstone release sites, by comparison to this site, were both relatively low. The navy blue arcs on Figure 6 represent the approximate limit of upstream penetration of 2009-2010 stocked fish. Penetration of fish upstream into minor tributaries of the South Johnstone River and beyond the upper stocking locations appears limited. Due to poor accessibility, no data were available on the upstream penetration of 2009-2010 stocked fish beyond the Nerada release location in the main North Johnstone River.

The distribution of wild, 2009-2010 age class *L. calcarifer* followed a similar pattern to that of the stocked fish. The highest CPUE for this age class was also in the lower North Johnstone River in freshwater close to the limits of tidal penetration (Figure 6). The maximum CPUE for stocked fish was lower than that obtained for wild fish of the same age class.

Other year classes of L. calcarifer

Recaptured *L. calcarifer* stocked prior to 2005 (n = 53) were found to have dispersed along the entire length of both the North and South Johnstone Rivers that were sampled (Figure 7). The relative abundance of stocked fish was highest in the lower and middle reaches of the North Johnstone River, particularly adjacent to the location where they were originally released. Prior to the current project, this location was stocked annually from 1993 until 2005.

The relative abundance of stocked *L. calcarifer* at sampling sites in the South Johnstone River was comparatively low and restricted to only three sites (Figure 7). A similar dispersal pattern was observed for wild fish, but their relative abundances were considerably higher than for stocked fish. Wild barramundi in these age classes had a much higher relative abundance at sites in the lower North Johnstone River than at other sites in the catchment (Figure 7).

Table 3. Length and maximum size of all age classes, except 2009-2010, of stocked and wild L. calcarifer in the Johnstone River.

	Stocked	*Wild*
Number	53	1443
Average size (TL, mm)	654	456
Standard error (mm)	26	2.79
Maximum size (TL, mm)	1030	1225

The maximum sizes of *L. calcarifer* sampled in this study were 1225 mm TL and 1030 mm TL for wild and stocked fish respectively (Table 3). The average size of wild fish (456 mm TL) was significantly smaller than that of the stocked fish (654 mm TL) ($t = -7.59$, $df = 53.22$, $P < 0.001$). This was probably (at least partly) due to a cessation of stocking between 2005 and 2009, thereby resulting in a progressive increase of the average size of stocked *L. calcarifer* in the river.

Comparative CPUE

Figure 8 shows the distribution of CPUE values for stocked and wild fish at sampling sites in Lake Tinaroo and the Johnstone River. Wild fish in the Johnstone River had higher maximum CPUE than stocked fish at either of the other two locations, but comparing the three groups using a Kruskal-Wallis test suggests that there is no significant difference between the distributions of the three groups ($H=0.3184$, 2 df, $P=0.853$).

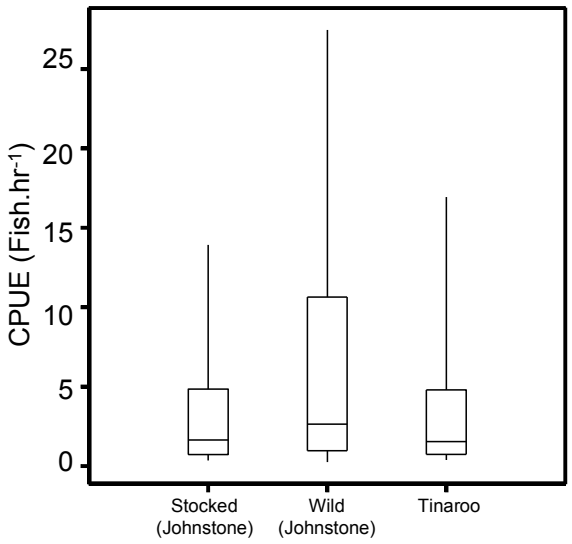


Figure 8. Box and whisker plot for CPUE for wild and stocked barramundi from Lake Tinaroo and the Johnstone River.

Johnstone River L. calcarifer are 2009-2010 age class and the fish stocked into Lake Tinaroo were spawned in mid 2009.

Discussion

In this current study, some riverine stocking locations were intentionally selected to give juvenile *L. calcarifer* ready access to tributary streams where wild fish are rarely or not found. This included near or in streams that drain undeveloped areas, including the rainforests of the Queensland Wet Tropics World Heritage Area. Even when fish were released directly into such areas, their relative abundances declined very rapidly with stocked fish presumably either not surviving or moving into other areas. This may have been due to the habitat at the stocking location being unsuitable or due to the absence of favourable prey items, or a combination thereof (Russell *et al.*, 2004).

During this study, *L. calcarifer* were stocked directly into the North Johnstone River at Nerada and the adjacent Rankin Creek and also into the upper South Johnstone River (near the township of South Johnstone) and into the adjacent Utchee Creek (see Figure 3). Subsequent electrofishing surveys at these locations found no evidence of concentrations of 2009-2010 stocked (or wild) *L. calcarifer*, nor was there any evidence of further upstream movements of these fish.

In the case of the fish stocked into the South Johnstone River (which could be discriminated from other 2009-2010 fish stocked into the North Johnstone River because they were double tagged), very few were ever recaptured. This suggests that rather than moving to, and becoming established in other more suitable areas (e.g. the North Johnstone River), these fish were likely subjected to a high post-release mortality. The reasons for this are unclear, but may be related to localised, unfavourable environmental conditions. For example, soon after these fish were released in December 2009, there were a series of large flow events in both the North and South Johnstone Rivers. Conditions in the narrow, higher gradient sections of these rivers would have been particularly harsh, thus further stressing these newly stocked fish and making acclimation to local conditions difficult.

Other studies have also suggested that newly released juvenile barramundi are very sensitive to local environmental conditions including poor water quality (Russell and Rimmer, 1997; Rimmer and Russell, 1998). The presence of small numbers of 2009-2010 age class stocked and wild *L. calcarifer* in the middle reaches of the North Johnstone River suggests less favourable habitat and/or limited mobility either upstream (from the lower freshwater reaches) or downstream (from Nerada).

Suitability of release habitat for *L. calcarifer* is likely to have a significant impact on their survival. In this study, *L . calcarifer* that were stocked directly into more benign habitats showed both a greater capacity for survival and a higher degree of site fidelity. For example, barramundi that were stocked into the lower freshwater reaches of the North Johnstone River appeared to remain in this area for at least two years and some much longer. An earlier study investigating stock enhancement of *L. calcarifer* in the Johnstone River (Russell *et al.*, 2004) found that the recapture rate of fish stocked into the lower freshwater site on the North Johnstone River was about four times that of fish stocked into the Nerada site. Russell *et al.*(2004) suggested that this may be explained by the presence of suitable nursery habitat, particularly the presence of extensive macrophyte beds (predominantly *Vallisneria* sp.) that offer the newly released fish both cover and abundant prey. These conditions were absent at both the Nerada and South Johnstone River release sites where post-release relative abundances were low.

Similar conclusions were reached by Rozas and Odum (1988) who noted that for a number of species submerged macrophyte beds afforded both protection from predators and an abundance of prey. Further evidence of the suitability of this habitat type as a nursery area is the abundance of 2009-2010 age class wild *L. calcarifer* at this site. After spawning in estuarine and coastal areas (e.g. Dunstan, 1959; Moore, 1982; Davis, 1986, 1987; Russell and Garrett, 1988), young-of-the-year *L. calcarifer* moved up the North Johnstone River where they settled in relatively high abundances in the macrophyte beds in its lower freshwater reaches. In 2010, these fish began to appear in this area from about February. By the end of sampling for this current project in late 2011, only small numbers of wild 2009-2010 year class barramundi had penetrated further upstream into the middle reaches and Nerada sites of the North Johnstone River and into the South Johnstone River.

Stocked *L. calcarifer* that were released into the Johnstone River pre-2006 as part of earlier studies (e.g. Russell and Rimmer, 1997, 1999, 2000; Russell and Rimmer, 2001; Russell *et al.*, 2002; Russell and Rimmer, 2002, 2004; Russell *et al.*, 2004) and older age class wild fish were also recaptured in the river. A number of very large barramundi were caught, including a stocked fish that was 1030 mm TL and a wild fish that was 1220 mm TL. Whilst these fish and other large individuals may have remained in the river and never moved to higher salinity areas to spawn, it is also possible that they made this migration one or more times and then returned. This is at odds with the findings of earlier studies in the Northern Territory (e.g. Davis, 1986), which found no evidence of mature barramundi returning to freshwater after they had moved into coastal areas to spawn. However, in Papua New Guinea, Moore and

Reynolds (1982) found evidence of movements of mature fish from coastal spawning grounds into freshwater. Evidence of stocked fish moving between river systems suggests that juveniles, at least, do have the capacity to move from freshwater to saltwater and then back into freshwater. Techniques such as otolith microchemical analyses may assist to answer this question.

In general, the relative abundance (CPUE) of pre-2006 stocked fish were lower than that of 2009-2010 age class stocked fish, suggesting that most of the former group had made the general seaward migration into coastal areas (Davis, 1986; Griffin, 1987). Many of these fish have subsequently been recaptured in the coastal inshore gill net fishery (D.J.Russell, unpublished data).

Whilst there was evidence from previous studies (see Appendix 3) of small numbers of stocked *L. calcarifer* making sometimes extensive inter-riverine movements including to and from the Johnstone River, to date there was no evidence of this happening with fish stocked as part of the current study. This is probably because none of the 2009-2010 fish would not have yet reached maturity and most would still be resident in riverine habitats whether freshwater or estuarine.

Juvenile, 2009 age-class *L. calcarifer* stocked into strategic locations in Lake Tinaroo also showed no evidence of moving upstream into smaller tributary streams that drained the rainforests and other areas of the adjacent Wet Tropics World Heritage Area. There was some evidence, however, of limited site fidelity around the stocking locations, particularly around the Kauri Creek release location (see Figure 5). Overall, there was a general pattern of drift by juveniles towards the main body of the impoundment over an increasing time from release. As witnessed in the Johnstone Rivers, site fidelity in Lake Tinaroo appeared to be predominately coupled to the suitability of available habitat and favourable prey items. This was supported by high CPUEs at locations like Kauri Creek where shallow beds of macrophytes and grasses provided nursery habitat and ample food. Spot sampling in the southern section of the impoundment suggests that, at the time of sampling, 2009 age class had not yet dispersed into these areas of the impoundment.

Stocking of *L. calcarifer* in rivers of the Queensland Wet Tropics World Heritage Area that also contain conspecific wild populations, does not appear to result in a range expansion of individuals into areas where natural populations did not previously exist. This is despite stocked barramundi being presented with opportunity to move into such areas through the judicious selection of stocking sites.

A major factor that determines the distribution of both wild and stocked *L. calcarifer* appears not to be the presence of vacant habitat, such as in lower order tributary streams or even in main river channels, but rather the presence of suitable habitat and food availability. Where there is suitable habitat, such as in the lower freshwater reaches of the North Johnstone River, at current stocking densities juvenile stocked and wild *L. calcarifer* will readily coexist. Similarly, in impoundments such as Lake Tinaroo, stocked *L. calcarifer* appear to prefer lentic waters rather than lower order, tributary streams where there is a paucity of suitable habitat and prey species. It is unlikely that stocking of fish into Wet Tropics streams of similar hydrology to the Johnstone River poses a significant threat unless fish are specifically released in areas upstream of a significant barrier that contains vacant, suitable habitat. Such areas would appear to be few. Similarly, it is unlikely that *L. calcarifer* stocked into impoundments will move into lower order tributary streams draining either directly into the impoundment or into larger rivers and streams that feed into it.

Diet

Introduction

The Wet Tropics bioregion of north-eastern Australia is home to many endemic aquatic species including some fishes (Pusey *et al.*, 2008) and amphibians (Gillespie, 2001) of high conservation importance. Stocking of fish into selected streams and impoundments in the Wet Tropics has occurred regularly since the development of mass hatchery rearing techniques in the mid 1980s (Burrows, 2004). Burrows (2004) estimated that at the time of his report, at least two million fish, including the barramundi *Lates calcarifer*, have been stocked into Wet Tropics streams. Prior to this, stocking in the area was restricted to the translocation of native fishes generally by local fish acclimatisation societies (Russell, 1987) and several unsuccessful releases of salmonids (Grant, 1975). The largest impoundment in the Wet Tropics region, Tinaroo Falls Dam, has received over half a million *L. calcarifer* since large scale stocking began in 1985 (Burrows, 2004). The Johnstone River on the north–eastern Queensland coast near the township of Innisfail was the most heavily stocked river in the region, receiving over 290 000 fingerlings between 1993 and the cessation of regular stocking in 2005 (D.J. Russell, unpubl. data).

Due to the popularity and intensity of fish stocking in the region, questions have been raised by some government agencies, community groups, conservationists and the general public regarding the potential impacts native species introductions may be having on local stocks and recipient ecosystems (Burrows, 2004). Similarly, there are concerns in many parts of the world about the impacts that introduced exotic and native species are having on endemic species (McDowall, 1968; Tilzey, 1976; McDowall, 2003; Lintermans, 2004; McDowall, 2006; McDowall, 2007; Corfield *et al.*, 2008).

Dietary interactions between stocked and endemic species have been shown to have a potentially major impact. In New Zealand, McDowall (2003) suggested that behavioural and dietary interactions between introduced salmonids and native species must be understood if potentially irreversible impacts of fish stocking are to be avoided. There are few published studies on the possible impacts of stocking Australian native fishes, particularly *L. calcarifer* on the recipient ecosystems. However, Morgan *et al.*(2004) compared the dietary niches of native fish resident in a large artificial impoundment in Western Australia (Lake Kununurra) to that of *L. calcarifer* populations located downstream below the impoundment and in a nearby river. Whilst they detected no significant dietary overlap between *L. calcarifer* and the other species, these authors noted the potential of *L. calcarifer* to impact on the local fish community through competition and predation if it was introduced into the impoundment. Any such effects would likely be minor (Morgan *et al.*, 2004).

In this current study, we address the lack of scientific information on the potential impacts of *L. calcarifer* fish stocking into areas of the Queensland Wet Tropics. In particular, we examine situations where they were introduced as novel predators and where they can potentially interact with conspecifics, species of high conservation importance and/or naive prey that don't have well developed anti-predator mechanisms. This included:

1) the potential for barramundi to impact on species of conservation importance by examining stomach contents of stocked and wild riverine *L. calcarifer* for the presence of any threatened or endangered species
2) the relative condition and growth of similarly aged stocked and wild *L. calcarifer* from different habitat types and/or different stocking locations.

Methods

Site descriptions, stocking and sampling protocols, quantification and identification of stomach contents, and statistical techniques are detailed in the 'General methods' section of this document.

Results

Dietary composition

Johnstone River (North and South)

In total, 984 wild and 276 stocked *L. calcarifer* were caught and stomach-flushed during this current project and of these, 62 (45 wild and 17 stocked) fish were recaptured and sampled on more than one occasion. No noteworthy variation in diet was evident between fish sampled in different reaches of the Johnstone River (Table 4). Crustaceans of the families Palaemonidae and Atyidae had the highest I_p (54.93 and 29.89 respectively), followed by unidentifiable fish remains ($I_p = 13.16$) (Table 4).

Tinaroo Falls Dam

Some 301 stocked *L. calcarifer* were stomach-flushed, with 164 of these fish sampled on up to three occasions. These fish were taken from the stocking of 2009 fish as well as other stockings and included a wide range of size classes. At this location, fish made up the major component of the diet of *L. calcarifer* (Table 4). *Nematalosa erebi* (Clupeidae), a common schooling fish in the dam, was the most significant prey species ($I_p = 36.58$). Other known prey items, by decreasing order of dietary importance (I_p), included tilapia (*Oreochromis mossambicus* and *Tilapia mariae*: Cichlidae), *Glossamia aprion* (Apogonidae), *Craterocephalus stercusmuscarum* (Atherinidae) and the crustacean *Cherax quadricarinatus* (Parastacidae). All these prey items all had an $I_p < 10$.

Cannibalism and interaction with rare species

The only evidence of cannibalism, even at the stocking locations where *L. calcarifer* were initially found at high densities, was a 74 mm TL stocked fish that was regurgitated by a larger (443 mm TL) *L. calcarifer* in the live fish well of the electrofishing boat prior to it being stomach-flushed. No evidence of consumption of rare or threatened species was found in the gut contents of any of the fish sampled during this study. The possible exceptions were unidentifiable anuran (frog) bones recovered from a 519 mm TL *L. calcarifer* sampled in the middle reaches of the South Johnstone River. Given the degraded, predominantly agricultural, habitat surrounding this site (sugar cane fields and cow paddocks) and its depauperate riparian zone, it is unlikely (though not certain) that this individual was a species of conservation concern. A positive identification of the specimen was unable to be obtained due to the condition of the specimen recovered.

Table 4. Index of Preponderance (Ip) showing importance of different prey items in the diet of L. calcarifer *from each of the stocking locations.*

Site codes are given in Figure 3.

Prey Items	SJL	SJM	NJL	NJM	NJU	Tinaroo
Ambassidae	0.0617	0.0000	0.0001	0.0000	0.0000	0.0000
Anura	0.0000	0.0307	0.0000	0.0000	0.0000	0.0000
Aytidae	37.1685	6.8498	57.3477	22.7208	0.0137	0.0000
Apogonidae	0.0000	0.0000	0.0000	0.0000	0.0000	1.7960
Atherinidae	0.0000	0.0000	0.0000	0.0000	0.3497	1.1631
Cichlidae	0.0023	0.0088	0.0002	0.1207	0.0000	3.9259
Clupeidae	0.0000	0.0000	0.0000	0.0000	0.0000	36.5760
Decapoda	0.0000	0.0000	0.0545	0.0000	0.0000	0.0006
Diptera	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000
Eleotridae	0.0002	0.0066	0.1621	0.5007	0.0000	0.0115
Ephemeroptera	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000
Flabellifera	0.0000	0.0000	0.0003	0.0000	0.0000	0.0000
Gastropoda	0.0013	0.0615	0.0643	0.0224	0.0000	0.0006
Gobiidae	0.1709	4.5577	0.0261	0.0000	0.4011	0.0000
Grapsidae	0.0293	0.0000	0.1470	0.0000	0.0000	0.0000
Inorganic Material	0.0207	0.0000	0.0001	0.0045	0.0343	0.0036
Melanotaeniidae	0.0000	0.0439	0.0000	0.6438	0.2057	0.8197
Mollusca	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
Odonata	0.0000	0.0000	0.0000	0.0000	0.0000	0.0006
Organic material	0.0000	0.0044	0.0007	0.0045	0.0000	0.0085
Orthoptera	0.0000	0.0000	0.0004	0.1699	0.0000	0.0000
Palaemonidae	58.1888	40.2766	39.5895	42.8878	22.4392	0.1990
Paratiscidae	0.0000	0.0000	0.0000	0.0000	0.0000	1.2256
Plant Material	0.3278	5.8803	0.6033	1.9046	0.0309	2.3372
Plotosidae	0.0000	0.0000	0.0000	0.0000	26.7427	0.0000
Poeciliidae	0.0036	0.0878	0.0000	0.0894	0.0000	0.0000
Pseudomugilidae	0.0000	0.0395	0.0042	0.0358	0.0000	0.0000
Thiaridae	0.0000	0.0000	0.0023	0.0000	0.0000	0.0000
Unidentified material	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
Unknown Fish	4.0245	42.1524	1.9970	30.8952	49.7826	51.9319
Contributing Stomachs	289	94	624	116	54	186

Variation in diet between sampling zones

A Detrended Correspondence Analysis (DCA) was used to investigate dietary differences of *L. calcarifer* between the different geographic zones in the lower Johnstone River (both the North and South Johnstone Rivers) and in Tinaroo Falls Dam (Figure 9). The distribution of the zones in ordination space along Axis 1 in the DCA was approximately geographically correlated, with zones located at progressively greater distances from Axis 2 with increasing distance from the sea. Tinaroo Falls Dam, which is located some 50 km to the north-west of the most westerly Johnstone River site (NJU, Nerada), was the furthest from Axis 2. This suggests that the diet of its *L. calcarifer* was quite different to the fish in the Johnstone River zones. Axis 1 explained 89% of the variation (eigenvalue of 0.66) (Figure 9). Coastal dietary fauna, for example atyid shrimps, grapsid crabs and unidentified decapoda, were distributed in ordination space closer to Axis 2, while freshwater fish including Cluepeidae, Paratiscidae and Atherinidae that were more closely associated with the diets of *L. calcarifer* from Tinaroo

Falls Dam, were distributed towards the other end of the axis. Sites in the middle and upper zones of the Johnstone River were most closely associated with gobiids, introduced poeciliids and palaemonid shrimp.

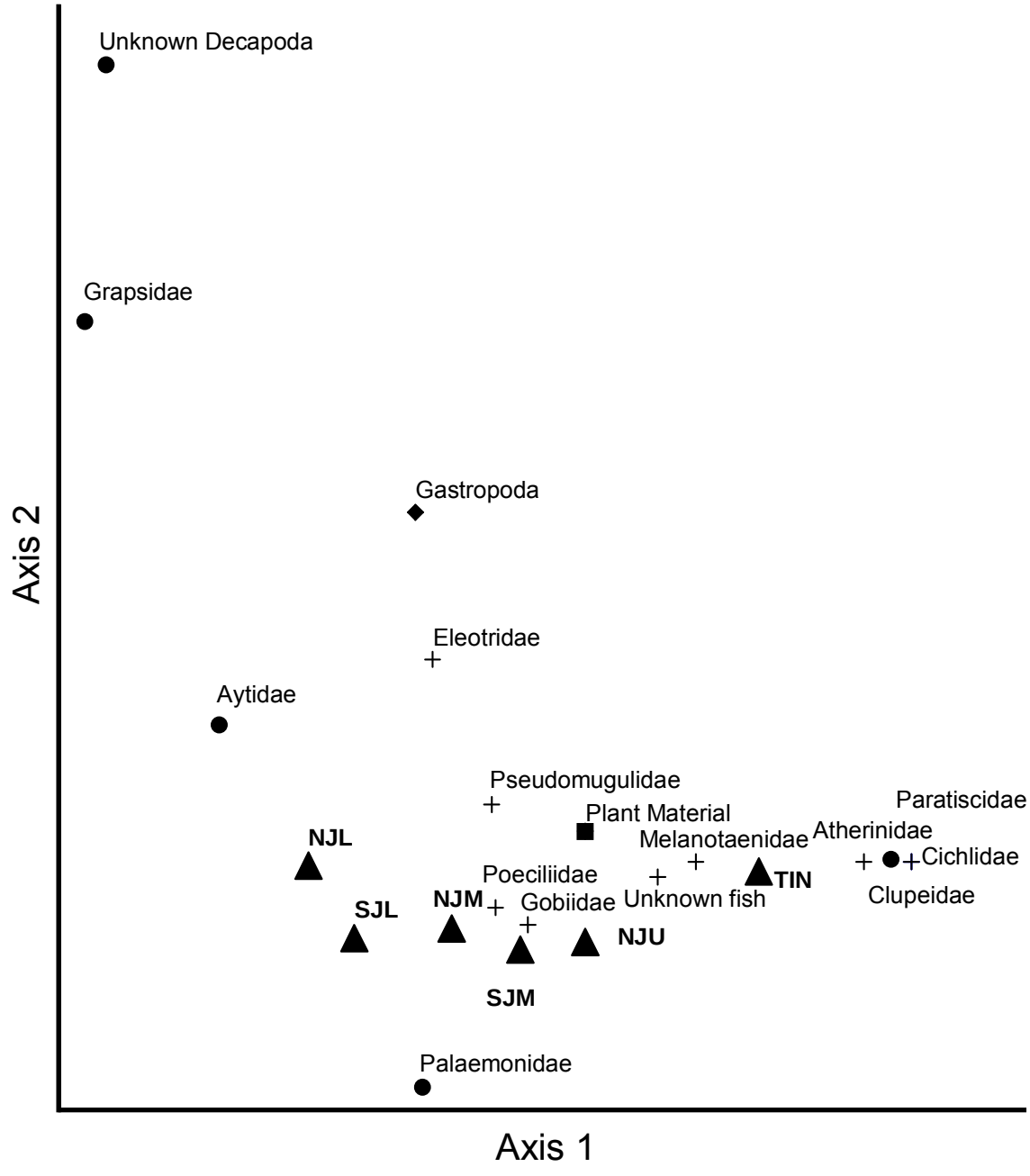


Figure 9. Detrended Correspondence Analysis of broad dietary groups of barramundi and sampling zones.

Positions of sampling zones (▲) are shown on Figure 3. General food items are ● crustaceans; + fish; ♦ molluscs; ■ miscellaneous plant material.

Condition factor

The combined condition factors for stocked and wild barramundi (2009 cohort) from the Johnstone River and Tinaroo Falls Dam captured over the study are shown in Figure 10. When the condition factors of the 2009 stocked cohort and other wild fish (2007, 2008 and 2009 cohorts combined) were compared, there was no significant difference in condition factors ($F_{1, 1579} = 1.3$, $P > 0.05$). Further, there were no significant differences between the individual year classes (2007, 2008 and 2009 cohorts) of wild and stocked (2009 cohort) fish ($F_{3, 1372} = 1.0$, $P > 0.05$).

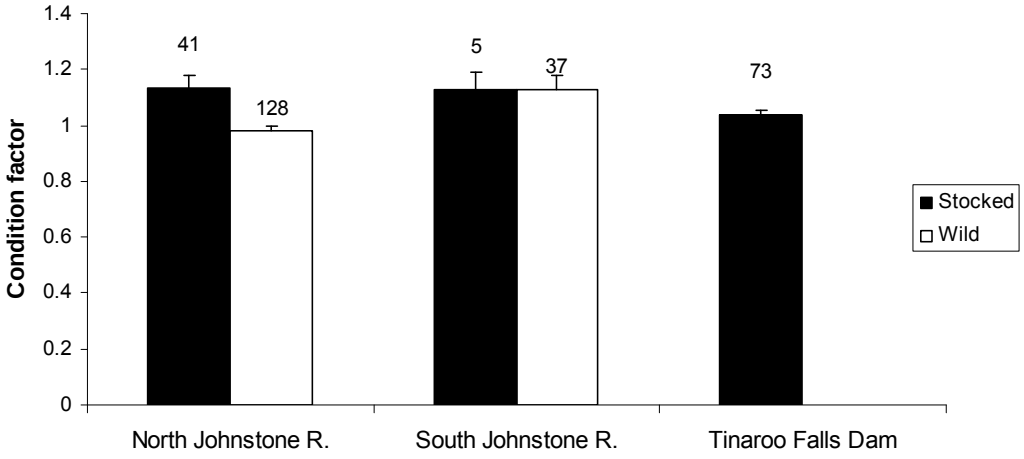


Figure 10. Condition factors of stocked and wild fish at the study locations.

Error bars are standard error

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However, when geographic location was considered, there were differences in condition factor between stocked and wild fish in some zones. Both stocked and wild fish from the 2009 cohorts, when compared across all the Johnstone River zones (see Figure 3), showed a significant difference in condition factors ($F_{6, 1372} = 7.4$, $P < 0.05$). Least significant difference (LSD) pairwise multiple comparisons suggest that there were no significant differences ($P > 0.05$) between stocked and wild fish in the lower North Johnstone (NJL), lower South Johnstone (SJL), North Johnstone middle zone (NJM) and stocked fish in Tinaroo Falls Dam. The condition factor of wild fish caught in the North Johnstone River middle zone (NJM) was similar to stocked fish ($P > 0.05$) in that zone, but significantly different to condition factors for both stocked and wild fish in all other zones ($P < 0.05$).

Growth

Figure 11 shows the progressive mean monthly sizes of stocked (2009/10) and same age wild barramundi in the Johnstone River and for similar aged stocked fish in Tinaroo Falls Dam. Even taking into consideration that they were released at a larger size, stocked fish in Tinaroo Falls Dam grew at a much faster rate (particularly in 2011) than did either the wild or stocked fish in the Johnstone River. The growth of stocked and wild fish in the Johnstone River, particularly after April 2011, was quite similar. The average (± 95% CI) daily growth rate for stocked *L. calcarifer* in Tinaroo Falls Dam that had been at liberty for more than 30 days was

0.57 (0.54, 0.60) mm day^{-1} compared with 0.26 (0.20, 0.32) and 0.23 (0.18, 0.28) mm day^{-1} for stocked and wild fish respectively in the Johnstone River.

These data show that there was no significant difference between the average daily growth rates for stocked and wild fish in the Johnstone River, but both were significantly less than that recorded in Tinaroo Falls Dam. By October 2011, the maximum size of stocked barramundi in Tinaroo Falls Dam was 558 mm TL, close to the minimum legal size for *L. calcarifer* in eastern Queensland of 580 mm TL. In December 2011, the maximum sizes of stocked and wild fish in the Johnstone River was 496 mm TL and 486 mm TL respectively.

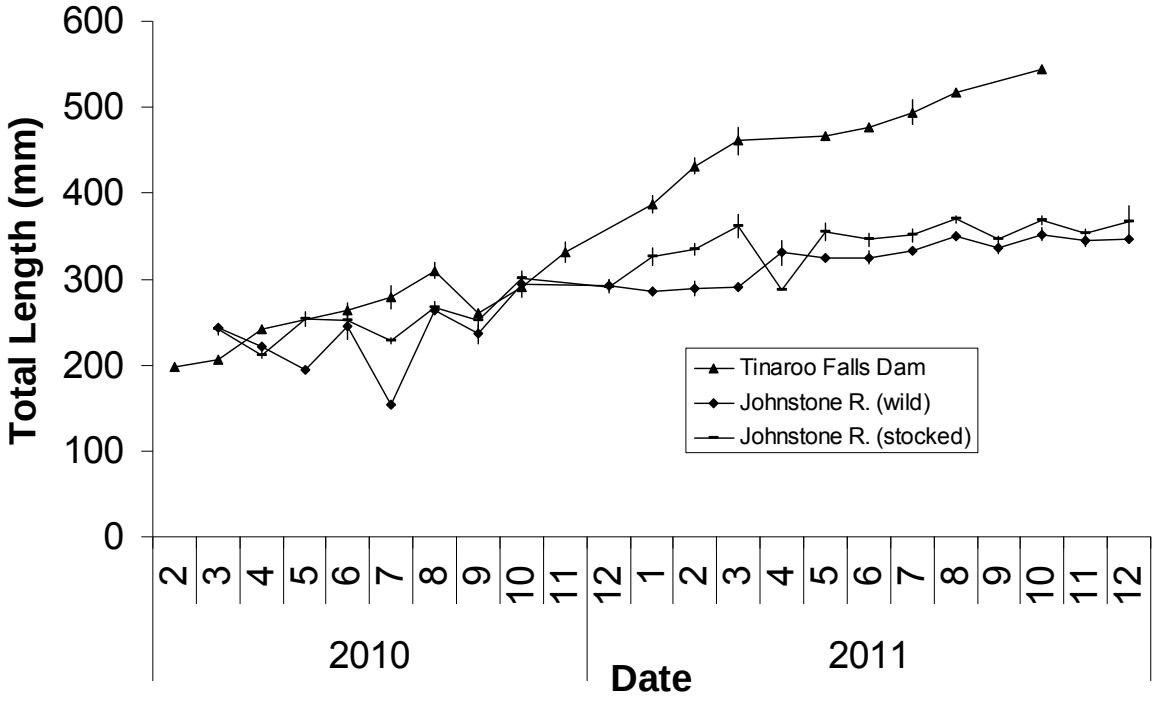

Figure 11. Mean monthly size (TL) barramundi of the same age cohort of stocked and /or wild barramundi from the Johnstone River and Tinaroo Falls Dam.

Error bars show standard error

Discussion

At the stocking rates used in this study, the release of hatchery-reared *L. calcarifer* into both the Johnstone River and Tinaroo Falls Dam appeared to have had minimal impact in terms of some of the specific ecological concerns raised regarding *L. calcarifer* stocking in northern Australia (e.g. Burrows, 2004). Specifically, no evidence was found to suggest that either stocked or wild *L. calcarifer* prey to any degree on species of conservation concern or have unforeseen effects on conspecific wild stocks in coastal streams of the Queensland Wet Tropics.

In this current study, there was no dietary evidence that stocked *L. calcarifer* preferentially consumed amphibians (or other sensitive species such as reptiles) resident in the littoral zone of upper tributary streams. For example, in this area there have been significant declines in the abundance and distribution of at least seven frog species during the 1990s (Laurance *et al.*, 1996; Gillespie and Hero, 1999; Burrows, 2004). However, during the course of the study,

the only anuran found in the stomach contents of *L. calcarifer* was from a fish captured in the main channel of the South Johnstone River. It is not known if this was one of the sensitive species referred to by Burrows (2004), but it is suspected that, because of the location where it was sampled, it was probably a more common species that was opportunistically consumed.

Dunstan (1959) noted that *L. calcarifer* favoured larger streams and still waters and appeared not to prefer small, faster flowing streams such as those occurring in the upper Johnstone River and other Wet Tropics systems. This is in agreement with the findings of the current study, which found little evidence (despite ample opportunity) of *L. calcarifer* moving into smaller, faster-flowing streams (stream order ≤ 3), and tributaries of major water bodies in the Wet Tropics World Heritage area where they would become either novel predators (Tinaroo Falls Dam tributaries) or where wild stocks are rarely, if ever, found (upper coastal sections of the Johnstone River). If *L. calcarifer* did consistently move into these upper tributary streams of the Wet Tropics bioregion, they would have come into regular contact with species of conservation concern and thus more evidence for the consumption of these species would have been expected.

While the findings of our study are unequivocal for adult amphibians, there is a possibility that larval amphibian stages are being eaten but not being detected. In a study of the predatory impact of trout on *Litoria spenceri*, Gillespie (2001) suggested that fish are likely to exert their greatest influence on frog populations by preying upon larval amphibian stages. Gillespie also noted that analysis of stomach contents may not be particularly informative because tadpoles are soft bodied animals with cartilaginous skeletons that digest quickly and may not be detectable in examinations of gut contents. Future studies could perhaps preclude this possibility by including other techniques for determining prey species such as genetic analyses or by examining the digestive rates of different prey items in the diet of *L. calcarifer*.

The Wet Tropics bioregion also supports a number of fish species that have limited distributions including *Guyu wujalwujalensis* and *Melanotaenia utcheensis* (Pusey and Kennard, 1996). The translocation of therapontid grunters (and other novel predators) is seen as a significant threat particularly to the existence of *G. wujalwujalensis* (Pusey and Kennard, 2001; Pusey *et al.*, 2004). The distribution of *Melanotaenia utcheensis* is restricted to the Johnstone River catchment including, amongst other places, in Utchee Creek. While *M. utcheensis* does not have an official conservation status (Pusey *et al.*, 2004), its distribution is restricted and McGuigan (2001) believed that it should be ranked as vulnerable. In this current study, no stocked *L. calcarifer* were found to have remained in Utchee Creek, which supports populations of *M. utcheensis* in its upper reaches. Indeed, few of the *L. calcarifer* stocked into the South Johnstone River (into which Utchee Creek drains) in 2009 were ever recovered, indicating very poor post-stocking survival (see 'Movements' section of this document).

The results from this current study suggest that the diet of *L. calcarifer* was variable and was closely related to both its geographic location (both within the catchment and between catchments) and habitat type. In Tinaroo Falls Dam, juvenile *L. calcarifer* preyed predominantly on a range of native teleosts, including those of the families Clupeidae, Melanotaeniidae and Atherinidae. Small numbers of the exotic cichlids *T. mariae* and *O. mossambicus* were also found in the stomach contents. In the lower reaches of the Johnstone River, shrimps and prawns in the genera *Caridina* and *Macrobrachium* were the dominant prey species. Further upstream, the dietary composition changed to include fish comprising members of the Gobidae and Eleotridae families, which are common in faster flowing rivers and stream habitats, preferring areas of fine sand and gravel (Pusey *et al.*, 2004; Thuesen *et al.*, 2011). *Caridina* shrimp species were observed to be associated with macrophytes (e.g. *Vallisneria* sp.) and littoral grasses (*Urochloa mutica*), particularly in the lower freshwater reaches of the river. While commonly consumed by juvenile *L. calcarifer* in the lower

freshwater zones, they were poorly represented in the stomach contents of *L. calcarifer* from the upstream sites in the Johnstone River.

This link between habitat type, geographic location and prey species would suggest that, as noted by Davis (1985) and later by Morgan *et al.*(2004), *L. calcarifer* are generalist in their dietary preferences, preying on a wide range of available species. Davis (1985), in a study of the diet of *L. calcarifer* in coastal and inland waters of the Gulf of Carpentaria and in Van Diemen Gulf, found that they were an opportunistic predator showing an ontogenetic progression in diet from micro-crustaceans to macro-crustaceans to fish. In fresh, non-tidal waters of rivers flowing into the Van Diemen Gulf of the Northern Territory, he found that crustaceans and fish made up 43% and 49% respectively of the diet of *L. calcarifer* between 200 and 400 mm TL. In Western Australia, Morgan *et al* (2004) found that *L. calcarifer* in the freshwater reaches of the lower Ord River and the Fitzroy River preyed primarily on teleosts (72%) and decapods (26%). They presented evidence suggesting that if *L. calcarifer* were stocked into a large impoundment in Western Australia they would most likely have minimal effects on the resident fish community through either competition or predation.

The opportunistic nature of *L. calcarifer* in prey selection (Davis, 1985) provides an explanation of the observed differences in diet between geographic areas and is most likely related to prey abundance in those systems. For example, Atyid shrimp are most abundant in the *Vallisneria* beds in the lower Johnstone River, but relatively low in abundance in upstream areas where this habitat type is absent. Similarly, some prey fish species that are abundant in Tinaroo Falls Dam are absent or only found in low numbers in the coastal section of the Johnstone River.

Despite opportunities that arise when smaller fish are stocked into areas that support populations of larger *L. calcarifer*, cannibalism was recorded only once during this current study. However, it has been reported in many studies of *L. calcarifer* in Australia (e.g. Davis, 1985), Papua New Guinea (e.g. Moore, 1980; Moore, 1982) and also in Asia (e.g. De, 1971). In the Van Diemen Gulf of the Northern Territory, Davis (1985) found that conspecifics comprised 5% of the fish species consumed by juvenile *L. calcarifer* in the 50-200 mm size range. He also found that, while cannibalism was generally rare in fish in Gulf of Carpentaria streams, conspecifics did comprise 11.4% of the diet of larger *L. calcarifer* in the size range 1001-1200 mm TL (Davis, 1985). In Tinaroo Falls Dam, McDougall *et al.*(2008) found no evidence of cannibalism in the stomach contents of larger fish, but even so, suggested that it may have been a cause of low survivorship of stocked juveniles in the recreational fishery in the late 1990s and early 2000s. Despite these views, the relative absence of evidence of cannibalism (at least of juvenile fish) at all sites, including Tinaroo Falls Dam, would suggest that stocking, (at current levels) is having minimal or no direct impact on the abundances of either conspecific wild stocks or on other stocked *L. calcarifer* cohorts. Davis (1985) speculated that the reason that cannibalism was so rare in the Gulf of Carpentaria was a reflection of prey availability rather than because of food preference. Consequently, a higher stocking rate than that used in the current study may result in increased instances of cannibalism in both stocked and wild fish.

The results of this current study suggest very little difference between the condition of stocked and wild *L. calcarifer* in the Johnstone River regardless of cohort or the geographic zone where they were recaptured. The exception were wild fish from the middle reaches of the North Johnstone River, which were different both from stocked fish in that zone and also from all the other zones including Tinaroo Falls Dam, and may have been related to factors such as food availability and competition. These results suggest that, at the stocking densities used in the current study, condition factor remains relatively constant between year classes and geographic zones regardless of whether the fish are stocked or wild. There is evidence from other studies (e.g. Li, 1999) to suggest that where some species are stocked in high

densities, adverse impacts may result, including higher than expected mortalities in the stocked species or ecological impacts on food sources. None of these impacts were evident in the current study.

The relatively fast growth rate of the *L. calcarifer* stocked into Tinaroo Falls Dam compared to that of fish in the Johnstone River may be due to a number of factors including the larger size of fish that were released into the impoundment. However, a different study has also found that *L. calcarifer* stocked into another impoundment (Lake Morris) also grew faster than Johnstone River fish (Russell and Rimmer, 1997). These authors speculated that this disparity in growth rates was probably due to a lack of competition and an abundance of prey in the impoundment (Russell and Rimmer, 1997), favouring faster growth. Despite a faster growth rate in Tinaroo Falls Dam than in coastal areas observed in this current study, the condition factor of *L. calcarifer* in that impoundment wasn't significantly different to that of stocked and wild fish in coastal areas such as the Johnstone River.

Of those potential ecological impacts that were examined in the current study, we found no significant demonstrable effects that could be attributed to the stocking of *L. calcarifer*. However, this result may differ with variations in stocking densities or in locations where there are special considerations like those in the Wet Tropics bioregion where endemic species exist. Moratoria on fish stocking may be politically hard to justify and perhaps even impractical, but the consequences of introductions, particularly outside of the fish's native range, need to be closely scrutinised in terms of their potential ecological and also genetic impact on wild populations (Burrows, 2004). Until these interactions are fully understood, stocking and or translocating native species to new areas, or increasing the number of individuals stocked into an existing stocked population, should be undertaken with caution.

Genetics

Introduction

Anthropogenic activities have undoubtedly had a severe impact on the health and vitality of many fish populations. Environmental degradation, over-exploitation, habitat destruction, and impedance of migratory behaviour have all been associated with the demise of important fisheries. In response to the decline of fish populations, augmentation, or stock enhancement based on hatchery–produced fish has been implemented on a massive and global scale (Araki and Schmid, 2010). Many of these programs have been quite successful in boosting fisheries biomass, especially for threatened or endangered species (Bell *et al.*, 2006; Uki, 2006). However, the practice of introducing large numbers of hatchery–reared fish into systems where natural population numbers are perceivably low is controversial and has raised concerns about the long–term genetic impacts that these practices may have on a population's evolutionary fitness. This is because aquaculture–produced fish commonly undergo a series of genetic bottlenecks, along with other processes, which can dramatically reduce allelic diversity and change the genetic profile from that of the receiving wild population. Consequently, fisheries managers are increasingly looking to understand the implications of long–term restocking programs on the genetic health of wild populations as part of their risk assessment strategies.

Fish destined for restocking often differ in their genetic profile (i.e. alleles represented and/or their frequencies) from that of wild populations as a result of a combination of aquaculture processes, including the 'founder effect', differential broodstock contribution, high levels of relatedness, and domestication selection. The 'founder effect' is often the most significant factor impacting on the amount of genetic diversity captured in the restocked fish, and occurs where a small number of broodstock from a wild population are brought into the hatchery and bred. Generally, given the difficulties in obtaining and holding large numbers of broodstock, coupled with a large fecundity in many fish species, hatcheries rely on only a few adults collected from the wild population as their broodstock breeding base. The founding broodstock therefore only capture a small fraction of the total genetic diversity present in the wild population and often results in a loss of rare alleles through a form of genetic drift (Frost *et al.*, 2006; Ferguson *et al.*, 2007). Additionally, as the only alleles that can be passed to progeny are those that the broodstock possess, hatchery fish often have highly skewed allelic frequencies from that evident in the wild population.

Compounding this 'founder effect' is that the limited genetic diversity represented by the foundation broodstock can then be reduced further, as not all broodstock captured undergo spawning, with those that do often differentially contributing to the resulting progeny generations (Allendorf and Phelps, 1980; Frost *et al.*, 2006). This differential spawning and contribution has the potential to dramatically reduce the effective population size (*Ne*) of hatchery populations. For example, Frost et al. (2006) showed that in barramundi (*Lates calcarifer*) hatcheries (despite the mass spawning behaviour of the species) only several male and female broodstock breed. This then results in the genetic contribution of each broodstock in the resulting progeny cohort being generally highly skewed towards one to two males and one female individual. They found that this differential spawning behaviour resulted in a significant lowering of N_e (genetic effective population size) from that expected under the assumption that all hormonally induced broodstock in the spawning tank would breed. Coupled with the low number of broodstock that were contributing alleles was the fact that progeny within the cohorts exhibited a high level of relatedness (i.e. were all half– or full–siblings).

The hatchery environment also plays a role in the genetic divergence of hatchery and wild fish. This is because of domestication and/or artificial selection, which is the selection of traits that are better adapted to the hatchery environment (Gross, 1998). Domestication selection is often an inadvertent process beyond the control of aquaculture practitioners and occurs as a result of some genotypes providing a higher fitness for survival than others under the specific culture conditions, while artificial selection is where traits are purposely selected for higher productivity. Artificial and domestication selection results in fish that are not only genetically distinct, but also leads to behavioural and phenotypic differences between hatchery and wild fish populations (Gjedrem and Thodesen, 2005; Weir and Grant, 2005). All of these factors combined lead to a lower genetic diversity and higher rates of genetic drift (changes in allele frequencies) in hatchery fish (Allendorf and Phelps, 1980; Bentsen and Thodesen, 2005; Araki and Schmid, 2010).

As a result of changes often observed in the genetic profile of hatchery fish from that of their wild progenitors, fingerlings released into natural populations have the potential to likewise change the genetic profile of the receiving population. They can do this by swamping the genotypes represented in the natural population by introducing vast numbers of co-related individuals. Depending on the size of the population undergoing augmentation, shifts may occur in allelic frequencies and overall heterozygosity in the population from that evident prior to stocking. Hatchery fish also tend to be highly related and originate from few families. Consequently, if they breed with each other and/or wild fish, the population can experience increases in inbreeding levels, and possibly fitness consequences associated with inbreeding depression. The actual magnitude of the effect will, however, depend on several factors, such as the number of fish stocked relative to overall population size, their reproductive success, and the survival of introgressed offspring (Bentsen and Thodesen, 2005).

Interestingly, despite the theoretical genetic concerns about long-term effects of stocked fish on natural populations, the actual negative impact of stocking hatchery fish has not been conclusively shown in many studies. Perhaps the best review undertaken on this issue is that of Araki and Schmid (2010), who summarised 266 peer-reviewed papers published in the last 50 years which described case studies on ecology and genetics of hatchery stocks and their effects on stock enhancement. From their analysis, they were able to only find 23 studies out of 70 which showed significantly negative impacts of hatchery rearing on the fitness of stocked fish (e.g. lower survival, growth rate, reproductive fitness) and 28 studies which showed reduced genetic variation in hatchery fish from that of wild populations. They concluded that there was accumulating evidence of negative effects of hatchery rearing in a variety of stocked fish species. However, in their analysis they also identified indications of successful stocking, where no or little negative effects were apparent. They found that the literature evaluation of the impacts of hatchery stocking on wild fish populations is highly species–biased towards salmonoids and suggest that hatchery practices may be very different for other fish species, resulting in a different impact. As a result, after their extensive review, they were left to state “that the answer to the question whether hatchery stocking is helpful or harmful to wild stocks depends on the goal of the hatcheries, species and the cases”. Consequently, it appears that the risk assessment of the overall impacts that augmentation programs may have on wild fisheries will need to be undertaken on a case–by–case species basis.

One fishery that has undergone a relatively long period of sustained augmentation using hatchery fish is the barramundi fishery in the Johnstone River, Queensland, Australia. The Johnstone River has been stocked with around 300 000 fingerlings since 1993 in a series of experiments to investigate stocking methodologies, efficacy and cost–benefits (Russell and Rimmer, 1997, 1999, 2000; Russell and Rimmer, 2002). Initially, stocking of the river was based on releasing fingerlings originating from broodstock collected and bred as part of a government program by the then Queensland Department of Primary Industries, but from the

early to mid 2000s there was a greater reliance on commercial hatcheries to supply fingerlings. This augmentation program represents the longest running, and best documented riverine barramundi stocking program in tropical Australia. Consequently, the Johnstone River represents an ideal model system to investigate concerns about the long–term ecological and genetic impacts of large–scale barramundi stocking. These concerns include if the population is exhibiting increased levels of inbreeding and loss of genetic diversity compared to the pre–stocking and surrounding populations.

Of additional interest is understanding the fate of these stocked barramundi juveniles, particularly in the case where several co–related families are represented in the cohort stocked. If stocked barramundi cohorts do contain several families of fingerlings, then a big driver on the possible impact of the stocked fish may be if all the families represented in the stocking survive and grow into adult fish that may then breed. Obviously, if there is evidence of differential survival of families post-stocking, then overall genetic diversity of the stocked cohort will be further reduced, potentially leading to a larger impact on the genetic diversity of the Johnstone River barramundi population. Finally, perceived negative genetic impacts of stocked barramundi will only be manifested if the stocked individuals survive to maturity and breed with wild fish. However, no instances of introgression between hatchery stocked barramundi and their wild counterparts have yet been identified in the literature.

Due to a lack of knowledge or evidence of the genetic impacts that long–term stocking of barramundi into the Johnstone River has had on the resident population, the objectives of the current project were three–fold:

(a) to compare historical genetic diversity represented by samples collected before stocking commenced in the Johnstone River with that of the contemporary population
(b) to undertake a genetic audit of hatchery–produced fingerlings to identify levels of familial genetic diversity represented in stocked cohorts of fish and to elucidate if families undergo differing levels of contribution and resultant survival post–stocking
(c) to use simulations based on hatchery–produced and wild fish genotypes to provide evidence for introgression resulting from hatchery and wild barramundi breeding within the contemporary Johnstone River barramundi population.

Methods

The genetic analyses in this project as described below were based on four individually collected genetic sample sets. Firstly, otoliths originating from fish that were collected prior to the commencement of stocking in the Johnstone River, or from fish that were identified as being born before the first stocking events through otolith ageing and/or total length analyses (Davis and Kirkwood, 1984; Stuart and McKillup, 2002), were used to provide a historical genetic profile of the barramundi population in the Johnstone River prior to stocking activities. Secondly, barramundi were sampled either through targeted electrofishing or from commercial fishing from the contemporary population to provide baseline genetic data on genetic diversity. As all barramundi stocked in the Johnstone River before our study had been marked with a coded wire tag (Russell *et al.*, 1991), we were able to identify within the contemporary population fish that had a hatchery origin and those originating from natural spawnings. Thirdly, to understand how many barramundi families usually comprise stocked cohorts of fish, and whether these families undergo differential survival post-stocking, 9423 fingerlings sized at 50–70 mm originating from two commercial hatcheries were stocked into the North and South Johnstone Rivers in December 2009. These fish were also marked with CWTs to allow them to be discriminated from wild stocks in the river. Finally, fin clip samples originating from the last batch of broodstock used by the then Queensland Department of Primary Industries and Fisheries (DPI&F) in their barramundi stocking

program (circa 2000–2004), as well as broodstock from the two commercial hatcheries used to produce cohorts of fingerlings stocked into the river as part of this project, were genotyped to provide parental pedigree information for fingerlings stocked into the Johnstone River.

Genomic DNA extraction: Johnstone River samples (wild and CWT recaptures)

For barramundi samples caught in the Johnstone River during this current study, genomic DNA was obtained from small pieces of fin tissue preserved in 70% ethanol using a simple detergent cell lysis technique modified from Taris *et al*., (2005). Fin tissue (<1 mm^2) was digested overnight at 60 ºC in a cell lysis buffer containing 1 mg ml^{-1} proteinase K, 670 mM Tris-HCl pH 8.0, 166 mM ammonium sulphate, 0.2% v/v Tween20 and 0.2% v/v IgePal CA-630 (NP-40). Proteinase K was then heat inactivated (95 ºC at 10 min) prior to removal of undigested cell debris by centrifugation (1000 g for 1 min). Aliquots of the resulting supernatant were diluted 1:1 with 1x TE (10 mM Tris-Cl, 1 mM EDTA), stored at –20 ºC and used within one to two weeks. Genomic DNA within the supernatant was not quantified due to a lack of purification that results in a complex mixture of nucleic acids, proteins, lipids and other cell components. A maximum of 0.5 µl of the 1:1 diluted supernatant was used directly as the template in each of two multiplex microsatellite polymerase chain reactions (PCRs) as higher volumes inhibited PCR reactions.

Genomic DNA extraction: broodstock and pre–release project fingerlings

Archival quality genomic DNA extracts of high purity were obtained from DPI&F broodstock tissue samples (80% ethanol preserved fin tissue), previously collected as part of a genetic audit of Queensland barramundi hatcheries (Bartlett *et al.*, 2007). DNA samples were also taken from representative fingerlings from two commercial hatcheries used to provide the fingerlings destined for stocking into the Johnstone River as part of the project aimed at identifying differential contribution among families of stocked barramundi. These hatcheries are nominally known as hatchery 1 (n = 92 fingerlings genotyped) and hatchery 2 (n= 184 fingerlings genotyped). Genotypes from these pre–release fingerlings were ascertained to enable us to (a) establish the number of barramundi families represented within each hatchery batch (and therefore the number of brood stock contributing to spawning) and (b) the respective pre–stocking contributions of each of the families in the cohort of fingerlings.

High purity DNA was obtained for broodstock barramundi fin clips using a digestion buffer modified from Adamkewicz and Harasewych (1996) and containing 0.2 mg/ml proteinase K, 100 mM Tris-HCl pH 8.0, 1.4 M sodium chloride, 20 mM EDTA and 2% w/v hexadecyltimethylammonium bromide (CTAB). Samples were digested at 60 ºC overnight, followed by chloroform purification (performed twice) and isopropanol precipitation using standard laboratory techniques (Sambrook and Russell, 2001).

For the project fingerlings destined for stocking prior to release, high purity DNA was obtained following the methods of Elphinstone et al, (2003). The quality and quantity of DNA obtained was verified by agarose gel electrophoresis (0.8% agarose in 1x TBE buffer stained with GelGreen) by comparison to commercially prepared DNA standards (lambda DNA, New England Biolabs). DNA was diluted to ~5 ng/µl prior to use in PCR.

Genomic DNA extraction: otoliths

To obtain genetic profiles of barramundi resident in the Johnstone River prior to the commencement of fish stocking, DNA was sourced from otoliths that were collected for other projects (see 'General methods' section for selection methods and storage details).While a number of extraction protocols described in Hutchinson et al.(1999) were attempted in preliminary trials, the final protocol utilised the 'Nucleospin Tissue XS' extraction kit (Macherey-Nagel), and followed the recommendations for extraction from small laser–micro–dissected samples. In short, whole or fragmented otoliths were placed in 1.5 ml tubes and pre–

incubated for 3 hrs in Buffer T1/ proteinase K at 56 ºC followed by overnight incubation in the supplied Buffer B3. Each otolith was handled with clean forceps, and large otoliths were fragmented by snapping them by hand while covered in laboratory wipes (Kim Wipes) to minimise cross–contamination. Following cell lysis, the resulting DNA was bound to nucleospin tissue XS columns, washed and eluted in 20 µl of elution buffer BE (Macherey-Nagel). The DNA recovered was not quantified due to the low volumes and quantities. Following initial empirical trials, 4 µl was used directly as the template in each of the two multiplex microsatellite polymerase chain reactions (PCRs).

PCR amplification and scoring of microsatellite marker

The two microsatellite PCR reactions utilised for all barramundi genetic samples were the P1 suite and the G suite (see Supplementary Information A), and these reactions amplified 9 and 8 microsatellite markers respectively. A maximum of 17 microsatellite loci were therefore genotyped in most fish, however, one locus of the modified G suite (Lca287) was subsequently dropped from the final analysis due to difficulties in scoring consistently (see error–checking below). Due to degraded DNA from otolith samples, Lca058 was also dropped from the analyses as a result of difficulties in amplification.

All PCR reactions were performed in low–profile microtitre plates (Biorad or Fisher Biotech) sealed with microseal B film (Biorad) and contained multiple fluorescently labelled PCR primers as described in Supplementary Information A. Both P1 and G suite multiplex PCR reactions contained 1x TypeIT microsatellite PCR master mix (Qiagen), 1 x P1 or G primer mix (see Supplementary Information A) and 0.5 µl of 1:1 diluted cell lysate supernatant (or 5 ng/µl high-quality DNA) in a 10 µl total volume. Thermal cycling was conducted in a Biorad C1000 or S1000 thermal cycler. For the P1 suite cycling, conditions consisted of a step-down program including one cycle of 95 ºC for 5 min followed by 10 cycles of 95 ºC for 30 s, 57 ºC for 90 s, 72 ºC for 30 s followed by 20 cycles of 95 ºC for 30 s, 55 ºC for 90 s, 72 ºC for 30 s and concluding with a 60 ºC incubation for 30 min to maximise non–template adenylation of PCR products.

A slightly modified program was utilised for the G suite and this consisted of one cycle of 95 °C for 5 min followed by 11 cycles of 95 °C 30 s, 57 °C for 90 s and 72 °C for 30 s, then 21 cycles of 95 °C for 30 s annealing temperature at 55 °C for 90 s and 72 °C for 30 s, then a final step of 60 °C for 45 min.

Successful PCR amplification was verified by agarose gel electrophoresis (1.5% agarose in 1x TBE including 0.3X GelGreen DNA stain (Invitrogen) prior to column purification of PCR products over Sephadex G50 resin (GE Healthcare Life Sciences) in 350 µl Whatmann filter plates. Purification was by centrifugation at 770 g for 2 min. Purified PCR products were size separated by capillary electrophoresis using a MegaBACE™1000 DNA Analysis System (GE Healthcare Life Sciences) at the Genetic Analysis Facility, James Cook University. Included with each PCR was the MegaBACE ET550R ladder (GE Healthcare Life Sciences) and final allele scoring was undertaken using Fragment Profiler™ software (GE Healthcare Life Sciences). All allele calls were checked manually. Alleles were labelled based on the relative number of microsatellite repeat units as determined from raw fragment sizes using the Microsoft Excel add-in FlexiBin (Amos *et al.*, 2007).

Genotyping error checking

To ensure consistency of scoring between genotyping runs and between individual technicians, a total of 140 fish were genotyped at least twice, and in some cases three times, from separate PCR reactions and the genotypes compared. These genotyped individuals included fish from the Johnstone River that were sampled on multiple sampling dates, individual stocked (CWT) or broodstock fish with missing data for one or more loci that were

re–run to obtain missing data, and a random selection of stocked fish from across all genotyping runs that were not missing data on initial runs. Where there were corresponding data from across two runs, the genotyping error rate was typically low (<0.5%), with most errors detected due to sample labelling/data entry errors.

To check for labelling or sampling errors, an identity check was performed on all project CWT genotypes using the software Cervus 3.0.3 (Kalinowski *et al.*, 2007). Multiple instances of identical genotypes under different tag numbers/sample IDs were identified (n = 58 instances) and these represent possible tag loss/re–tagged individuals, or duplicated samples with sampling/labelling errors. Only one representative genotype was retained in each instance and this corresponded to the sample ID/tag number with the most recent date in the case of possible re–tags, and in the case of putative labelling errors during genotyping, the genotype with corresponding phenotypic data in the project's coded wire tag database was retained.

A total of 841 non–CWT barramundi genotypes were obtained from the contemporary Johnstone River population, while a total of 332 CWT barramundi were successfully genotyped after removing individuals with poor quality data (fewer than 14 markers), and those with identical genotypes as indicated above.

Parentage analysis: pre–release project fingerlings

To establish family contributions to the cohort of fingerlings from each hatchery prior to stocking, samples from both hatcheries were analysed for parentage by comparison of 16 microsatellite genotypes against their respective candidate broodstock (see above) using Cervus 3.0.3 (Kalinowski *et al.*, 2007). In each case, broodstock genotypes were used to determine allele frequencies prior to simulation of 100 000 progeny genotypes from broodstock with known sex, and all possible candidate broodstock included. The assignment of the single most likely candidate mother (dam) and father (sire) was then undertaken using Cervus 3.0.3, and DELTA scores were used to determine the probability of correct parent assignment. A conservative approach was used in accepting assignments. Only assignments with a parent pair–progeny trio confidence of $\geq$ 95% and zero mismatches across at least 14 microsatellite loci were accepted.

Parentage analysis: post–release fingerlings recovered as CWT fish

To establish family contributions within the project CWT fish recaptured from the Johnstone River, all CWT fish genotypes were included in one of two separate Cervus 3.0.3-based parentage analyses:

(a) against Hatchery 1 broodstock used to generate fingerlings and (b) against Hatchery 2 broodstock used to generate fingerlings.

Parentage analyses for all CWT fish were conducted separately for both broodstock groups to ensure that allele frequencies and simulated progeny genotypes used to determine probabilities of correct parent assignment were realistic and reflected likely combinations of parental genotypes. As for the pre–release fingerlings, parentage analysis was again conducted using simulations of 100 000 progeny genotypes generated from broodstock of known sex. The proportion of candidate parents sampled for the two analyses involving project hatchery broodstock was set to 1.0 to reflect complete sampling of all possible candidate dams and sires.

In the post–release parentage analysis, a conservative approach was used in accepting parental assignments. Only assignments with zero mismatches across at least 14 loci and a parent pair–progeny trio confidence of $\geq$ 95% were accepted. As all CWT individuals were included in

each analysis, the final strict assignments were merged and only a single instance of a CWT individual assigned with equal confidence and no mismatches against both Hatchery 1 and Hatchery 2 broodstock was detected, and this individual was discarded from the dataset.

Pre– vs post–stocking genetic diversity

This project had access to samples from barramundi that were born before stocking had significantly begun in the Johnstone River. As a result, it was possible to examine how genetic diversity had changed between pre– and post–stocking eras. The genetic material available to establish the genetic profile of the Johnstone River barramundi population prior to stocking comprised of (a) otoliths collected from fish that had been aged as being born before stocking began, (b) relevant wild DPI&F broodfish collected from the Johnstone River and used to produce fingerlings for stocking, and (c) current wild fish sampled in the project that were of a size where conservative interpretation of age–growth studies suggested that they would have been born before restocking. The contemporary genetic profile of the Johnstone River barramundi population was derived from fish sampled in the current project. Genetic diversity indices (heterozygosity, F_{IS}, $F_{ST)}$ of pre– and post–stocking populations were calculated using the programmes GenAlex 6 (Peakall and Smouse, 2006) and Arlequin (Excoffier and Lischer, 2010), as were exact tests for population differentiation and Analysis of Molecular Variance (AMOVA). Statistical confidence on the estimates of population differentiation and AMOVA were derived from 10 000 permutations of the data. The Garza-Williamson Index (Garza and Williamson, 2001) was used to indicate if the contemporary barramundi population had undergone a bottleneck in genetic diversity compared to pre–stocking levels.

Identification of putative progeny resulting from stocked/wild matings

To identify if any of the wild fish (those without CWTs) sampled in the current project may have been the product of a mating between hatchery–reared fish and wild parents, a procedure that combined genotypic simulations and Bayesian admixture analysis of molecular genetic data was used (Schwartz and Beheregaray, 2008; Sanz *et al.*, 2009). The analyses proceeded in the following way. Firstly, to identify barramundi sampled in the contemporary population that may have been the product of a mating between a hatchery and wild fish, there was a requirement to predict what the genotypic profile of such an introgressed fish would represent. Unfortunately, no genetic material from actual cohorts of stocked fish had ever been preserved, so there were no baseline data using real genotypes to establish the genetic profile of a batch of previously stocked fingerlings, or what the genotype of a hatchery/wild introgressed F1 barramundi would look like.

To overcome this problem, hypothetical populations of hatchery, wild and F1 fish were simulated to produce three genetic clusters of genotypes against which we could compare the genotypes of wild fish sampled in the current project. Here, the genotypes of DPI&F broodfish from three known spawnings used to stock the Johnstone River, and from which we had genetic material, were used in the program HYBRIDLAB (Nielsen *et al.*, 2006) to simulate 5000 progeny genotypes at 15 of the 17 microsatellites. This represented the simulated hatchery fish genetic pool. Similarly, allele frequency information representing that observed in the population of pre–stocking barramundi were used to simulate 5000 progeny under conditions of random mating and Hardy–Weinberg equilibrium to create the wild fish genetic pool.

HYBRIDLAB was again used to simulate the genetic profile of fish resulting from a F1 cross between these simulated hatchery and wild fish. This was achieved by simulating the mating of 5000 hatchery fish with 5000 wild fish to produce another progeny batch of 5000 simulated F1 introgressed genotypes. After simulation of the three possible types of genetic pools, the Bayesian assignment programme Structure (version 2.3.3) (Pritchard *et al.*, 2000) was used to

infer the proportion of the genome with ancestry to either hatchery or wild simulated genetic clusters (k = 2). The posterior probabilities of the data were determined with 300 000 iterations (after a burn–in period of 100 000 iterations) under a genetic admixture model, assuming independent allele frequencies and using prior information on the genetic cluster that fish originated.

For each individual fish, Structure infers a Q-value ranging from one to zero that denotes the mean posterior proportion of the genome with ancestry to the genetic clusters defined. Based on our simulated mating groups, Structure analysis statistically defined the Q-value represented by the genetic clusters of hatchery ($q \geq 0.99$), wild ($q \leq 0.01$) or F1 ($0.40 \geq q \geq 0.40$) fish, respectively. These simulated Q–values were used to establish a statistical benchmark against which real genotypes of fish collected from the Johnstone River could be compared. As proof that this technique can correctly assign genome ancestry, analyses were also conducted where simulated progeny from the three genetic clusters were not given a population identity and then assigned to the two genetic clusters. All simulated fish were correctly assigned to their appropriate cluster. Accordingly, the genotypes of 841 wild barramundi sampled from the Johnstone River were added into a third Structure analysis under the same program conditions and their Q–value determined to establish the probability of genome ancestry to a wild/wild fish, or wild/hatchery fish mating.

Results

Summary parentage assignment of project stocked barramundi fingerlings: Hatchery 1

A total of 89 Hatchery 1 (H1) pre–release fingerlings were assigned with strict confidence and no mismatches to broodstock, while 74 of the CWT recaptured fish from the Johnstone River were assigned against H1 broodstock with the same stringency (Figure 12). Only a single dam contributed to both pre–release fingerlings and recaptured CWT fish assigned to H1 families (Figure 12). In contrast, 17 different sires were detected with variable contributions (Figure 12, Supplementary Information B).

Family contributions were highly skewed ranging from 1.1% to 18% in the pre–release (stocked) fish and 1.4% to 16.2% in the recaptured CWT H1 fish (recovered). Five families initially detected in pre–release fingerlings were not recovered from the Johnstone River, although these families were initially represented in the cohort at low frequencies and may not have been detected due to sampling effects. One family at low frequency was detected in recaptured CWT fish, but not detected in the pre–stocked sampled batch (Figure 12).

Two families, family 1 and 6, exhibited a substantial drop in contribution from pre–release to recovery from 10.1% to 1.4% (family 1) and 18.0% to 6.8% (family 6), which may be suggestive of comparatively lower survival in the wild, although again due to low recapture numbers this drop in detection may be due to sampling error (Figure 12; Supplementary Information B). Another two families, family 5 and 15, exhibited a substantial increase in contribution from pre–release to recovery (Figure 12, Supplementary Information B), with family 5 increasing in relative abundance from 6.7% to 16.2%, while family 15 increased from 9.0% to 21.6%. Such increases may indicate relatively high survival in the wild, however sample sizes are small in each case and results may again be subject to sampling error.

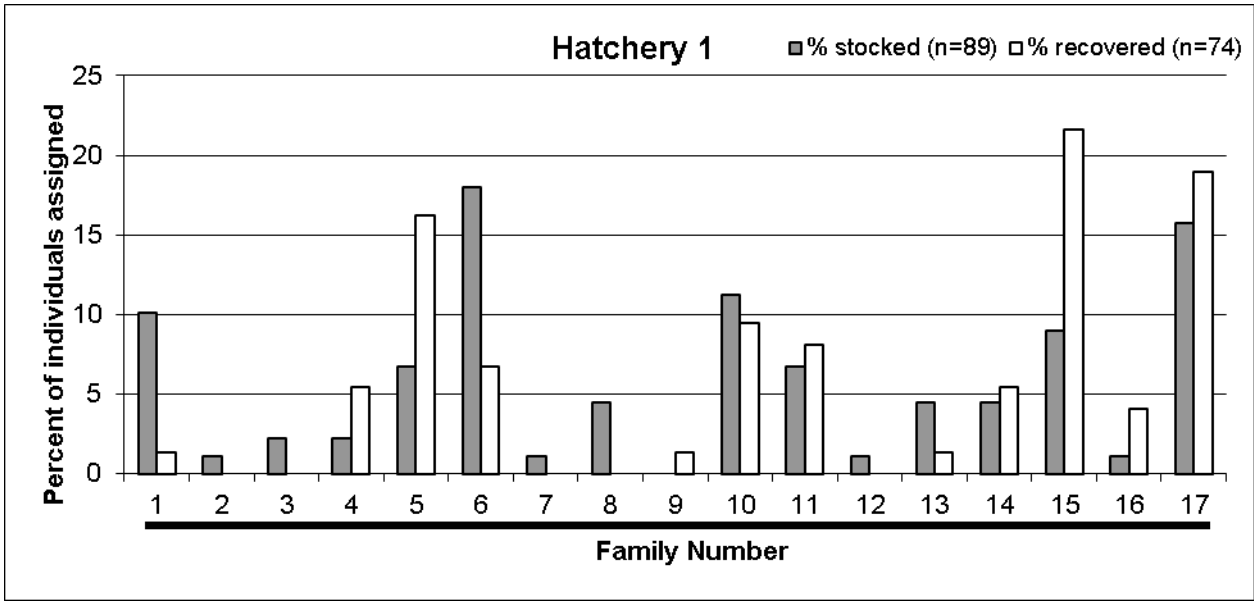

Figure 12. Half-sib family contributions as per cent of individuals assigned to Hatchery 1 broodstock with strict (> 95%) confidence and zero mismatches using 16 microsatellite loci.

Families detected at the time of stocking (stocked) are shown in solid bars, those recovered from the Johnstone River later as CWT fish are indicated by open bars, sample sizes are indicated in figure legend. The solid line along the X axis shows families with a common dam. Note some families were not detected initially (family 9) or were not recovered (families 2, 3, 7, 8 and 12).

Summary parentage assignment of project fish: Hatchery 2

A total of 174 Hatchery 2 (H2) pre–release fingerlings were assigned with strict confidence and no mismatches to broodstock, while 140 of the CWT recaptured fish from the South Johnstone River were assigned against broodstock with the same stringency (Figure 13). Although the total number of H2 families detected amongst pre–release and recaptured CWT fish (n = 18 families) was similar to that for H1 (n = 17 families), a greater number of contributing dams was detected with four candidate H2 mothers identified (Figure 13) compared to just a single dam in the H1 cohort. Only six candidate fathers (sires) were detected in the H2 cohort compared to 17 in the H1 cohort, which may reflect smaller male broodstock numbers held at H2, as well as differences in hatchery practices (i.e. differences in the number of females induced and the number of consecutive nights of spawning and egg collection).

Once again, family contributions were highly skewed ranging from 0.6% to 34.5% in the pre–release fingerlings and 0.7% to 23.6% in the recaptured CWT fish assigned to H2 broodstock (Figure 13,

Supplementary Information C). Two H2 families (family 2 and 12) were initially detected at low frequency (<5%) in pre–release fingerlings, and were not detected in the recovered CWT fish, while an additional two families (family 3 and 16) were recovered at low frequency (<1%), but were absent from the initial sample of CWT fish (Figure 13).

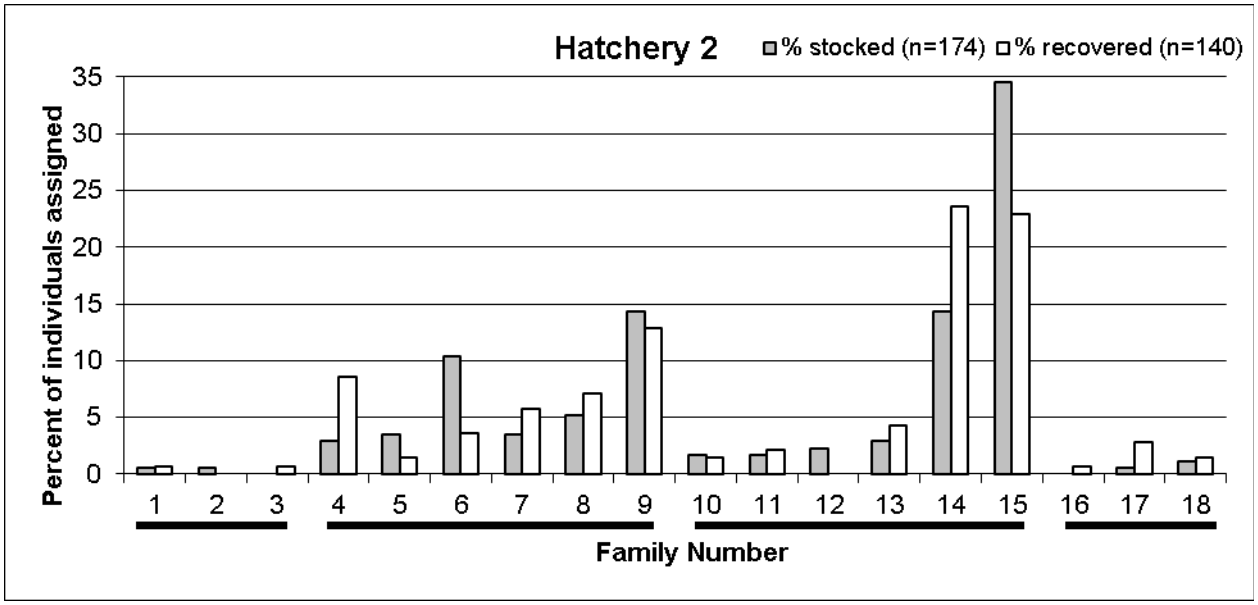

Figure 13. Family contributions as per cent of individuals assigned to Hatchery 2 broodstock with strict (> 95%) confidence and zero mismatches using 16 microsatellite loci.

Families detected at the time of stocking (stocked) are shown in solid bars, those recovered from the Johnstone River as CWT fish are indicated by open bars, sample sizes are indicated in figure legend. Solid line along the X axis shows families with a common dam. Note some families were not detected initially (family 3 and 16), or were not recovered (families 2 and 12).

There were some changes in H2 family contributions when comparing pre–release fingerlings to those recovered as CWT individuals and assigned against H2 broodstock. These changes included that of the two more abundant families, with family 14 increasing in frequency from 14.4% to 23.6%, while family 15 decreased in frequency from 34.5% to 22.9% (Figure 13,

Supplementary Information C). This may reflect slight differences in the relative survival between these two families, however both families remained relatively frequent, making up >20% of all recaptured H2 CWT fish.

Table 5. Allelic diversity, expected heterozygosity (He) and Garza–Williamson Index of pre–stocked and contemporary Johnstone River barramundi genotyped at 15 microsatellite loci.

	Pre-stocked (n = 45)		Contemporary (n = 841)	
Locus	Number of	He	Number of	He
Lca003	4	0.468	12	0.496
Lca016	25	0.336	29	0.224
Lca040	3	0.657	9	0.673
Lca057	6	0.681	7	0.606
Lca008	7	0.160	7	0.166
Lca154	3	0.500	4	0.483
Lca178	2	0.626	6	0.595
Lca020	4	0.565	7	0.538
Lca021	6	0.745	6	0.771
Lca371	2	0.499	6	0.511
Lca064	14	0.862	15	0.843
Lca069	2	0.444	2	0.513
Lca070	3	0.591	6	0.537
Lca074	15	0.357	15	0.344
Lca098	9	0.618	11	0.633
Mean	7	0.541 ± 0.176	9.5	0.529 ± 0.182

(n = number of barramundi genotyped for each population, FIS = Wright's inbreeding coefficient, ns indicates that estimate is not statistically different from zero).

Pre–stocking versus contemporary genetic diversity in the Johnstone River

All loci, except for Lca008, were found to be in Hardy–Weinberg equilibrium after correction for multiple comparisons (False Discovery Rate (Benjamini and Hochberg, 1995)). Mean allelic diversity was higher in the contemporary population, as was the Garza–Williamson index, indicating that the contemporary barramundi population has not undergone a loss of allelic diversity or experienced a genetic bottleneck post–stocking. Minimal differences were observed between pre–stocking and contemporary Johnstone River barramundi population genetic profiles. Both of these temporally separated populations exhibited statistically similar expected heterozygosity (prestocked H_e = 0.541 ±0.176; contemporary H_e = 0.529 ±0.182; P > 0.05), while the inbreeding coefficient (F_{IS}) for both populations, whilst slightly negative,

was not statistically different from zero (pre–stocking F_{IS} = -0.008, P > 0.05; contemporary F_{IS} = -0.052, P > 0.05) (Table 5). This suggests that there is no evidence for an increase in inbreeding levels (or lower heterozygosity) between pre–stocked and contemporary populations.

Figure 14. Structure program screenshot of the proportion of genome ancestry to either a simulated DPI&F progeny or wild Johnstone River genetic cluster (k=2) under an independent loci with prior population data, admixture model.

Representation of the genome to DPI&F hatchery and wild fish genetic clusters (on a scale of 0-1) are represented by the red and green colours respectively.

Likewise, in support of this contention that the two populations have not genetically changed after 18 years of barramundi stocking, the indices of population differentiation were unable to differentiate the two temporal populations (F_{ST} = 0.005 ± 0.001, whilst the among–population variance using an AMOVA was -0.001176 (Table 6).

Detection of introgressed F1 individuals between hatchery stocked fish and wild barramundi

Genetic admixture analyses based on simulated DPI&F hatchery and pre–stocked wild barramundi, and the subsequent F1 cross, failed to detect evidence of introgression within the 841 barramundi sampled as part of this current project. All contemporary barramundi had a q-value to the genetic cluster represented by wild fish of ≥ 0.80. This indicated that at least 80% of their genetic profile was similar to that seen in wild non–introgressed barramundi. While it is important to note that ≤ 20% of the genome of contemporary fish were also assigned to the genetic cluster of DPI&F progeny, this was expected given the broodstock used to simulate progeny obviously also had ancestry with the pre–stocked population used to simulate the wild genotypes. If they were true hybrids we would have expected the q–values to the two genetic clusters to be bound within the values of $0.40 \geq q \geq 0.40$.

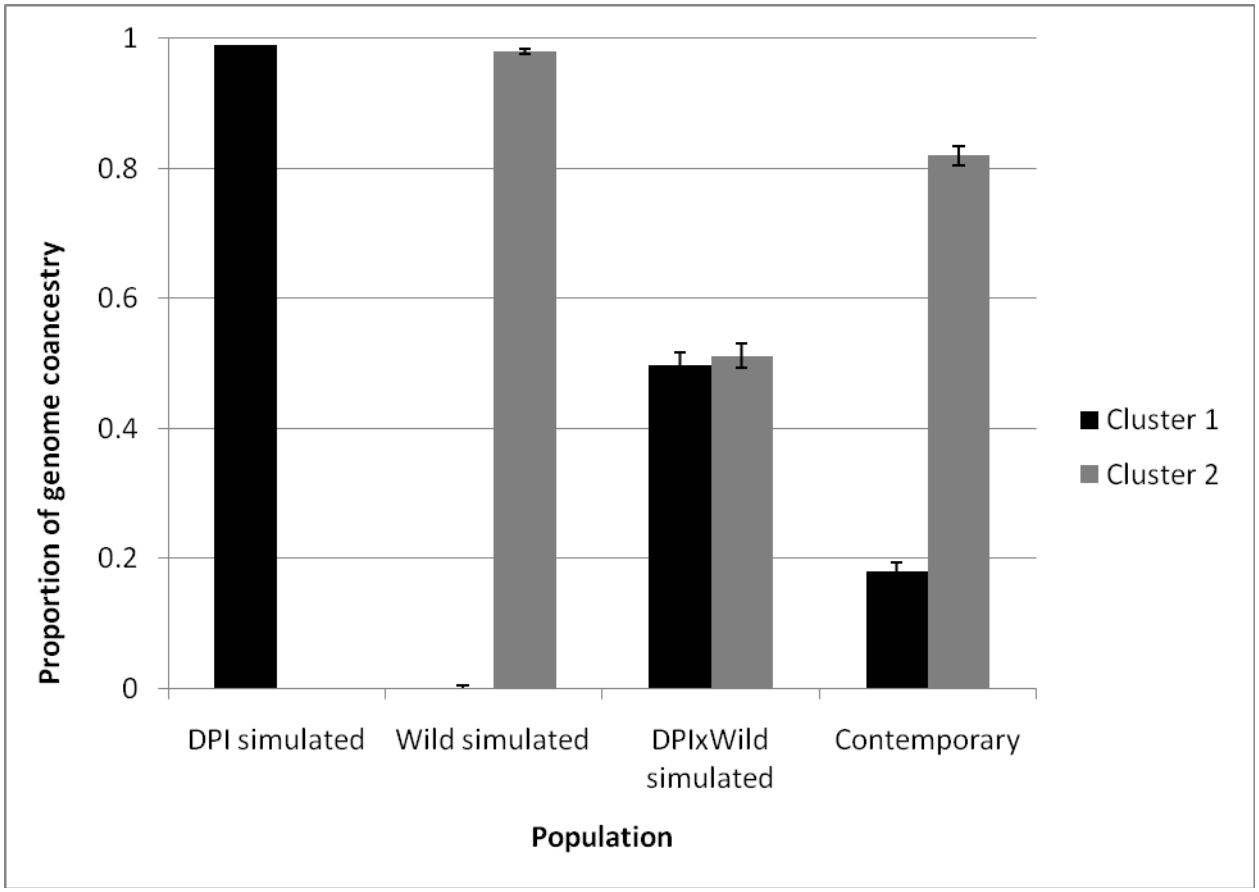

Figure 15. Mean (± S.D) proportion of genome coancestry (q-value) of simulated and contemporary barramundi to either a DPI&F hatchery progeny genetic cluster (Cluster 1), or wild Johnstone River progeny genetic cluster (Cluster 2).

Table 6. Analysis of molecular variance (weighted average over all loci) for pre–stocked and contemporary barramundi from the Johnstone River.

Source of variation	Sum squares	Variance	Percentage variation
Among populations	3.624	-0.0048	-0.1176
Among individuals within populations	3448	-0.0075	-0.1852
Within population	3468	4.0598	100
Total	6920	4.0476	

Discussion

Despite a long period of stocking fingerlings into the Johnstone River, no significant adverse genetic impacts were detected in this study. Comparisons between genotyped barramundi dated pre–stocking with that of individuals from the contemporary Johnstone River population did not detect any major losses in allelic diversity, heterozygosity, or an accumulation of inbreeding (F_{IS}). Additionally, based on simulations using grandparental genotypes of three broodstock pairings historically used to produce progeny stocked into the river, no evidence of introgression between hatchery and wild fish was discovered.

The influence that stocking of hatchery produced fingerlings will have on the genetic diversity of the receiving population will largely depend on the long–term survival and reproductive fitness of stocked individuals, their genetic profile, and importantly the proportion of stocked fish to the receiving population (Neff *et al.*, 2011). Since the commencement of stocking in 1992/93, nearly 300 000 barramundi fingerlings have been released into the Johnstone River (DJ Russell, pers comm). The proportion that captive–reared fingerlings represent when compared to annual natural recruitment within the river system and gene flow between adjacent river systems is unknown. Russell and Rimmer (1997) compared the abundance of CWT fish relative to the wild barramundi population in the Johnstone River and found that stocked fish made up between 10 and 15% of the 580-650 mm TL size cohort. This suggests that for this size cohort, natural recruitment accounts for at least 85% of the remaining genotypes in the population. Based on our genetic data, these high levels of natural recruitment appear to be sufficient to prevent swamping of the population with captive–reared genotypes.

However, if stocking levels were to increase then replacement of wild genotypes through the integration of captive–bred genotypes would be expected, particularly if natural recruitment of barramundi juveniles into the river population is density–dependent. In this type of situation, ecological competition induces density–dependent mortality, which can lead the stocked population to cause a net loss in the number of naturally spawned fish (Goodman, 2005). Such density–dependent mortality and resultant decline in wild fish abundance due to captive–reared fish has been reported in a chinook salmon population (Levin *et al.*, 2001). The effects from increased stocking rates become most prevalent where fitness differences in genes between captive–reared and wild fish arise. Modelling shows that higher stocking rates cause a reduction, rather than enhancement, of population size over the long term due to the fitness disadvantage of captive–bred fish and strong overcompensation at the recruitment stage in natural populations (Satake and Araki, 2011). Consequently, if supplementation of the Johnstone River barramundi population is to continue into the future, strict annual quotas on the number of fish put into the river at or below historical releases should be exercised.

In river systems which possess genetically unique and enclosed breeding populations, the magnitude of impact from stocking is expected to be greater than those river systems where fish have the opportunity to freely interchange genes with conspecifics from neighbouring populations. This is because in closed populations the process of genetic drift (i.e. sampling effects correlated with the representation of allele frequencies in the population) are not countered by the relatively strong genetic force of gene flow. Therefore genetic drift resulting from stocking consanguineous genotypes into the Johnstone River barramundi population would be expected to be countered by gene flow occurring from barramundi moving into the river from adjacent systems, as well as captive–reared fish moving out of the system.

Although across its entire species range barramundi is genetically structured in accordance with an isolation by distance model of gene flow, adjacent river systems are genetically analogous (Keenan, 1994; Chenoweth *et al.*, 1998; Doupe *et al.*, 1999; Marshall, 2005). This indicates adequate levels of local gene flow to prevent divergence. Although previous genetic

surveys have not specifically looked at genetic structure in the river systems in and immediately surrounding the Johnstone River, tagging studies have confirmed the presence of considerable movement, where tagged fish originating in the Johnstone River had been found to move along the coast and into adjacent watercourses (see 'Movements' section of this report). Fish were found to have moved distances of up to 38 km along the coast into the Russell River to the north and 20 km south into Mourilyan Harbour; one fish had moved 105 km between tagging and recapture from Trinity Inlet near Cairns, south along the coast into the Johnstone River. Tagging, therefore, highlights the potential for substantial movement and gene flow between neighbouring populations and may serve as a buffer to genetic change at the levels barramundi have been stocked. Hence it is very likely that gene flow between adjacent river systems is relatively frequent and has helped to modulate any genetic changes in the Johnstone River that may have resulted from restocking with fingerlings.

Theoretically, artificial breeding programs that rely on few broodstock and therefore a low effective population size (N_e) would lead to the stocking of highly related progeny per generation. Consequently, loss of genetic diversity in the population would stem from a reduction in effective population size mediated by an increase in variance in reproductive contribution among individuals (Ryman and Laikre, 1991; Wang and Ryman, 2001). Maintaining a high N_e is therefore essential for captive–breeding. If the DPI&F breeding program bred from sufficient broodstock to produce the captive–reared fingerlings stocked into the Johnstone River, and then rotated their broodstock regularly, N_e may have been sufficiently maintained to decrease overall variance in contribution of genotypes. This, in turn, would have limited the impact of stocking on the genetic profile of the Johnstone River wild population.

Spawning records from the DPI&F indicate that a minimum of 17 female broodstock and an indeterminate (but greater) number of males were rotationally spawned over the 13 years they captive–reared fingerlings to stock the Johnstone River. At a minimum, assuming single male and female contributions per spawn, the use of this number of broodstock would have provided an N_e of 34 and a rate of inbreeding of 1.5%, which is close to acceptable for long–term maintenance of genetic diversity (i.e. a N_e of 50 - Falconer and Mackay, 1996). However, since several males generally mate with females during Barramundi spawns (Frost *et al.*, 2006), the N_e is expected to be marginally higher (depending on the number of contributing males) than the above estimate. Thus the rotation of large numbers of broodstock appears to have aided the maintenance of genetic diversity in the DPI&F stocking program and would in itself partly account for low levels of genetic diversity change over the term of the program.

If the N_e represented by the number of broodfish used to produce progeny for stocking is going to aid maintenance of genetic diversity, there is an assumption that differential family survival post–stocking will not significantly lower the amount of genetic diversity captured in the spawning event through particular families going "extinct". Whilst we have a good understanding of the maintenance of family–specific genetic diversity early in the barramundi hatchery production cycle (Frost *et al.*, 2006; Wang *et al.*, 2008), what happens to family diversity once juveniles are stocked into aquaculture ponds, or open water bodies, and how N_e is affected, is uncertain. At the hatchery level, previous work shows that one or two broodstock normally dominate the genetic contributions to the pool of offspring, with other broodstock in the spawning tank contributing lower and highly skewed numbers of progeny (Frost *et al.*, 2006; Wang *et al.*, 2008). Consequently, cohorts are usually represented by full–sib maternal families (1 or 2) and numerous half–sib paternal families, dependent on the number of males in the spawning tank that engaged in the mass spawning. As the hatchery process proceeds, differential family survival erodes some of the familial genetic diversity initially captured in the spawn, particularly for those families poorly represented, and by the

time fish are ready for stocking the effective population size of the individual cohort is substantially lowered from that present immediately post–spawn.

For example, Frost et al., (2006) tracked changes in *Ne* of several barramundi progeny cohorts and observed a drop in N_e between 5 and 15% between 48 hr post–spawning and post–metamorphosis (day 27). Understanding if this process of differential survival continues once fish are released into open water bodies is important, as any further reductions in family diversity will ultimately lower N_e in the stocked population.

In order to examine if there was evidence for post–stocking family survival, the current study not only examined the familial composition of barramundi obtained from two hatchery cohorts destined for stocking, but went on to quantify the representation of these families in post–stocked fish recaptured from the Johnstone River. Like previous work with barramundi (Frost *et al.*, 2006; Wang *et al.*, 2008), we found that the hatchery cohorts were dominated by large numbers of half sibling families. Genetic contribution within each of these cohorts was skewed towards only a few parents. Nevertheless, at the time of stocking 17 half–sib families from Hatchery 1 and 18 half–sib families from Hatchery 2 were stocked into the Johnstone River.

Although sampling error associated with recapture of large numbers of tagged fingerlings from the broader population made it difficult to get any reasonable quantitative estimates on individual family survival over time, what was evident from the data was that most of the families initially stocked were persistent in the Johnstone River population up to two years after they were released. This suggests that the major selective pressures leading to differential family survival occur early on in the hatchery process and that by the time fish are large enough for stocking (~30 mm–100 mm), family survival has stabilised and that a large proportion of the genetic diversity captured within multi–family barramundi cohorts is subsequently retained and available to be incorporated into the wider genetic profile of the receiving population without further skewing variance in broodstock reproductive output. Consequently, adequate N_e in broodstock as outlined previously, coupled with the maintenance of within–cohort family genetic diversity in each of the numerous batches of fingerlings produced over the term of the Johnstone River restocking program, would have resulted in lowered opportunity for captive–reared juveniles genotypes to swamp genetic diversity of the resident population.

Ultimately, if the stocked fish are going to lead to a reduction in genetic diversity of the river population, the genotypes they carry have to be integrated into the genetic background of the receiving population (i.e. introgression needs to occur). Whether hatchery fish retain reproductive fitness and breed with wild populations is very contentious, with most examples of introgression reported in salmonids (i.e. Hansen *et al.*, 2000; Almodóvar *et al.*, 2001; Araki *et al.*, 2007).

One of the biggest problems in detecting evidence for introgression is predicting what the genotype of an introgressed fish within the broader population looks like. This difficulty is compounded when, as in our study, the genotypes of the fish that would directly breed with those in the wild population are not known. Nevertheless, if the genotypes of parental fish are available it is possible to simulate matings between brood fish and produce progeny genotypes. Likewise, using allele frequencies and assumptions of random mating under Hardy–Weinberg equilibrium, it is possible to simulate genotypes represented within the wild population. Subsequently, by undergoing a further round of simulation between these *in silico* progeny and wild fish genotypes, a set of *in silico* introgressed progeny with a distinct genetic signature can be produced. These can then be used through Bayesian Structure analyses as a benchmark to identify actual introgressed individuals. This type of methodology has been

successfully used to detect inter–specific fish hybrids (Schwartz and Beheregaray, 2008; Shaddick *et al.*, 2011).

Similarly, we used this approach in an attempt to identify whether any of the fish genotyped in the contemporary Johnstone River barramundi population were consistent with expectations of a mating between progeny of DPI&F broodstock and that of a wild fish. From the 841 wild barramundi genotyped from the Johnstone River in the current study, no evidence of an introgression genetic event was found. All fish genotyped had predominantly genome ancestry to wild genotypes (Figure 14 & Figure 15). Thus the proportion of the Johnstone River barramundi population that have some genetic origin to the particular DPI&F brood fish that simulations were based on is relatively low. This may mean that these captive–reared progeny are not commonly spawning with wild fish, or that the genetic contribution of this particular subset of fish to the total gene pool is relatively low and below that of our sampling capabilities.

Nevertheless, there are some caveats to our approach that limited the capacity for us to detect introgression events. First of all, the power of this type of analysis is highly dependent on the genotypes of the brood fish used to produce progeny groups. The restricted number of genetic samples of the DPI&F brood fish that we had access to meant that we could only simulate three spawnings. This is only a minor proportion of the 30+ spawnings undertaken over the 13 years DPI&F produced captive–reared fish for the program (DPI&F spawning records). Therefore, our analyses had no power to detect the presence of introgression between hatchery progeny originating from other broodstock used and wild fish. Secondly, detecting specific genotypes of barramundi among the broader genetic background of the river population is subject to considerable sampling effects. If introgressed fish represent only a small proportion of the wider barramundi population in the Johnstone River, then many thousands of fish may need to be captured and genotyped to detect a single individual. Such a massive sampling effort was beyond the resources of this project. As a result, no evidence for introgression was found, but we cannot rule out that captive–reared fish contribute to the broader barramundi gene pool in the Johnstone River. Further studies based on substantially larger sample sizes will be needed to address this issue.

In summary, genetic data indicate that the stocking of fingerlings from multiple broodstock over a sustained period has not significantly changed the background genetic profile of the Johnstone River barramundi population. Likely reasons for this are 1) that levels of augmentation have been too low to override the homogenising effects of gene flow from adjacent populations and 2) that, collectively, rotation of broodstock coupled with the mass spawning behaviour of barramundi has resulted in an acceptable high effective population size of fish being released into the river to prevent loss of genetic diversity and accumulation of inbreeding. To understand further just why stocking into this system has not resulted in genetic change, future studies should incorporate methods to estimate the magnitude of natural recruitment compared to stocking in the Johnstone River, as well as overall population abundance of the receiving wild population.

Supplementary Information A. Summary of microsatellite marker suites and associated primers indicating original source of primer sequences and relevant PCR conditions including final primer concentrations and fluorescent labels utilised in the present study.

* indicates primer concentrations which differ from source publication. [1]indicates markers with 1bp alleles due to likely indels, [2]indicates marker locus excluded from final analysis due to scoring reliability problems.

Locus GenBank Accession #	Source	Motif	Marker suite	Final primer concentration (μM)	Observed allele size range (bp)	Number of alleles detected	Primer Sequence (5′–3′) and fluorescent tag used
Lca03	(Yue *et al.*,	$(CA)_n$	G	0.1*	216- 239	4	**F:** TET-TCAAATCAGTTTGTGACACG
Lca08[1]	(Zhu *et al.*,	(GA)	P1	0.2	253-260	5	**F:** HEX-
Lca16[1]	(Yue *et al.*,	(CA)	G	0.2	255-283	6	**F:** FAM-ACAAGGGCTGCGCTCAGGTG
Lca20	(Zhu *et al.*,	(CA)	P1	0.8*	129-142	7	**F:** TET-TTGCCCACCCAAAGACC
Lca21	(Zhu *et al.*,	(CA)	P1	0.8*	183-195	6	**F:**HEX-GTGCCACCTGCCTGACC
Lca40	(Yue *et al.*,	$(GT)_n$	G	0.2	122-141	6	**F:**HEX-
Lca57	(Zhu *et al.*,	$(GT)_n$	G	0.2	207-222	8	**F:** HEX-
Lca58	(Zhu *et al.*,	$(GT)_n$	P1	0.24	402-459	15	**F:** HEX-
Lca64	(Zhu *et al.*,	(AC)	P1	0.2	279-313	17	**F:** FAM-
Lca69	(Zhu *et al.*,	$(GT)_n$	P1	1.28	356-361	3	**F:** FAM-
Lca70	(Zhu *et al.*,	(CAG	P1	0.64*	297-313	7	**F:** TET-
Lca74	(Zhu *et al.*,	(CA)	P1	1.28	163-170	4	**F:** FAM-
Lca98	(Zhu *et al.*,	$(TG)_n$	P1	1.2	189-213	8	**F:** TET-
Lca154	(Wang *et al.*,	$(TG)_n$	G	0.1*	144-154	5	**F:** TET-
Lca178	(Wang *et al.*,	(GA)	G	0.2	434-446	6	**F:** FAM-
Lca287[2]	(Wang *et al.*,	$(TC)_n$	G	0.2	169-213	14	**F:** FAM-
Lca371	(Wang *et al.*,	(CA)	G	0.2	375-387	5	**F:** TET-GGGCCGGTGATCAGAGACG

Supplementary Information B. Summary details of Hatchery 1 Barramundi families detected both pre-stocking and in CWT individuals recovered from the South Johnstone River (post–stocking). Assignments are based on 16 microsatellite loci.

			PRE-STOCKING ASSESSMENT			POST-STOCKING ASSESSMENT		
FAMILY	DAM ID	SIRE ID	COUNT STOCKED	Proportion STOCKED	% STOCKED	COUNT POST-STOCKING	Proportion POST-STOCKING	% POST-STOCKED
Family1	B14	B-01	9	0.101	10.1	1	0.014	1.4
Family2	B14	B-07	1	0.011	1.1	0	0.000	0.0
Family3	B14	B-08	2	0.022	2.2	0	0.000	0.0
Family4	B14	B-09	2	0.022	2.2	4	0.054	5.4
Family5	B14	B-10	6	0.067	6.7	12	0.162	16.2
Family6	B14	B-11	16	0.180	18.0	5	0.068	6.8
Family7	B14	B-12	1	0.011	1.1	0	0.000	0.0
Family8	B14	B-16	4	0.045	4.5	0	0.000	0.0
Family9	B14	B-17	0	0.000	0.0	1	0.014	1.4
	B14	B-21	10	0.112	11.2	7	0.095	9.5
	B14	B-22	6	0.067	6.7	6	0.081	8.1
	B14	B-23	1	0.011	1.1	0	0.000	0.0
	B14	B-24	4	0.045	4.5	1	0.014	1.4
	B14	B-25	4	0.045	4.5	4	0.054	5.4
	B14	B-26	8	0.090	9.0	16	0.216	21.6
	B14	B-28	1	0.011	1.1	3	0.041	4.1
	B14	B-29	14	0.157	15.7	14	0.189	18.9
		SUM	89	1	100	74	1	100

Supplementary Information C. Summary details of Hatchery 2 Barramundi families detected both pre–stocking and in CWT individuals recovered from the South Johnstone River (post–stocking). Assignments are based on 16 microsatellite loci.

			PRE-STOCKING ASSESSMENT			POST-STOCKING ASSESSMENT		
FAMIL	DAM	SIRE	COUNT STOCK	Proportion STOCKED	% STOCKED	COUNT POST-STOCKED	Proportion POST-STOCKED	% POST-STOCKED
Family	B-09/40	B-06/34	1	0.01	0.57	1	0.007	0.7
Family	B-09/40	B-27/39	1	0.01	0.57	0	0.000	0.0
Family	B-09/40	B-28/41	0	0.00	0.00	1	0.007	0.7
Family	B-11/44	B-02/38	5	0.03	2.87	12	0.086	8.6
Family	B-11/44	B-	6	0.03	3.45	2	0.014	1.4
Family	B-11/44	B-06/34	18	0.10	10.34	5	0.036	3.6
Family	B-11/44	B-25/33	6	0.03	3.45	8	0.057	5.7
Family	B-11/44	B-27/39	9	0.05	5.17	10	0.071	7.1
Family	B-11/44	B-28/41	25	0.14	14.37	18	0.129	12.9
Family	B-	B-02/38	3	0.02	1.72	2	0.014	1.4
Family	B-	B-	3	0.02	1.72	3	0.021	2.1
Family	B-	B-06/34	4	0.02	2.30	0	0.000	0.0
Family	B-	B-25/33	5	0.03	2.87	6	0.043	4.3
Family	B-	B-27/39	25	0.14	14.37	33	0.236	23.6
Family	B-	B-28/41	60	0.34	34.48	32	0.229	22.9
Family	B-	B-06/34	0	0.00	0.00	1	0.007	0.7
Family	B-	B-27/39	1	0.01	0.57	4	0.029	2.9
Family	B-	B-28/41	2	0.01	1.15	2	0.014	1.4
		SUM	174	1	100	140	1	100

Benefits

Benefits and beneficiaries

Currently, fish stocking around Australia delivers considerable social and economic benefits directly to recreational fishers. In addition, stocking has played an important role in the conservation of threatened species. Stocking programs have indirect downstream spin–offs to associated support industries that are dependent on the ongoing prosperity of the fishing industry. Rural and regional communities in particular have derived considerable benefits from the establishment of recreational fisheries in inland waters. This is particularly true in Queensland, where barramundi stocking has created new fisheries in impoundments around the state to the benefit of local communities.

If these benefits are to continue, then key data deficiencies related to fish stocking sustainability issues need to be addressed. This current program has investigated some of the major concerns of stakeholders that relate to barramundi fish stocking, particularly in the Queensland Wet Tropics Bioregion. This research will provide fisheries managers with some of the tools they need to apply world's best practice to future *L. calcarifer* stocking activities, thus promoting an ecologically sustainable fishery. Policies and guidelines developed from this research will directly benefit fish stocking groups, natural resource management groups, fisheries managers and conservation agencies. However, the ultimate beneficiaries from the application of this research will be the end users, *i.e.* the fishing industry, which may benefit through the development of protocols and guidelines that will protect stocks through sustainable fish stocking activities.

Parts of this research may potentially be applicable to other fisheries in other jurisdictions, and the methodologies developed can benefit other researchers planning similar research.

Most of the benefits and beneficiaries listed above were identified in the original application.

Further development

Whilst this current project has answered some of the major questions regarding barramundi fish stocking, there is a wide range of other activities that could be undertaken to further build on the outcomes and outputs of this project. Future research questions that could be addressed include:

- Are the results of this project applicable to other species or in other areas where there are different climatic and hydrological conditions and dissimilar land uses and catchment morphologies?
- Is stocking affecting predator/prey balances?
- How to measure and maintain species–specific N_e (effective population size)?
- How to estimate optimum stocking density and/or carrying capacity?
- How to incorporate physical and environmental parameters such as water level fluctuations (richness, relative abundance) when determining carrying capacity
- The need for modelling capacity to predict the effectiveness/impacts of stocking new species?
- Do stocked fish displace rather than enhance natural populations?
- Does stocking overcompensate for natural recruitment?
- What should post–stocking surveys do and can they be standardised across state borders?

While this current study addresses many of the environmental and genetic issues surrounding barramundi stocking, similar studies may be needed in the future for other Australian stocked species including Australian bass, golden perch and sooty grunter.

The results of this current project have already been disseminated through presentations to international forums including the 4th International Symposium on Stock Enhancement and Sea Ranching and at Austasia Aquaculture 2012. In addition, a stakeholder implementation workshop attended by a range of industry, fisheries and natural resources managers and stocking groups, as well as the project steering committee, was held in May 2012. At this workshop, the results of the current project as well as their implications for future stocking activities were discussed in detail. Articles on the genetic and ecological impacts of fish stocking are being prepared for publication in scientific and technical journals.

The Queensland Department of Agriculture, Fisheries and Forestry is currently developing a discussion document for fish stocking in Queensland, including topics such as hatchery practices and stocking protocols. This document will ultimately assist in the development of an updated policy for Freshwater Fishing in Queensland.

After the completion of the project, genetics data and samples will be stored at the James Cook University's Centre for Sustainable Fisheries and Aquaculture in Townsville. Information on tag recaptures will continue to be maintained and managed as part of the Suntag recreational fisheries database.

Planned outcomes

This project has delivered the principal planned outcome by 'providing fisheries managers with the quantitative data needed to assess the ecological and genetic impacts of barramundi stocking'. Specific outputs that have contributed to this key outcome are:

- an expanded knowledge of the movements of stocked fish in the freshwater reaches of tropical coastal streams and impoundments through the implementation of a series of planned releases of *L. calcarifer* at strategic locations. In both the Johnstone River and in Tinaroo Falls Dam, stocked *L. calcarifer* show little inclination to move into small, often fast–flowing tributary streams that drain relatively pristine forests in the surrounding catchment. This suggests that the stocked *L. calcarifer* are unlikely to encounter many of the species (e.g. rare amphibians) that are of conservation concern.
- In support of this, the diet of *L. calcarifer* in both stocking locations was opportunistic and directly related to the abundance of prey species in the habitats they occupied. No evidence was found that the *L. calcarifer* sampled in this study either in the Johnstone River or in Tinaroo Falls Dam preyed on species of conservation concern.
- The studies of the stocked *L. calcarifer* indicated an apparent poor post–stocking survival of fish released into the relatively unproductive, fast–flowing waters of the South Johnstone River. This result confirms previous work suggesting a preference for slower, more productive water bodies. During this study, in the Johnstone River the highest densities of stocked and juvenile wild fish were in the same areas where there was an abundance of suitable habitat and prey species. Similarly, in Tinaroo Falls Dam, stocked *L. calcarifer* were not sampled in the small and/or fast–flowing tributary streams, but rather in the main body of the dam where there was adequate cover and plentiful prey species.

- Whilst the potential for impacting on other species of conservation concern should be foremost in consideration of stocking sites, managers should not exclude stocking locations on unsubstantiated presumptions that these fish will move upstream and impact on unspecified amphibians and fish. However, stocking should not be undertaken upstream of natural barriers where there are known rare, threatened or endangered species. An example of these circumstances is the vulnerable Bloomfield River cod (*Guyu wujalwujalensis*) whose distribution is entirely limited to the upper Bloomfield River.

- The lack of evidence of significant changes in either genetic diversity, inbreeding levels or evidence of F1 introgression would suggest that the historic stocking densities that have been used in the Johnstone River (~ 281 000 between 1993 and 2005) is below the threshold that is likely to cause problems. While these data should not prevent the development of sustainable hatchery protocols, they could potentially be used by managers to develop guidelines for issuing stocking permits to community groups seeking permission to stock other similar Wet Tropics Bioregion streams that support conspecific wild stocks.

Project results have been communicated widely including through:

- an implementation workshop to disseminate the results of the project to stakeholders held in May 2012 in Townsville. Those participating in the workshop included representatives from the fishing industry and community fish stocking groups, fisheries and natural resource managers, Suntag, geneticists and research scientists.

- articles in scientific journals, popular fishing magazines, newspapers and industry newsletters.

Conclusions

This study is the first comprehensive work undertaken on the potential long–term genetic and ecological impacts of barramundi stocking in coastal rivers and impoundments in northern Australia. Prior to this study being undertaken, the benefits of stocking activities to local communities and to the fishing industry were well known, but there was only speculation as to any potential negative impacts on recipient ecosystems. Most of this speculation was based on the results of overseas studies (generally on salmonids) that may or may not have been particularly relevant in an Australian context.

An earlier FRDC project (2007/057) identified the major management concerns related to fish stocking in Australia. During the study, the views of a wide range of stakeholders including fisheries and conservation managers, community groups and industry, were canvassed. Concerns included:

- Does leakage of stocked fish into sensitive environmental habitats occur?
- Do stocked fish threaten vulnerable species?
- Have historic stocking programs caused changes in the genetic population structure of wild stocks?
- Are there other ecological impacts including those on survival and growth and displacement of wild fish populations?
- Is stocking density of barramundi in rivers and impoundments at sustainable and/or optimal levels?

The first four of these concerns were addressed in the objectives of the current project.

Objective 1 of the current study (assess movements and ecological impacts of stocked *L. calcarifer* in a model river and impoundment) addressed many of these stakeholder concerns. In coastal rivers, stocked juvenile barramundi showed little propensity to move into smaller tributary streams or into areas where wild fish weren't normally found. This included tributary streams of the Johnstone River that drained the pristine forest locations in the adjacent Wet Tropics World Heritage Area and was in spite of stocked fish being released directly into, or adjacent to, such streams. Wild fish were found in the same locations as stocked fish as well as at other sites within the coastal reaches of the Johnstone River system. Stocked barramundi in Tinaroo Falls Dam showed similar movement patterns with none found to move into upstream tributary streams even though fish were released at locations that facilitated their movement into such areas.

The limited movements of stocked *L. calcarifer* into these smaller rainforest streams would suggest a low predation rate on some of the amphibians and other rare species that are endemic to that habitat. This is supported by the dietary studies of *L. calcarifer* in both the river and impoundment study areas that showed that *L. calcarifer* is an opportunistic predator, consuming a wide range of different prey items including even invasive fish species such as tilapia. In the Johnstone River, there was little evidence that stocked and wild juvenile *L. calcarifer* were having any substantial effects on each other, with only one instance of cannibalism observed and both groups having similar condition factors and growth rates. Similarly, in Tinaroo Falls Dam, cannibalism was not observed during this current study and the condition factor of the stocked *L. calcarifer* was similar to that observed in both wild and stocked fish in the Johnstone River. The growth rate of stocked *L. calcarifer* was faster in Tinaroo Falls Dam than that observed for either stocked or wild fish in the Johnstone River, probably reflecting an abundance of prey species and suitable refuge habitat.

Under the current stocking strategies and densities, it is likely that stocked (and wild) *L. calcarifer* are having minimal impact on species of conservation concern in the Johnstone River and Tinaroo Falls Dam catchments. While each case needs individual assessment, this is likely to hold true in other catchments in the Queensland Wet Tropics Bioregion with similar hydrology patterns, catchment morphologies and land uses. Translocation of fish outside their natural range is not permitted in Queensland and stocked fish should not be introduced into streams, for example above natural barriers, where they are likely to become novel predators affecting vulnerable, threatened or restricted species that may not have developed anti–predator mechanisms. An example of this is the Bloomfield River cod (*Guyu wujalwujalensis*), which has a distribution restricted to the upper catchment of the Bloomfield River, above Bloomfield River falls, in the Queensland Wet Tropics Bioregion. The introduction of a novel predator such as *L. calcarifer* has the potential to severely impact on this species' population.

Objective 2 of this study (determine if barramundi stocking had any discernable adverse genetic impacts on wild populations in a previously stocked river system) addressed the concern of managers and other stakeholders that stocking would have deleterious genetic effects on wild stocks in the recipient ecosystem. Despite *L. calcarifer* stocking being undertaken in the Johnstone River more or less continuously since 1993, no evidence was found during genetic analyses that any notable changes in genetic diversity of wild stocks or increased inbreeding levels have occurred in this population. Furthermore, there was also no evidence of introgression of original F1 broodstock genes into the wild population. While these results suggest that there is no evidence of genetic damage to Queensland barramundi stocks as a result of past fish stocking activities, there is nevertheless a need to develop and implement appropriate hatchery protocols to ensure the future viability of commercial and recreational fisheries in Queensland.

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Appendix 1

Intellectual property

The results of this project will be published, widely disseminated and promoted and therefore available in the public domain. No patentable inventions or processes have been developed during this project.

Appendix 2

Project staff

Name	Position	Institution
John Russell	Principal Investigator, DAFF	DAFF
Dean Jerry	Associate Professor, James Cook University	JCU
Fiona Thomson	Fisheries Biologist, DAFF	DAFF
Paul Thuesen	Fisheries Biologist, DAFF	DAFF
Trent Power	Fisheries Technician, DAFF	DAFF
Carolyn Smith-Keune	Geneticist, James Cook University	JCU
Richard Saunders	Fisheries Biologist, DAFF	DAFF

Appendix 3

Movements of stocked barramundi (Lates calcarifer) in Australia: a desktop study[1]

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Introduction

In Queensland, barramundi (*Lates calcarifer*) are now widely stocked into impoundments to improve recreational fishing opportunities, but there have also been stockings into rivers and estuaries for stock enhancement purposes (McKinnon and Cooper, 1987; Russell *et al.*, 2002; Russell and Rimmer, 2004; Russell *et al.*, 2004; Rutledge *et al.*, 1990). As barramundi need access to high salinity waters to complete their breeding cycle, impoundments can only support 'put and take' recreational fisheries. Despite this, impoundment stocking, which commenced in Queensland in the mid–1980s, has proved to be highly successful and popular (McKinnon and Cooper, 1987). Outside Queensland, there have been only limited stockings of barramundi into an impoundment in the Northern Territory (Russell *et al.*, 2004). Estuarine and river stockings are much more contentious because of possible adverse impacts on the genetics of the wild fisheries and on the environment (Gillanders *et al.*, 2006). In the Wet Tropics, Burrows (2002) raised concerns that stocking of novel predators outside their natural range could place at risk significant amphibian and crustacean communities.

Despite the popularity and economic value of barramundi to the Queensland economy (Rutledge *et al.*, 1990), there are few published studies documenting the post-stocking movements of barramundi released into impoundments and estuaries. These studies have been mostly dependent upon either tag–recapture information recorded in the Suntag recreational fishing database (see www.info-fish.net), or on a series of studies done by the Queensland Department of Employment, Economic Development and Innovation (DEEDI) over the period 1993 to 2005 when nearly 287 000 microtagged stocked barramundi were released into the Johnstone River in north Queensland. Much of the recapture data from this latter study are also included in the Suntag database.

[1] This document was prepared earlier as a project milestone and was submitted to FRDC in July 2010.

Understanding the movements of barramundi stocked into impoundments and rivers, particularly the potential for leakage into areas of environmental significance where they would not be found naturally and also inter–riverine movements, is an important prerequisite if fish stocking programs are to have maximum social and economic benefit, but minimum environmental impact. This current study aims to provide a concise synopsis of the available publications on the movements of stocked barramundi in open and closed systems in Australia. Further, the information contained in these publications is supplemented with more recent data (2001–2010) on movements of stocked barramundi extracted from the Suntag and DEEDI databases.

Methods

A literature search was undertaken to locate existing published information on the movements of stocked barramundi in Australia. Additional data was also sourced from the Suntag recreational fish tagging database and from previous DEEDI research programs. Other sources of data, for example observations of untagged barramundi in locations where wild fish would not be expected to be found, were not included in the analyses because of the uncertainty of their origin.

All recapture data on barramundi stocked between 2001 and 2010 that had moved 5 km or more from their original release location were sourced from the Suntag database. These included both fish that were tagged prior to stocking and stocked fish that were subsequently caught, tagged and then released by recreational fishers. Recaptures of fish in the Suntag database were made by recreational fishers (and some from commercial fishers and research organisations) who were requested to collect these data in accordance with the recapture protocol on the Infofish website (see www.info-fish.net). To help eliminate possible errors associated with the size of the reporting grid or point location, only recaptures that had moved 5 km or more from the stocking or release location were included in the analyses. Data recorded included number of days at liberty, growth (mm), direction of movement (upstream, downstream, coastal) and type of system (closed or open) where the original release and subsequent recapture took place (i.e. an impoundment versus riverine environment). Analyses were conducted of the movements of stocked fish resident in easterly flowing catchments from five regional areas of Queensland. These were the Wet Tropics of the north–eastern coast, Townsville, the Burdekin and Fitzroy River catchments and Gladstone. Because of the small numbers involved, data on fish from the Gulf of Carpentaria and the Mackay regions were ignored in this study.

An analysis was also made of data on the movements of stocked fish collected by DEEDI as part of its Johnstone River microtagging research programs between 1993 and 2005. Much of these data were also included in the Sunfish database.

Data retrieved from both of these sources suffer from a number of limitations including lack of definition of fine scale movements, particularly into smaller tributary streams. There is often a reluctance or inability on the part of the recreational and commercial fishers who recapture tagged fish to report the exact recapture location. Furthermore, because of selected targeting, it is more likely that recreational and commercial fishers caught larger fish approaching or beyond the minimum legal size (580 mm total length). Despite these limitations, for larger scale movements including inter–riverine or coastal movements, these data are extremely useful.

Results and discussion

Recaptures of stocked fish from the Suntag database

Between 2001 and 2010, analyses found 389 stocked barramundi that had moved 5 km or more were recaptured between Gladstone and the Wet Tropics of North Queensland, and in the Gulf of Carpentaria (Figure 16). Of these regions, the Wet Tropics, followed by Townsville and the Fitzroy River localities had the highest number of recaptures (Figure 16). The Wet Tropics recaptures include microtagged fish stocked as part of the DEEDI research program previously mentioned. Out of the total number of fish recaptured, 230 were stocked into open systems (i.e. rivers and inlets), while 159 were stocked into closed systems (impoundments). The low number of recaptures from impoundments may simply reflect a lower fishing effort in many of these systems (e.g. Lake Dalrymple) than occurs in open systems (B. Sawynok, pers comm.).

Other possible contributing factors include 1) most of the movements within impoundments, because of their confined nature, were less than 5 km and therefore automatically excluded from the dataset and 2) there is now a larger number of identifiable stocked fish in open river systems because many community stocking groups are now routinely tagging barramundi prior to their release. Sawynok (2009) notes that, depending on area, there can be some considerable variations from the overall recapture rate of 7% for barramundi. For example, in the Fitzroy River estuary he documented a recapture rate of 19.5%.

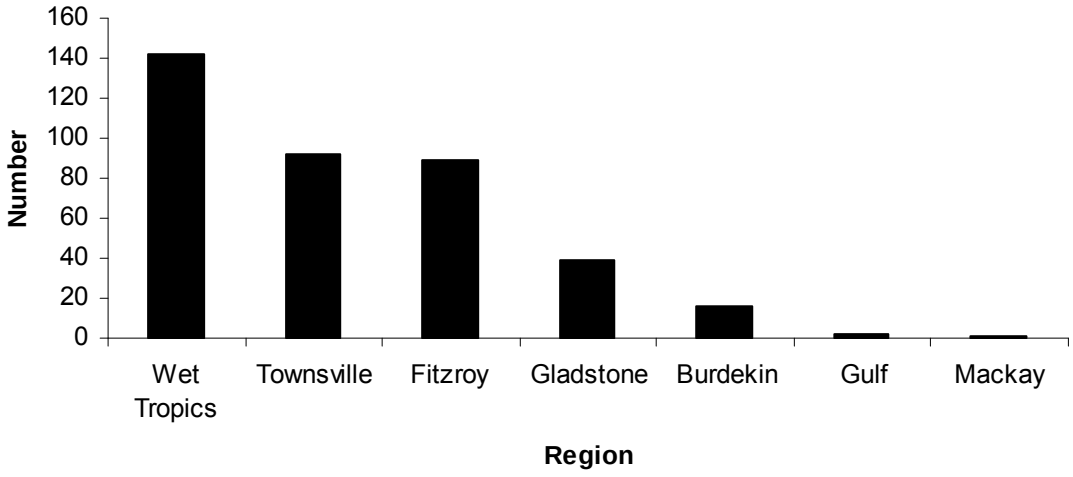

Figure 16. Frequency of recaptured stocked barramundi movements by region in Queensland.

Movement trends

For distances of 5 km or greater, the ratio of upstream to downstream movements for stocked barramundi from all regions pooled was 0.22:1. The majority of stocked fish that moved downstream and out of impoundments probably traversed a weir or dam wall although there may be some situations where extensive flooding created alternate routes around these stream barriers. Some of these fish moved considerable distances downstream into estuarine and coastal areas or even into other rivers (n= 121). Very few fish (n = 36) made movements of 5 km or more within an impoundment and only one fish moved upstream into an impoundment

(Figure 17). This latter movement occurred in the Ross River (Aplin Weir to Gleeson Weir). The majority of fish were recaptured in the same river systems where they were released.

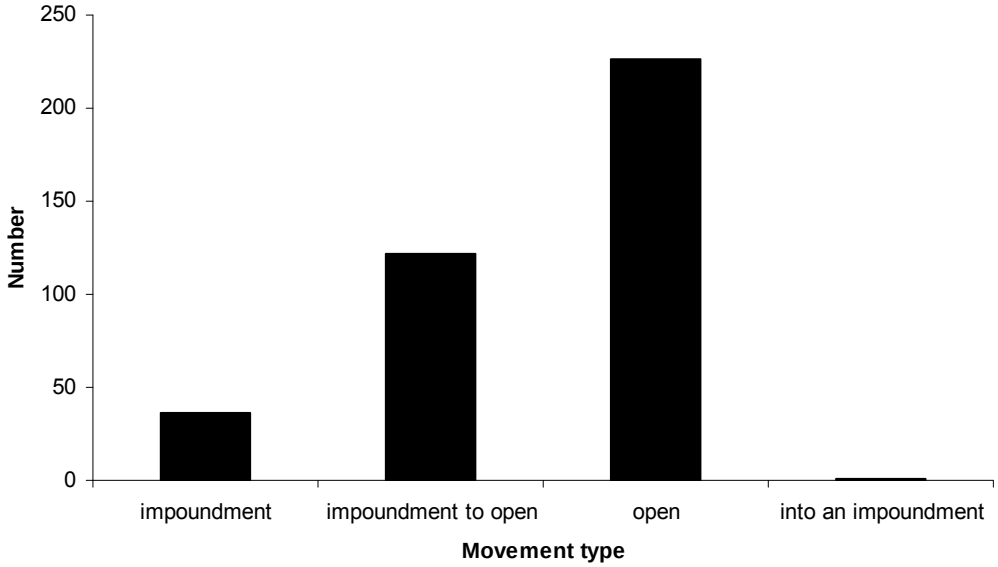

Figure 17. Types of movement by stocked barramundi in Queensland.

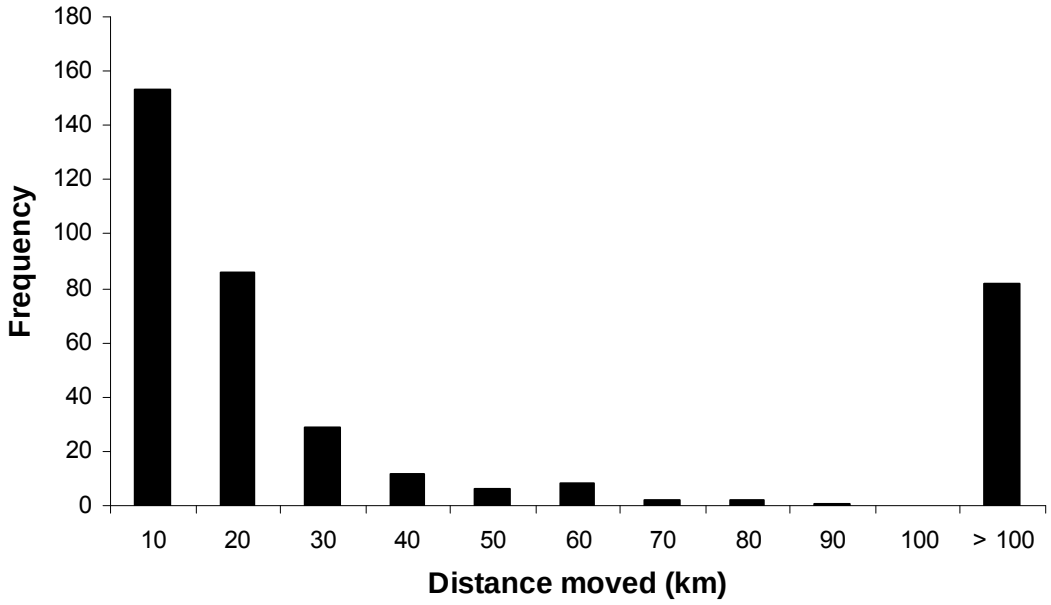

Figure 18. Frequency of stocked barramundi movements within Queensland. Fish moving < 5 km are not included.

The distances moved downstream by stocked fish are shown in Figure 18. The majority had moved between 5 and 20 km from their release locations, but the fish that had made very large movements (>100 km) were generally those that were stocked into impoundments in

either the upper Fitzroy or Burdekin River catchments and had subsequently moved downstream. These downstream movements were most likely associated with a general migration to estuarine and coastal spawning grounds (Davis, 1986; Dunstan, 1958; Moore and Reynolds, 1982), and are associated with flooding and high flows (Sawynok and Platten, 2009). Fish that were released into impoundments on shorter coastal streams generally needed to move fewer kilometres to downstream spawning areas than those released into impoundments on the larger river systems (i.e. Fitzroy and Burdekin systems) (Figure 19).

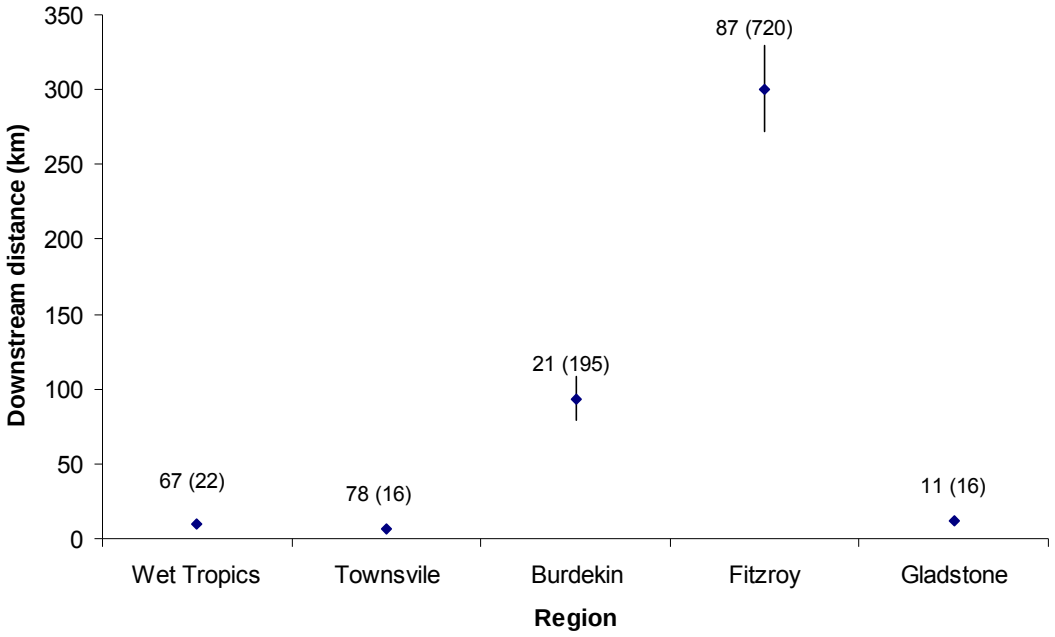

Figure 19. Mean distance (± S.E.) travelled by stocked barramundi in eastern Queensland that moved downstream from point of stocking or tagging.

Sample sizes are given above each data point along with the maximum distance moved in parentheses.

Movements of stocked fish within impoundments were generally limited by the size of the impoundment and were relatively small. Only small numbers of stocked fish were recaptured upstream from where they were either originally stocked or tagged (Figure 20). As a general trend, the larger the river and the more diverse the freshwater stocking sites, the larger the movements that occurred (i.e. the Burdekin River, see page 97). However, with tagged wild fish in the Fitzroy River estuary, juveniles (up to 580 mm TL) predominantly moved upstream in flow events, while adult fish primarily moved downstream (Sawynok and Platten, 2009).

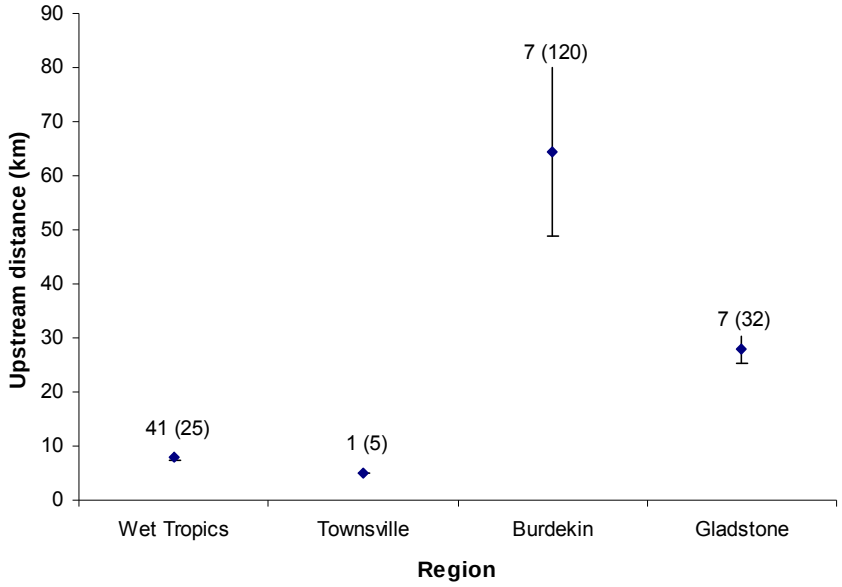

Figure 20. Mean distance (± S.E.) of stocked barramundi in north Queensland that travelled upstream from point of stocking or tagging.

Sample sizes are given above each data point along with the maximum distance moved in parentheses.

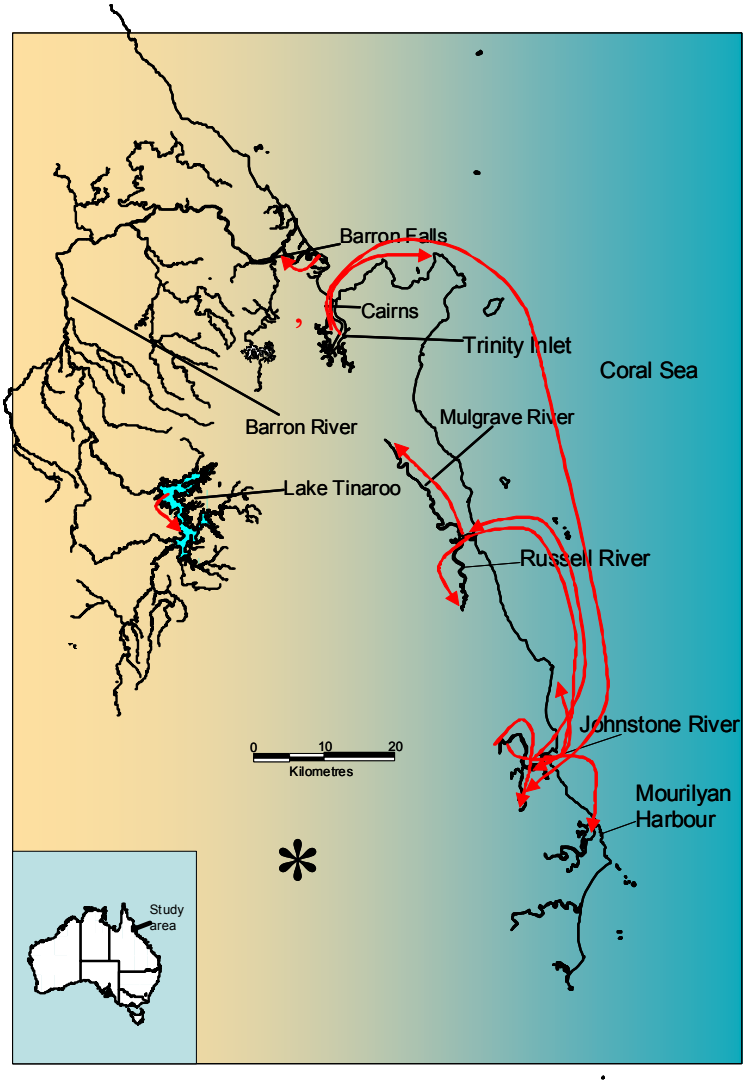

Figure 21. Wet tropics watercourses. Red arrows show examples of fish movements.

Movements by region

Wet Tropics

Since 2002, local community fish stocking groups have released many thousands of tagged barramundi into coastal waterways in the Wet Tropics including the Mulgrave, Russell and Barron Rivers and into Trinity Inlet and associated feeder streams (Sawynok and Pearce, 2006) (Figure 21). These community fish stocking groups are increasingly purchasing larger fish (> 200 mm total length) in order to mark the fish with conventional dart or anchor tags before they are released. In addition, DEEDI routinely released juvenile barramundi marked with CWTs into the Johnstone River near Innisfail. Between 1993 and 2005, over 287 000 fish were released into this river system, with most recaptures occurring within 3 km of their original release sites (Russell and Rimmer, 1997). While this stocking research program was not intentionally designed to determine fine scale movements of barramundi (for example, movements from the main river into smaller feeder creeks), it produced evidence of intra–riverine movements of up to 37 km (Russell and Rimmer, 1997). Later tag returns showed that small numbers of stocked fish had moved along the coast and into adjacent watercourses to the north and south of the Johnstone River. For example, some fish moved distances of up

to 38 km along the coast into the Russell River to the north and south into Mourilyan Harbour (Figure 21).

The highest number of recaptures (n = 145) of stocked fish in this Suntag database subset was recorded in the Wet Tropics region. This included at least seven inter–riverine movements; for example, one fish traveled 105 km from Trinity Inlet, south along the coast into the South Johnstone River (Figure 21). The Wet Tropics region had a relatively high number of upstream movements (n = 41). These included upstream movements in the Mulgrave, Russell and Barron Rivers, with the two largest movements (20 km and 13 km respectively) occurring in the Mulgrave River. About half of all the upstream movements recorded in the Wet Tropics region were from Tinaroo Dam rather than in rivers, creeks and other watercourses. None of the recaptures from stocked fish released into Tinaroo Dam moved out of the dam, either upstream into tributary streams or downstream over the spillway into the Barron River. It is possible that barramundi, particularly juveniles, may occasionally move into upstream areas, but remain undetected because the recreational fishing sector almost exclusively target the main body of the dam rather than associated tributary watercourses. The Tinaroo Fish Stocking Group now routinely installs a spillway net barrier prior to the dam overtopping to prevent stocked barramundi from being washed over the wall.

An earlier report analysing tag–recapture data from the Suntag database also found that stocked barramundi in Tinaroo Dam only moved within the impoundment (Sawynok and Pearce, 2007). These authors found no evidence of fish moving upstream into any of the tributary streams or surviving the drop over the spillway to move into the downstream reaches of the Barron River.

Townsville

As with fish stocking activities in the Wet Tropics region, community groups working under DEEDI permits were responsible for most of the releases of hatchery–reared barramundi in the Townsville region. These fish form the backbone of a recreational fishery in the weirs of the Ross River, with many fish subsequently tagged and released as part of the Suntag program.

The general direction of movement of stocked fish in the weirs of the Ross River (Figure 22) appears to be downstream. In an earlier study, Sawynok and Platten (2009) noted that 131 of 3145 barramundi tagged in the Ross River weir pools had moved away from their original release location. They found that 9.9% of all recaptured fish had shifted into a downstream weir pool and 21.7% had moved into the tidal reaches of the river or into river systems and coastal areas to the north or south of the Ross River. One fish had moved a net distance of 130 km south into Groper Creek near the mouth of the Burdekin River, while another had moved to a beach to the north of Townsville (Sawynok, 2004). Sawynok and Platten (2009) suggest that in most years there are sufficient seasonal flows in the Ross River to give fish resident in the weir pools the opportunity to move into downstream habitats.

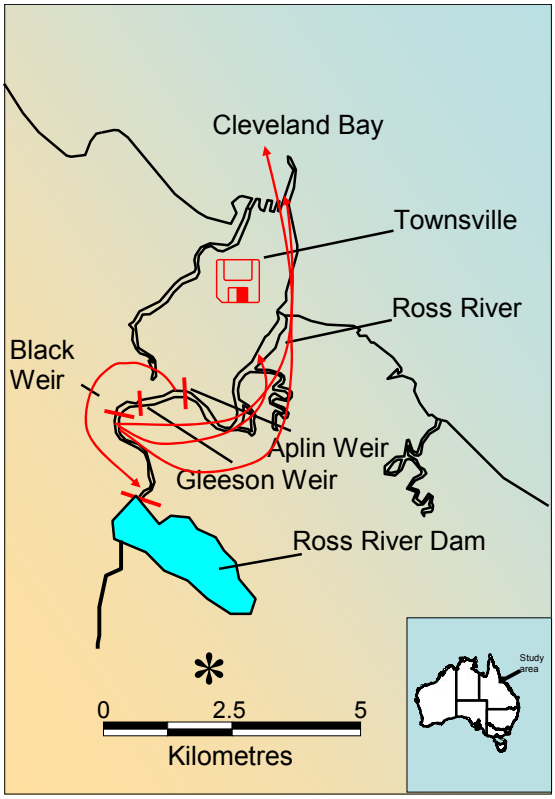

Figure 22. Sites in the Townsville area. Red bars show weirs and impoundments and red arrows show selected fish movements.

There were 79 recaptures recorded in the Sunfish database for fish tagged in the freshwater reaches of the Ross River between 2001 and 2010 that had moved 5 km or more from their release location. Most of the fish in this dataset were released in the Black Weir (n = 76) with the remainder (n = 3) tagged downstream in Aplin Weir. Between 2001 and 2010, all recaptures but one occurred downstream from the original stocking location, with 78 of these individuals recorded as moving over at least one of the Ross River weirs. A single fish moved 5 km upstream from its release location in Aplin Weir into Gleeson Weir, presumably when the latter weir was submerged during wet season flooding. With the exception of one fish which was caught outside the river mouth in Cleveland Bay, all of the recaptured fish were caught in the tidally influenced reaches of the Ross River. The maximum distance travelled was 16 km (Black Weir downstream to the Ross River), while the mean distance moved (± SE) was 6.51 ± 0.30 km.

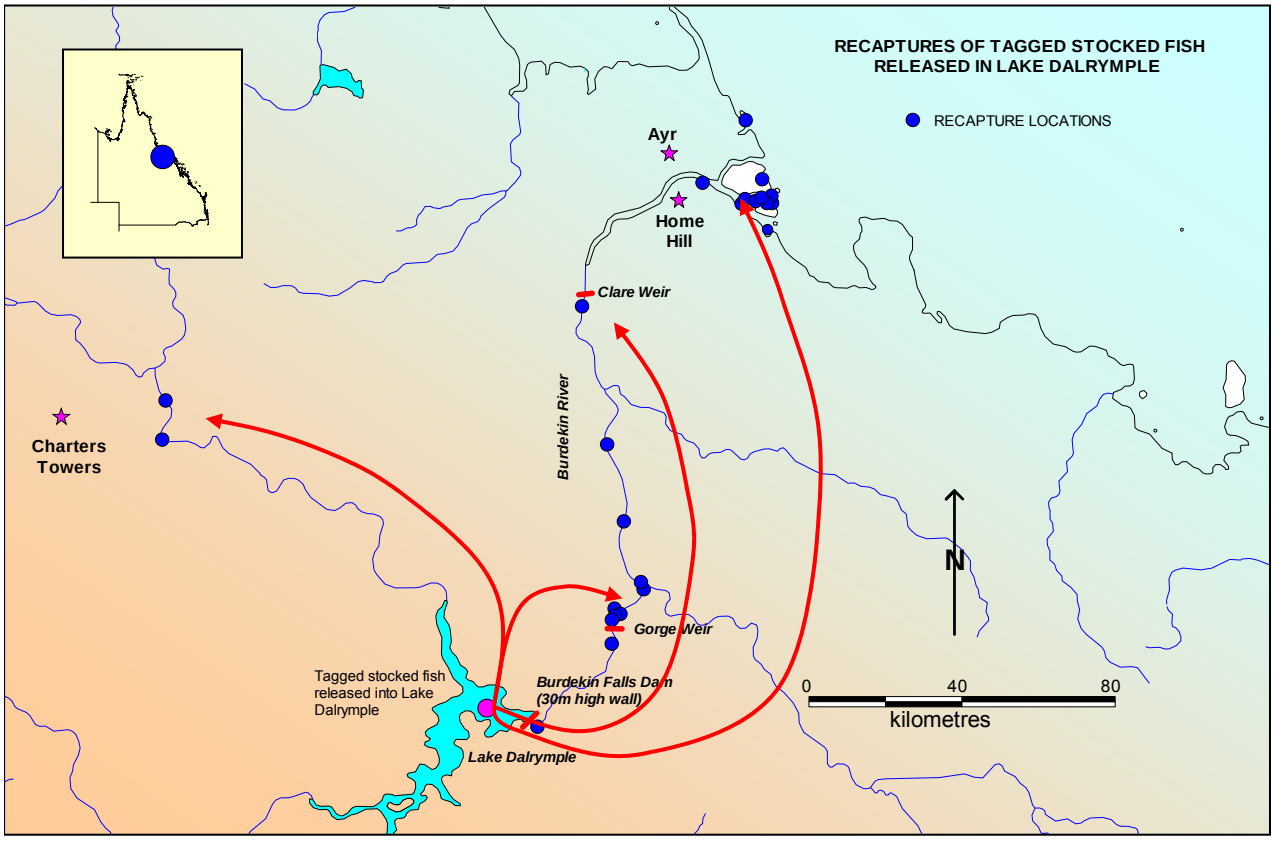

Figure 23. Movement trends of tagged barramundi stocked into Lake Dalrymple on the Burdekin River.

(Map courtesy of Bill Sawynok, Infofish). Red bars show weirs and impoundments and red arrows show selected fish movements.

Burdekin

The Burdekin River basin has an area of about 130 000 km^2 and contains nine surface water storages, the largest of which is Lake Dalrymple (1860 GL storage capacity) (http://adl.brs.gov.au/water2010/pdf/catchment_120_0_summary.pdf). There were two major release locations for tagged stocked fish in the Burdekin catchment, the Clare Weir and Lake Dalrymple (Figure 23). Between 2001 and 2010 there were 28 recaptured barramundi recorded in the Suntag database that had moved 5 km or more. Of these, 17 were originally stocked in Lake Dalrymple, with the remainder released into the Clare Weir. The majority of fish (n = 21) made downstream movements, with the mean distance travelled (± SE) of 93.57 ± 14.9 km. Sixteen fish (all stocked in October 2007) were recorded as surviving the 37 m drop over the dam wall of Lake Dalrymple and moving into the lower Burdekin River or along the coast (Figure 23). There was complete information on growth and period at liberty for 15 of these fish in the Suntag database. The average lengths (± SE) at which these fish were stocked into Lake Dalrymple and then subsequently recaptured downstream was 282.7 ± 1.8 mm and 756.7 ± 15.9 mm respectively. Most recaptures were from the commercial fishery, which is restricted to operating in estuarine and coastal areas. The average time at liberty (± SE) for all recaptures was 2.12 ± 0.07 years, but as the dam had overflowed every year since construction (Lewis *et al.*, 2009) it is difficult to estimate the residency time of the stocked barramundi in Lake Dalrymple. It is probable that, given the extended average period at liberty, for most of this time these fish remained either in Lake Dalrymple or downstream in the freshwater reaches of the Burdekin River.

Seven stocked barramundi made upstream movements in the Burdekin River including two fish released into Lake Dalrymple that moved up to 120 km upstream (Figure 23). These tagged fish were at liberty for 765 and 671 days and grew from 280 mm to 750 mm and 780 mm respectively. Five fish stocked into the Clare Weir also made upstream movements of between 13 km and 65 km.

Fitzroy

With a catchment area of nearly 150 000 km2, the Fitzroy River system is the second largest in Australia. Within the catchment there are 24 surface water storages (http://adl.brs.gov.au/water2010/pdf/catchment_130_0_summary.pdf), a number of which are stocked with hatchery–reared barramundi by community fish stocking groups (Figure 24).

The river is straddled by a tidal barrage near the city of Rockhampton and downstream there is an active commercial gill net barramundi fishery. Many of the tag recaptures come from this estuarine commercial fishery and Milton *et al.*, (2008) highlight the importance of maintaining minimum flows in the system to allow for barramundi stocked in upstream barrages to migrate to the lower catchment. Overall recapture rate for barramundi in this system is generally 8.2%, however it is as high as 20.1% in the Fitzroy River estuary (Sawynok, 2007; Sawynok and Platten, 2009).

Figure 24. Movement trends of tagged barramundi stocked into the Fitzroy River system.

(Map courtesy of Bill Sawynok, Infofish).

Given the size of the catchment, the level of fishing activity and the number of remote, upstream stocking locations, it is not surprising that there are a number of large net movements of stocked barramundi recorded in the Suntag database for this area. In the Fitzroy River, there were 83 recaptures of stocked barramundi between 2002 and 2009 that had moved 5 km or more from their original release location. Of these, 28 involved movements over dam walls or river barrages with fish travelling a mean (± SE) distance of 543 ± 53 km. For example, a fish moving downstream to the estuary from the Moura Weir (Figure 24) would need to negotiate five stream barriers (Sawynok and Platten, 2009). The largest net movements recorded over all the regions were from this system, with 23 individuals travelling at least 700 km before being recaptured. No upstream movements were detected in the region during the study period. Sawynok and Platten (2009) established a relationship between stream flow events and downstream movements of stocked fish from various parts of the catchment.

Gladstone

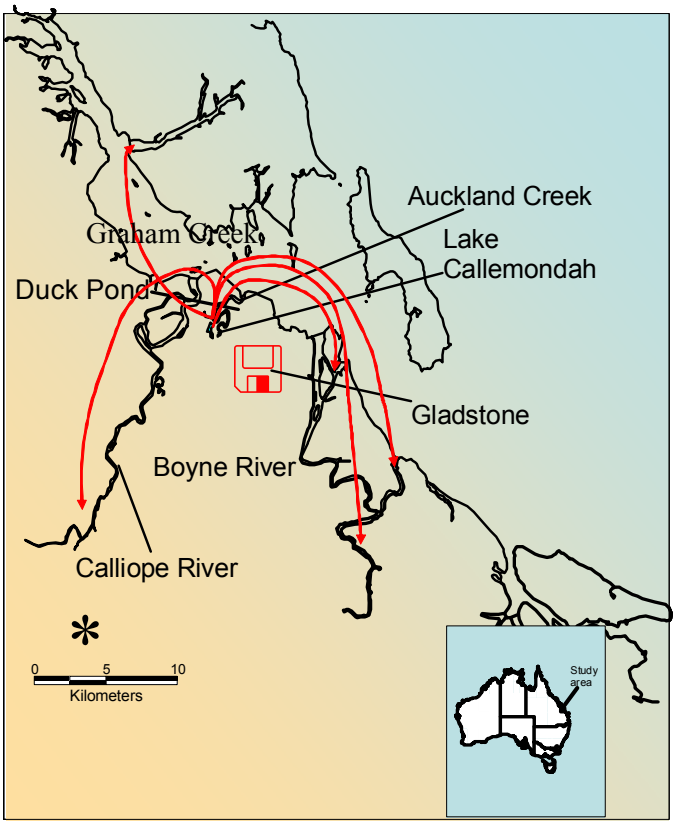

Figure 25. Movement trends of barramundi stocked into Lake Callemondah and the Duck Pond. Red arrows show examples of fish movements.

Lake Callemondah is a small freshwater impoundment situated on Auckland Creek in the urban environs of Gladstone in Central Queensland (Figure 25). The lake is fed by stormwaters flowing from the western residential suburbs of Gladstone, and is separated from

the tidally influenced Auckland Creek by a low concrete and rock wall (Sawynok and Platten, 2007, 2009).

Between 2001 and 2010, there were 39 recaptures in the Suntag database of barramundi that had moved 5 km or more. These recaptures were from fish that had either been stocked into Lake Callemondah (n = 33) or the nearby Duck Pond (n = 6) and occurred in nearby watercourses (i.e. the Calliope and Boyne Rivers, Auckland, Keppel, Graham and Pacific Creeks and South Trees Inlet) (Figure 25). The majority of movements out of these impoundments were downstream and into coastal habitats (n = 21). Seven barramundi made upstream movements into the Calliope and other adjacent coastal streams.

Analyses of the Suntag database by Sawynok and Platten (2009) noted that 59 (57.3%) of the 103 recaptures of stocked fish tagged in Lake Callemondah came from outside of the Lake. These fish had moved into adjacent marine and estuarine habitats, or had subsequently made upstream movements in the adjacent Calliope and Boyne Rivers (Figure 25). Sawynok and Platten (2009) found that one fish had moved some 36 km, first downstream and then along the coast before moving up into the Boyne River. These authors suggest that the egress of stocked barramundi from Lake Callemondah is closely related to high flow events such as occurred in February 2003 and February 2008.

Table 7. Mean total lengths (± S.E.) at release and recapture, growth and period at liberty for stocked fish from the Suntag database.

	Wet Tropics	*Townsville*	*Burdekin*	*Fitzroy*	*Gladstone*
Length at tagging (mm)	298.0 (2.8)	309.4 (4.1)	255.3 (7.0)	359.0 (18.6)	475.9 (18.5)
Recapture Length (mm)	536.5 (14.2)	625.1 (16.2)	738.4 (26.8)	769.4 (14.3)	767.6 (18.7)
Days out	700.8 (43.6)	597.0 (27.2)	483.0 (27.4)	886.3 (30.0)	925.4 (79.8)
Growth (mm)	238.5 (14.1)	315.7 (15.9)	793.0 (57.8)	371.2 (23.9)	300.1 (21.6)

Growth of stocked fish

Figure 26 shows the growth of all recaptured stocked fish from all regions. A number of fish from the Wet Tropics and Gladstone area appear to have grown relatively slowly compared to the majority of fish from other regions. Sawynok and Platten (2009), who used a larger dataset that included records of all stocked barramundi, made a similar observation of slow growth rates in the Wet Tropics region. There is also some evidence of variability of growth rates over time within the same location that may be due to different environmental conditions between years or to the genetics of the broodstock used (Sawynok and Platten, 2009). In Figure 26 there were two fish released in the Townsville area that had grown more rapidly than the majority of fish from other areas. These outliers may either be the result of natural variations in growth or could be the result of measurement or data recording errors. The mean daily growth rate of stocked fish in all areas is shown in Figure 27. Mean daily growth rate varies from between 0.35 mm day^{-1} in the Gladstone area to 0.67 mm day^{-1} in the Burdekin River.

There was considerable variation in the mean lengths of stocked barramundi at time of tagging (Table 7) and this was probably due to a number of reasons. For example, some community fish stocking groups are now choosing to release barramundi with an average size of around 300 mm, while others still release smaller (but cheaper) size classes of fish. Further, some hatchery–reared fish that were not tagged prior to being stocked have been subsequently caught, tagged and then released by anglers. It would be expected that fish in this category would be larger, as smaller individuals would be unlikely to be targeted by recreational fishers.

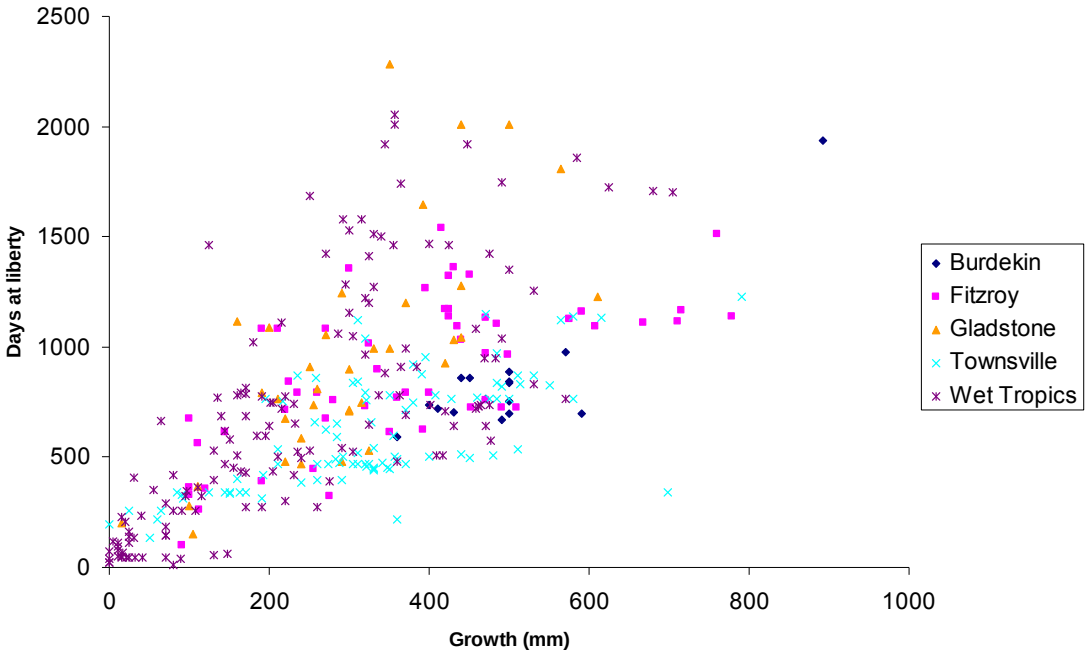

Figure 26. Relationship between time at liberty and length of stocked barramundi by region.

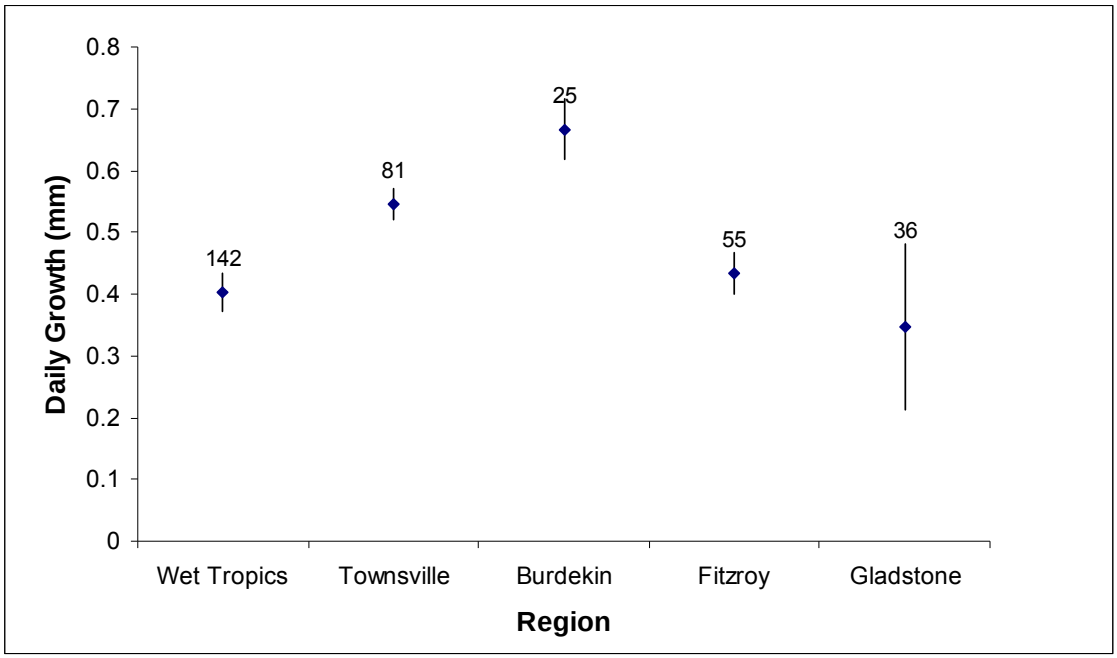

Figure 27. Mean growth (± S.E.) of stocked barramundi in north Queensland. Samples sizes (n) are above each data point

With the exception of the Wet Tropics region, the slower average daily growth rates (Figure 27) were generally from areas where fish were larger at the time of tagging (e.g. Gladstone). These relatively slower growth rates may be because of a number of reasons, including cooler temperatures in more southern areas and allometry (Bagenal and Tesch, 1978) in growth patterns whereby juveniles increase in length quicker than larger fish. The slower growth in the Wet Tropics may be because the streams in this region are generally short, fast flowing and therefore less productive than larger rivers like the Burdekin and Fitzroy. Gillanders and Kingsford (2002) and Milton *et al.*, (2008) have highlighted the importance of the productivity in freshwater systems like the Fitzroy River to the growth of barramundi.

Conclusions

The existing data suggest that while most recaptured fish were caught in the vicinity of where they were either originally stocked or tagged and released, they are capable of travelling extraordinary distances downstream, over weirs and other barriers into estuaries and coastal areas. These types of movements are primarily related to spawning activity, which occurs in saline waters and there is little doubt these stocked barramundi interbreed both with each other and with wild stocks, thereby contributing to the overall gene pool in that system. The full genetic implications of this inter–mixing are unclear, but are being specifically investigated in the current FRDC project *Fish stocking programs - assessing the benefits against potential long term genetic and ecological impacts*. Fish stocked in coastal rivers are also capable of coastal and inter-riverine movements but leakage to other systems is estimated to be only about 5% (Sawynok and Platten, 2009). These authors suggest that this low leakage means that any genetic impacts on wild stocks are likely to be limited initially to the system(s) where Barramundi were originally stocked.

Whilst much less common, stocked fish were also found to move considerable distances upstream and out of impoundments (e.g. Lake Dalrymple). From the publications and data

that have been reviewed in this study, there is little, if any, evidence of movements of stocked barramundi into environmentally sensitive areas. However, neither of the tagging programs mentioned above were designed to elicit finer scale movements of juvenile barramundi, particularly young–of–the–year fish. The current FRDC project mentioned above is designed to complement these programs by giving information on the likelihood of movements into certain environmentally sensitive areas, especially those movements of smaller fish (75-250mm TL) in the period immediately (up to 12 months) after stocking.

Acknowledgements

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