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Is stocking barramundi (*Lates calcarifer*) in north-eastern Queensland a threat to aquatic biodiversity?

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Abstract. The stocking of predators can have significant consequences on recipient aquatic ecosystems. We investigated some potential ecological impacts of stocking a predatory fish (*Lates calcarifer*) into a coastal river and a large impoundment in north-eastern Australia. *L. calcarifer* was mostly found in slower-moving, larger reaches of the river or in the main body of the impoundment where there was abundant suitable habitat. In the tidally influenced freshwater reaches of the coastal river, *L. calcarifer* predominately consumed aytid and palaemonid shrimp that were associated with local macrophyte beds or littoral grasses. In this area the diets of juvenile stocked and wild *L. calcarifer* were similar and stocked fish displayed a high degree of site fidelity. Further upstream in the river, away from tidal influence, and in the impoundment, fish were the main prey item. Cannibalism was uncommon and we suggest that, at the current stocking densities, there was little dietary evidence of predatory impacts from *L. calcarifer* on species of conservation concern. We caution against introducing novel predatory species such as *L. calcarifer* in or near areas that are outside their natural range and are known to support rare, threatened or endangered species.

Additional keywords: conservation, fish stocking, predation, Queensland Wet Tropics, sea bass, stock enhancement, tropical impoundment.

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Introduction

The stocking of exotic and translocated fishes into freshwater systems is a common and widespread global practice (Cowx 1998). Fish stocking to enhance fisheries is arguably a common goal of most stocking programs (Cambray 2003), although it may also be used to mitigate the loss of stocks, restore fisheries or to create new fisheries (Cowx 1994). In Australia, fish stocking has taken place since the arrival of Europeans, especially of salmonids in temperate regions (McDowall 2006), and has resulted in some well documented adverse impacts on native fishes (Tilzey 1976; Ault and White 1994; Lintermans 2000). More recently, fish stocking in Australia has been expanded to include a range of native fishes in an effort to create new 'put and take' fisheries, bolster the populations of threatened or endangered species, as well as for the enhancement of existing wild fisheries (Cadwallader and Kerby 1995; Rowland 1995, 2009; Holloway and Hamlyn 1998; Harris 2003). Stocking of *L. calcarifer* is currently met with broad community support and is undertaken mainly to enhance wild riverine stocks and to establish and maintain sports fisheries in major freshwater impoundments where previously none existed (McKinnon and Cooper 1987; Rutledge *et al.* 1990). Impoundment fisheries for this species are regarded as 'put and take' and are reversible over time as *L. calcarifer* needs to access estuaries and coastal areas to spawn (Dunstan 1959; Russell and Garrett 1985). This is not the case with the limited, open river stockings that commenced in Queensland around 1990 (Russell and Rimmer 1997), which were primarily undertaken to enhance existing recreational and commercial *L. calcarifer* fisheries. Available data suggests that stocked *L. calcarifer* in these open systems make up a relatively high proportion of targeted size classes of both recreational and commercial fisheries (Rimmer and Russell 1998). There is also evidence that stocking *L. calcarifer* is of considerable economic value to the local community in terms of both direct and indirect benefits (Rutledge *et al.* 1990).

There is ample evidence to suggest that the introduction of non-endemic native or exotic species into natural water bodies may threaten native fishes (Crowl *et al.* 1992) and perhaps disrupt ecosystem processes (Simon and Townsend 2003). In particular, there are concerns about the impacts of translocating or directly stocking predatory fishes outside of their natural range, including their potential impact on predator-naïve species including amphibians, invertebrates and other fishes (Burrows 2004). Crowl *et al.* (1992) suggested that if potential prey species have evolved in isolation from predatory fish, they may be significantly impacted by the introduction of novel predators. There is evidence showing that introduced and/or translocated fishes in Australia and elsewhere have been responsible for severe reductions in the numbers of, or even local extinctions of, amphibians (Bradford 1989; Townsend 1996; Hero *et al.* 1998, 2001; Gillespie and Hero 1999; Pope 2008), native fishes (Barlow *et al.* 1987; Crowl *et al.* 1992; Arthington and McKenzie 1997; Lintermans 2000; McDowall 2003; Pusey *et al.* 2006) and crustaceans (Concepcion and Nelson 1999). Significant declines in the abundance and distribution of at least seven frog species were recorded in Queensland during the 1990s, some of those in places where translocated fishes were concurrently present (Laurance *et al.* 1996; Gillespie and Hero 1999; Burrows 2004). In the Queensland Wet Tropics bioregion, illegal translocations resulted in the loss of *Melanotaenia eachamensis* from its type locality (Barlow *et al.* 1987). Another vulnerable species with a restricted distribution, *Guyu wujalwujalensis*, is also considered potentially threatened by any future translocations (Pusey and Kennard 2001; Pusey *et al.* 2004).

To date in Australia, only one study (Morgan *et al.* 2004) has specifically investigated the potential ecological impacts of stocking the large native piscivore, *L. calcarifer*. In this study, Morgan *et al.* (2004) compared the dietary compositions of native fish species 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. While they detected no significant dietary overlap between *L. calcarifer* and the other species in the lake, 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, although they suggested that the effects would likely be minor.

The Wet Tropics bioregion of north-eastern Australia contains some habitats of high conservation importance and supports endemic species including some fishes (Pusey *et al.* 2008) and amphibians (Gillespie and Hero 1999; Gillespie 2001). In the current study, we investigated some of the potential ecological impacts of stocking *L. calcarifer* in the Wet Tropics bioregion of northern Australia. Specifically, we examined:

(1) the stomach contents of stocked fish (in Tinaroo Falls Dam and the Johnstone River) and similar-sized wild fish (in the Johnstone River) to determine the predominant prey groups, to compare the diets of stocked and wild *L. calcarifer* and to detect the consumption of any species of conservation importance or evidence of cannibalism; and
(2) the movements of stocked juvenile *L. calcarifer* in a coastal river (in the Johnstone River), and a large impoundment (the Tinaroo Falls Dam) to determine whether, after release, they moved from the traditional release locations into potentially environmentally sensitive areas.

Materials and methods

Study area

Johnstone River

This river rises on the Atherton Tableland (maximum elevation ~1365 m above sea level) and flows into the Coral Sea near the township of Innisfail (~17°32′S, 146°02′E) in north-eastern Queensland (Fig. 1). About 5 km upstream from the mouth its two major branches, the North and South Johnstone rivers, merge to form the main Johnstone River. It has a small (~1630 km^2), predominantly agricultural catchment with a narrow coastal plain (less than ~30 km wide) and an escarpment that prevents the upstream movement of most fishes (including *L. calcarifer*). The middle and coastal sections of the North and South Johnstone rivers are characterised by mostly good, dry-season water quality, perennial flows, a relatively high gradient and riverine habitats with a generally elevated water velocity and coarse or sandy substrates (Russell and Hales 1993). Since stocking began in 1993, over 290 000 *L. calcarifer* fingerlings (D. J. Russell, unpubl. data), each individually marked with either a coded wire tag or a dart or anchor tag (Russell and Rimmer 2004; Russell *et al.* 2004; Russell 2005, 2008), have been released into the river.

In the current study, stocking release locations for *L. calcarifer* in the North Johnstone River were in (1) the tidally influenced, lower freshwater reaches of the main channel, and (2) further upstream (~25 km) at Nerada in the mouth of Rankin Creek and in the adjacent main river channel (Fig. 1). In the South Johnstone River catchment, stocking took place only in the bottom reaches of a lower tributary, Utchee Creek, and in the main river channel downstream of the confluence with Utchee Creek, adjacent to the township of South Johnstone. Both the South and North Johnstone rivers and many of the tributary creeks in the lower catchment (stream order ≤3) partially drain the environmentally sensitive Wet Tropics World Heritage Area (WTWHA), a refuge for some species of conservation concern (e.g. regionally endemic fish and amphibians). Stocking locations were (1) within the natural range of *L. calcarifer* in the freshwater reaches of the Johnstone River where stocking had occurred in the past, and (2) in or near the lower reaches of WTWHA feeder streams. At all locations, *L. calcarifer* were released into areas that provided immediate refuge from predators (i.e. macrophyte beds, rock piles and woody snags). Post-stocking sampling locations were selected to cover as much of the coastal section of the river as possible within the limitations of site accessibility and salinities suitable for electrofishing operations. Three locations were chosen in the North Johnstone River to represent the lower freshwater (NJL), the middle coastal reaches (NJM) and the upper coastal sections (NJU) of the river, all of which were in the natural range of *L. calcarifer* (Fig. 1). Above this site, the gradient of the river increased, becoming relatively shallow and fast flowing and unsuitable for sampling. Similarly, the South Johnstone River had three coastal sampling locations, the lower, freshwater sections of the river (SJL), the middle reaches (SJM) and an upstream location (SJU) that covered most of the remaining navigable sections of the river.

Tinaroo Falls Dam

This large impoundment (~17°10′S, 145°33′E) is situated on the Barron River, which is arguably the most heavily regulated of all north Queensland streams (Russell *et al.* 2000). It has a storage capacity of 436.5 GL, a surface area of 33.7 km^2, a shoreline of ~209 km, a surface elevation of 670 m and is primarily used to supply irrigation water to the surrounding agricultural district. The perimeter of this impoundment is characterised by a dendritic-like system of inlets or arms and their associated feeder tributaries (Fig. 1). Of these, the Severin, Robson and Kauri creek arms were chosen as fish release locations (Fig. 1) primarily because they contained extensive fish refugia and their feeder streams drained intact, complex notophyll vine forest (Tracey 1982) in the adjacent WTWHA or State forest. Both were also considered likely to support

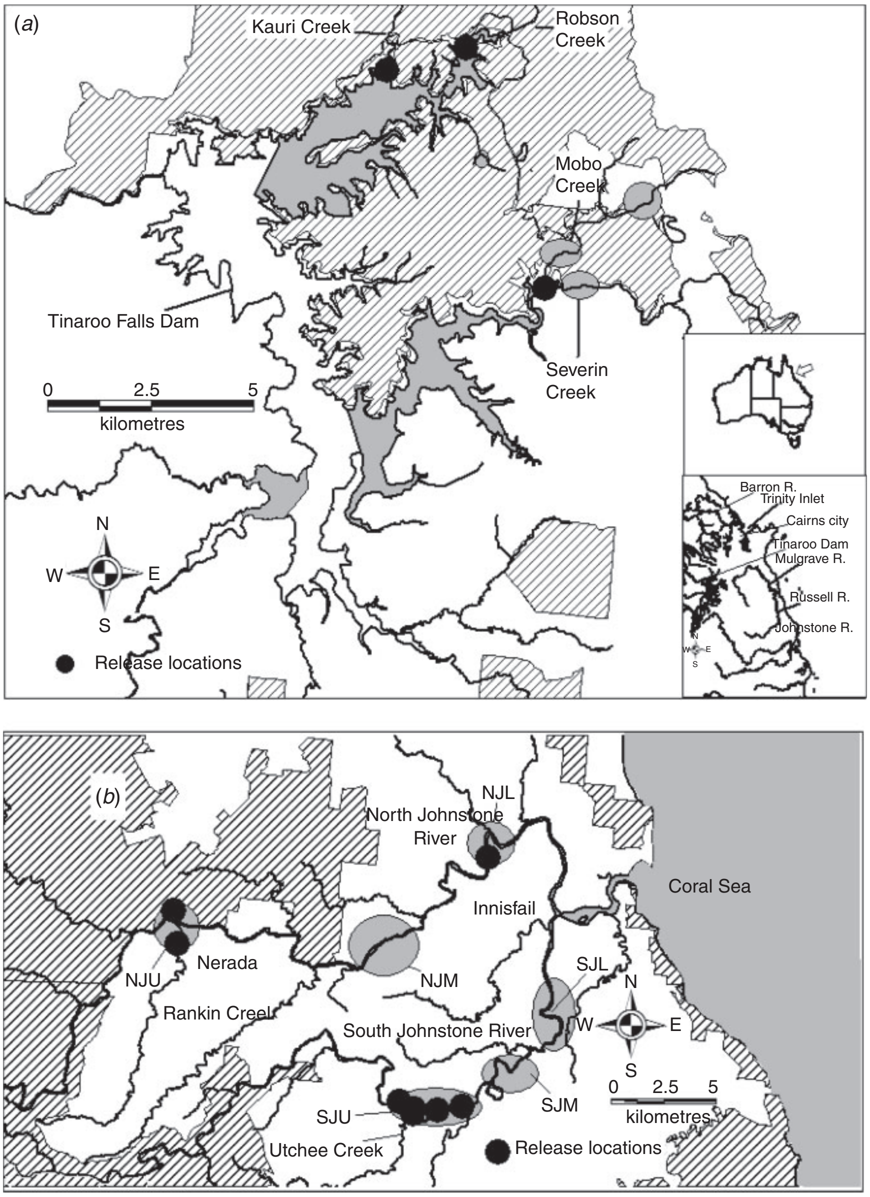

Fig. 1. Release locations for *L. calcarifer* in (*a*) Tinaroo Falls Dam and (*b*) the Johnstone River catchments. Shaded areas are sampling zones and oblique-hatched areas show the boundaries of the Wet Tropics World Heritage management area. Insets show locations of study areas.

regionally endemic species (i.e. crustaceans, fishes and amphibians). Tinaroo Falls Dam is the largest impoundment in the region and annually receives stocked *L. calcarifer*. Over half a million individuals were stocked since fish releases began in 1985 until the early 2000s (Burrows 2004), with stocking continuing until the present day.

Tagging program

Lates calcarifer that were stocked both into Tinaroo Falls Dam and into the Johnstone River were sourced from three commercial *L. calcarifer* hatcheries that used broodstock obtained from the local genetic subpopulation (Keenan 1994). Batches of fish 50–70 mm total length (TL) were stocked during November and December 2009 into the Johnstone River catchment. In Tinaroo Falls Dam, larger fish (mean size $\pm$ s.d. $= 194 \pm 12.5$ mm TL) were released in February 2010 in conjunction with the local Tablelands Fish Stocking Society. Details of numbers of *L. calcarifer* stocked by site and date are given in Table 1.

Johnstone River

Before release, hatchery-reared fish were marked with internal coded-wire microtags (CWT) measuring 1.1×0.25 mm. All CWTs and associated equipment were sourced from North-west Marine Technologies Inc. (NMT; Shaw Island, WA, USA; www.nmt.us). Each CWT is laser etched with a unique binary

Table 1. Numbers of *L. calcarifer* experimentally stocked in the Johnstone River catchment and in Tinaroo Falls Dam in 2009–10
See Fig. 1 for release locations

Release location	November 2009	December 2009	February 2010
North Johnstone (Nerada) R.	1625	1724	
North Johnstone (upper tidal) R.	2183	1854	
South Johnstone R.	0	2037	
Tinaroo Falls Dam			2996
Total	3808	5615	2996

code or number representing a particular batch of tags. Tags were inserted under the skin and into the muscle layer of the cheek using an NMT automatic tag injector (model MK IV). To allow for the later, non-destructive identification of the origins of recaptured stocked fish, *L. calcarifer* released into the North Johnstone River were tagged in either the left or right cheek while those stocked into the South Johnstone River were double tagged in both cheeks. Following tagging, fish were passed through a NMT Quality Control Device (model QCD) to confirm successful tag insertion. *L. calcarifer* were then returned to the flow-through holding tanks for at least 48 h and then passed through the Quality Control Device for a second time before release. This ensured that the CWTs had been retained. A handheld NMT wand detector was used to determine whether fish recaptured during field electrofishing surveys (see below) were either wild or stocked. If recaptured stocked fish had grown larger than ~165 mm TL, they were also externally tagged with a Hallprint type TBF-2 (45 mm long) fine anchor T-bar tag (Hallprint Pty Ltd, Hindmarsh Valley, SA; www.hallprint.com) by locking the T-bar behind the pterygiophores of the second dorsal fin. These tags were marked with a unique number on the flag end to allow non-destructive identification of individual fish. Fish greater than ~350 mm TL were tagged with a Hallprint 85-mm-long plastic-tipped dart tag, which was inserted at the same location as described above for the T-bar tags. To assist with movement studies, any wild or stocked *L. calcarifer* caught during the surveys were also externally tagged using the same tags, depending on the size of the fish.

Tinaroo Falls Dam

The dam contains an all-stocked *L. calcarifer* fishery and therefore there was no need to distinguish between wild and stocked fish using CWTs. Since the minimum size of fish that were released in Tinaroo Falls Dam was ≥165 mm TL, they were all externally tagged with Hallprint anchor tags, as described above. Any untagged *L. calcarifer* from other stockings that were captured during the electrofishing surveys were also tagged with a dart or anchor tag, as described above.

Fish sampling

Lates calarifer were primarily collected using a variety of electrofishing apparatus manufactured by Smith-Root Inc. (Vancouver, WA, USA; www.smith-root.com). In the lower and mid-river sections (stream order ≥4) of the Johnstone River and in Tinaroo Falls Dam, surveys were conducted using a 4.5-m aluminium boat, which was mounted with a Smith-Root 7.5 GPP electrofisher. This vessel was manned by three crew: the coxswain and two persons netting fish. If site access or stream size (i.e. stream order ~<4), were unsuitable for launching the 4.5-m vessel, a smaller 3.5-m aluminium boat mounted with a Smith-Root 2.5 GPP electrofisher with only one person netting stunned fish was used. In very small systems that were unnavigable by boat, (i.e. headwater and feeder streams of Tinaroo Falls Dam), a Smith-Root backpack (model LR-24) electrofisher was used. Electrofishing power time-on (seconds) was recorded on sampling occasions as a measure of effort, enabling the calculation of catch per unit effort (CPUE) as an indicator of relative abundance. After capture, all fish were anesthetised in either an aerated fish-bin (60 L) or an onboard live fish tank (90 L) using AQUI-S^{TM} (Aqui-S NZ Ltd, Lower Hutt, New Zealand; www.aqui-s.com) with dosages of 20–40 mg L^{-1}. Once sedated, captured fish were processed as described below.

Post-capture processing

The total lengths (TL ± 1 mm) of the anesthetised fish were measured and most were weighed (±1 g) using Arlec digital scales (Arlec Australia Pty Ltd, Melbourne, Australia; www.arlec.com.au). The foregut contents of these fish were then removed using stomach flushing (gastric lavage). Hartleb and Moring (1995) found this to be an effective non-destructive technique to remove 100% of the stomach contents of a range of species in fish >14 cm long. Stomach flushing was undertaken by inserting polypropylene tubing (tube diameter varied depending on fish size) into the upper gut of *L. calcarifer* and then pumping small volumes of freshwater using a 12-V pump through the oesophagus of the fish and into the stomach. Once the stomach had visibly expanded with water, gentle pressure was applied and prey items were regurgitated into a fine sieve. The stomach contents of individual fish were subsequently preserved in vials containing 70% ethanol.

Prey items collected were identified in the laboratory to the lowest taxonomic level possible. To assist in this process, a reference collection of common prey species collected at each of the sampling zones was maintained for the duration of the study. The frequency of occurrence of individual taxa within all fish containing prey was then recorded (Hyslop 1980).

Movement monitoring program

Between October 2009 and November 2011, routine electrofishing sampling for juvenile *L. calcarifer* was undertaken at approximately six-week intervals at locations in the lower Johnstone River and in Tinaroo Falls Dam (Table 2). This sampling targeted *L. calcarifer* stocked as part of this current program and similar-age-class wild fish in the Johnstone River. As well as the release locations, site selection included a range of habitat types that were physically available for *L. calcarifer* to colonise. These included individual freshwater sampling sites on the main channels in the lower and upper coastal freshwater sections of the Johnstone River catchment and associated tributaries and headwater streams. In Tinaroo Falls Dam, monitoring took place in the main arms of the impoundment, particularly at or near the original release locations, and their associated tributary streams. The positions (latitude and longitude) of sampling locations were recorded using a hand-held Garmin model 60CSx GPS (Garmin Australasia, Sydney, Australia; www.garmin.com) in both the Johnstone River and Tinaroo Falls Dam.

Dietary analyses

Frequency of occurrence (O_i), whereby the number of stomachs in which one or more of a given food item was found, expressed a percentage of all non-empty stomachs, was used to analyse diet (Windell and Bowen 1978; Hyslop 1980). Only data from fish stocked as part of the current project or wild fish less than 450 mm TL were used in the analyses. For stocked and wild *L. calcarifer*, the ratios of total number of fish that had ingested a given food category to the total number of fish containing one or more prey items were individually compared using a binomial proportion test. Because of smaller sample sizes at other locations, only 2009–10 stocked and wild fish from the NJL location were used in the analyses. To minimise the possibility of the results being affected by the diets of *L. calcarifer* changing as the fish aged, only data from fish caught in 2010 were used.

Results

Movements

Tinaroo Falls Dam

While *L. calcarifer* released as part of this current study dispersed from the release locations into other parts of the dam, there was no evidence of upstream movement into either protected areas or other areas of potential conservation significance. A small number ($n = 6$) of *L. calcarifer* from previous stockings (not part of this current program) were captured in parts of the impoundment where summer rainfall had caused dam water inflows to back up into the lower ends of the tributary streams. In these cases, the *L. calcarifer* were mostly large fish up to 1180 mm TL and were not caught in the flowing sections of the tributary streams. The relative abundance of stocked *L. calcarifer* (as measured by electrofishing CPUE) was higher at the release locations than in other parts of the dam (Table 3). While the average time at liberty was higher at the release locations than in the main dam, overlapping 95% confidence limits suggest no significant difference.

Table 2. Number of individual sites, number of sampling events and electrofishing effort expressed as fishing time during those events between November 2009 and November 2011

7.5 GPP and 2.5 GPP are the two types of units used in boat electrofishing. BP, back-pack; n.a., not available

Locations	Sites	Sampling events			Effort (h)		
		7.5 GPP	2.5 GPP	BP	7.5 GPP	2.5 GPP	BP
North Johnstone R.	26	142	19	1	60.2	4.7	0.3
South Johnstone R.	20	93	13	n.a.	36.3	4.9	n.a.
Tinaroo Falls Dam	25	127	n.a.	9	56.4	n.a.	2.5
Total	71	362	32	10	152.9	9.6	2.8

South Johnstone River

Wild fish, including pre-2009 age classes, dominated *L. calcarifer* catches in all sampling zones, both upstream of, and below, the original release locations in the South Johnstone River (see Fig. 1). No stocked *L. calcarifer* released into the South Johnstone River in late 2009 as part of the current program were found either in the main channel of that river or in associated tributary streams upstream from the original release locations (Table 4). Only 13 wild fish were recaptured at these upstream sampling sites during the entire program and these were mostly larger fish up to 740 mm TL. The CPUE for all wild fish caught with the 7.5 GPP electrofisher progressively decreased with increasing distance upstream from the river mouth but the CPUE for all stocked fish was equal in the SJM and SJL zones. In the freshwater reaches of the upper tidal zone (SJL), most fish (both stocked (all years) and wild) were caught in, or adjacent to, instream macrophyte beds. Only three of the *L. calcarifer* that were stocked into the South Johnstone River in late 2009 as part of the current program were ever recaptured. However, the remaining stocked fish in the 2009 year class ($n = 15$) that were caught in the South Johnstone River were identified by their tagging location (left or right cheek) as having been originally released in the North Johnstone River. This suggests that they had moved downstream from their release location and then up into the South Johnstone River, some in as little as about three months after they had been originally stocked.

North Johnstone River

In the lower, freshwater reaches of the North Johnstone River, both the number and CPUE of wild *L. calcarifer* recaptured decreased with increasing distance upstream from the river mouth (Table 4). For all stocked and wild fish, CPUE was highest in the lower tidal reaches of the river (Zone NJL) and most of these fish were caught in the main river channel where there were extensive instream macrophyte beds that provided

Table 3. Number of fish, electrofishing effort and CPUE (fish h^{-1}) of fish stocked as part of this study in Tinaroo Falls Dam and upstream tributaries

Fish recaptured more than once are not included. Key to electrofisher types is given in Table 2

Location	No. of fish	Effort (hr)	CPUE (fish h^{-1})	Mean days at liberty (±95% CI)
Upstream of release locations (7.5 GPP)	0	6.5	0	n.a.
Upstream of release locations (BP)	0	2.5	0	na
At release location (7.5 GPP)	116	18.7	6.2	220.7 (±48.2)
Other parts of dam (7.5 GPP)	75	31.3	2.4	174.2 (±32.1)

Table 4. Electrofishing effort, CPUE (fish h^{-1}) and numbers of stocked and wild fish caught in sampling zones of the South Johnstone and North Johnstone Rivers during this study

Fish recaptured more than once are not included. Sampling zones are shown in Fig. 1

Zone	Effort (hours)	All Stocked (CPUE)	All Wild (CPUE)	2009–10 Stocked (CPUE)	2009–10 Wild (CPUE)
South Johnstone River					
SJU (7.5 GPP)	4.3	0	8 (1.9)	0	0
SJU (2.5 GPP)	4.1	0	5 (1.2)	0	0
SJM (7.5 GPP)	13.9	9 (0.7)	85 (6.1)	8 (0.6)	9 (0.6)
SJM (2.5 GPP)	0.8	0	1 (1.3)	0	0
SJL (7.5 GPP)	18.1	13 (0.7)	202 (11.2)	10 (0.6)	41 (4.8)
North Johnstone River					
NJU (7.5 GPP)	7.6	26 (3.4)	19 (2.5)	20 (2.6)	1 (0.1)
NJU (2.5 GPP)	4.7	9 (1.9)	25 (5.3)	9 (1.9)	4 (0.9)
NJU (BP)	0.3	0	0	0	0
NJM (7.5 GPP)	18.1	18 (1.0)	192 (10.6)	6 (0.3)	53 (2.9)
NJL (7.5 GPP)	34.5	233 (6.8)	696 (20.2)	218 (6.3)	263 (7.6)

the juvenile *L. calcarifer* with cover. At the upstream stocking site (Zone NJU), 29 fish stocked in 2009 as part of this current program were subsequently recaptured. Of these, 12 were recaptured in tributary streams, with the remainder from the main river. None of those stocked *L. calcarifer* moved up Rankin Creek into a deep-water hole ~3.25 km from its confluence with the North Johnstone River, although two CWT fish (886 and 850 mm TL) stocked before 2005 and six wild fish (293–821 mm TL) were caught at this location. Despite releasing 3349 *L. calcarifer* at the Nerada location (Zone NJU) in 2009, the CPUE of 2009–10 stocked fish caught with the 7.5 GPP electrofisher at this location was less than half of that obtained downstream in Zone NJL where just over 4000 fish were stocked.

Diet

Dietary composition

Johnstone River (both North and South). In total, 984 wild and 276 stocked *L. calcarifer* were stomach flushed during this current project; of these, 62 fish (45 wild and 17 stocked) were recaptured and flushed on more than one occasion. Only data from fish stocked as part of the current project or wild fish less than 450 mm TL were used in the analyses. Of the food items that could be identified in *L. calcarifer* sampled from all the Johnstone River sites, crustaceans from the families Palaemonidae and Aytidae had the highest O_i. Fish, including unidentifiable fish remains ($O_i = 40.74$), were also present in a high proportion of the stomachs examined, particularly from the North Johnstone Upper (NJU) site. A breakdown of the O_i by individual sites is given in Table 5. The occurrence of Palaemonidae was relatively constant across all Johnstone River sites (O_i ranging from 24.14 to 34.26). However, for Aytidae the O_i values were highest at the North Johnstone Lower (NJL) and South Johnstone Lower (SJL) sites (47.12 and 34.95 respectively), decreasing to 3.7 at the North Johnstone Upper (NJU) site.

Tinaroo Falls Dam. In all, 301 *L. calcarifer* stocked as part of this current program were stomach flushed, with 25 of these fish being sampled on more than one occasion. At the sites sampled in Tinaroo Falls Dam, fish (including those from

Table 5. Frequency of occurrence of prey items ingested by *L. calcarifer* in the Johnstone River sampling zones and Tinaroo Falls Dam

Locations of sampling zones are shown on Fig. 1

Food items	SJL	SJM	NJL	NJM	NJU	Tinaroo
Insecta						
Diptera	0.35	0.00	0.00	0.00	0.00	0.00
Ephemeroptera	0.35	0.00	0.00	0.00	0.00	0.00
Odonata	0.00	0.00	0.00	0.00	0.00	0.54
Orthoptera	0.00	0.00	0.16	0.86	0.00	0.00
Mollusca						
Gastropoda	0.35	2.13	1.60	0.86	0.00	0.54
Mollusca (unknown)	0.00	0.00	0.16	0.00	0.00	0.00
Thiaridae	0.00	0.00	0.32	0.86	0.00	0.00
Crustacea						
Áytidae	34.95	13.83	47.12	25.86	3.70	0.00
Decapoda (other)	0.00	0.00	1.28	0.00	0.00	0.54
Grapsidae	0.69	0.00	0.64	0.00	0.00	0.00
Flabellifera	0.00	0.00	0.32	0.00	0.00	0.00
Palaemonidae	34.26	27.66	26.76	24.14	33.33	2.69
Parastascidae	0.00	0.00	0.00	0.00	0.00	2.15
Osteichthyes						
Ambassidae	1.04	0.00	0.16	0.00	0.00	0.00
Apogonidae	0.00	0.00	0.00	0.00	0.00	2.69
Atherinidae	0.00	0.00	0.00	0.00	5.56	4.30
Cichlidae	0.35	1.06	0.00	2.59	0.00	12.90
Clupeidae	0.00	0.00	0.00	0.00	0.00	9.68
Eleotridae	0.35	1.06	1.12	1.72	0.00	0.54
Gobiidae	1.73	6.38	0.80	0.00	5.56	0.00
Melanotaeniidae	0.00	1.06	0.00	2.59	1.85	3.76
Plotosidae	0.00	0.00	0.00	0.00	1.85	0.00
Poeciliidae	0.35	1.06	0.00	1.72	0.00	0.00
Pseudomugilidae	0.00	1.06	0.32	0.00	0.00	0.00
Unknown fish	11.76	25.53	8.49	24.14	40.74	46.77
Amphibia						
Hylidae	0.00	1.06	0.00	0.00	0.00	0.00
Other						
Inorganic material	1.73	0.00	0.16	0.86	1.85	1.08
Organic material	0.00	1.06	0.48	0.86	0.00	1.08
Plant material	11.42	17.02	9.78	12.93	5.56	10.75
Unidentified material	0.00	0.00	0.16	0.00	0.00	0.00
No. of stomachs	289	94	624	116	54	153

unknown taxa) made up the major component of the diet of *L. calcarifer* (Table 5). Invasive tilapia species (*Oreochromis mossambicus* and *Tilapia mariae*: Cichlidae) ($O_i = 12.9$) and bony bream (*Nematalosa erebi*: Clupeidae) ($O_i = 9.68$), a common schooling fish in the dam, were the most significant prey species. Other identified prey items listed included the fish *Craterocephalus stercusmuscarum* (Atherinidae), Melanotaeniidae, *Glossamia aprion* (Apogonidae), and the crustaceans *Cherax quadricarinatus* (Parastacidae) and *Macrobrachium* sp. (Palaemonidae). All these individual prey items were relatively minor dietary components with an $O_i < 5$.

The only evidence of cannibalism was in the North Johnstone River where a 74-mm-TL stocked *L. calcarifer* was regurgitated by a larger (443 mm TL) *L. calcarifer* in the live fish well of the electrofishing boat before being gut flushed. This occurred soon after a stocking in December 2009 at the NJL release location. Furthermore, no evidence of the consumption of rare or threatened species was found in the gut contents of any of the *L. calcarifer* (either stocked or wild) sampled during this study. The partial skeleton of an anuran was recovered from a 519-mm-TL *L. calcarifer* in the middle reaches of the South Johnstone River adjacent to heavily cultivated agricultural land. While the specimen was too digested to identify unequivocally, it was most likely either *Litoria caerulea* or *Litoria xanthomera* (S. Donnellan, South Australian Museum, pers. comm.), both of which are currently not regarded as threatened species in northern Australia.

Dietary comparison between stocked and wild fish

The diets of both stocked and wild 2009–10 cohort *L. calcarifer* were both limited in their diversity, with the majority of prey of both groups coming from the crustacean family Aytidae. Other prey common to both stocked and wild *L. calcarifer* from the NJU location included a small number of species from the family Palaemonidae and unidentifable fish. Other components of the diet were relatively minor, with some, e.g. plant material, probably consumed accidently. When the ratios of the number of fish that ingested each of the prey groups to total number of *L. calcarifer* with gut contents of any type were compared between stocked and wild *L. calcarifer*, no significant differences were found ($P > 0.05$).

Discussion

No evidence was found during this study to suggest that stocked *L. calcarifer* prey upon species of conservation concern, naturally move into areas that are outside the normal range of wild fish, or have unforseen effects on conspecific wild stocks. At the stocking rates used in this study, the release of hatchery-reared *L. calcarifer* into both the Johnstone River and Tinaroo Falls Dam appears to have had minimal impact in terms of some of the specific ecological concerns raised in the literature regarding *L. calcarifer* stocking in northern Australia (e.g. Burrows 2004 and others).

In the catchments of the Wet Tropics bioregion, threatened, vulnerable or endangered aquatic species are more likely to inhabit small, lower-order and headwater streams (e.g. Barlow 2000; Pusey *et al.* 2008) than main river channels or artificial impoundments. In the current study, there was no evidence of stocked fish moving upstream into the smaller feeder streams of Tinaroo Falls Dam but rather they were initially mostly caught close to their original release location and then slowly dispersed into the main body of the dam. This was despite stocked *L. calcarifer* being deliberately released into the impoundment close to the mouths of feeder streams. There were, however, some larger *L. calcarifer* from previous stockings captured at or near the confluence of these streams and the main dam. These larger individuals were sampled when water levels in the dam were quite high and the mouths of these feeder streams were considerably wider, deeper and faster flowing than during drier times of the year. Similarly, in the hinterland of Papua New Guinea, Moore and Reynolds (1982) noted that in periods of rising water levels, larger *L. calcarifer* may move from deeper rivers and lakes into large off-stream swamps whilst water levels are high.

In the Johnstone River, availability of suitable habitat appears to be a major factor determining both stocking success and site fidelity of stocked juvenile *L. calcarifer*. Dunstan (1959) noted that *L. calcarifer* favoured larger streams and still waters and appeared not to prefer small, faster-flowing streams. In the normally relatively quiet waters of the lower Johnstone Rivers near the limits of tidal influence, Rimmer and Russell (1998) observed that aquatic macrophyte beds contributed to enhanced survival and a high relative abundance of juvenile stocked *L. calcarifer*. Similarly, during the current study there was a relatively high CPUE of both stocked and wild juvenile *L. calcarifer* at the same lower North Johnstone River stocking location, suggesting an initial high degree of site fidelity for stocked fish, again probably due to suitable habitat and adequate food availability. However, some *L. calcarifer* (mostly larger fish) moved into, or close to, tributary streams, presumably when requisites such as suitable habitat and adequate food availability were met (e.g. during higher flow as observed in Tinaroo Falls Dam). Some upstream tributaries in the Johnstone Rivers did contain small, relatively deep (~3 m) waterholes where small numbers of mostly larger *L. calcarifer* (up to 886 mm TL) were sampled. However, fish stocked as part of the current study were not caught in these locations despite release sites being, in some cases, only short distances downstream.

In the North Johnstone River (which is larger and, in its lower reaches, has a gentler gradient than the corresponding reaches of the South Johnstone River) some of the locally stocked *L. calcarifer* remained resident at both the upper (NJU) and particularly the lower (NJL) release locations. Small numbers of earlier year classes (pre-2009) as well as wild *L. calcarifer* were also sampled at these two locations. The relatively low recapture CPUE at the upper (NJU) release site when compared to that of the upper tidal zone release location (NJL), would suggest either poorer survival or that the stocked fish were moving into other areas, in this case, almost certainly downstream. While there was no evidence of downstream movement from the current study, fish released earlier at this location as part of other studies were recaptured at the upper tidal zone site (Russell and Rimmer 1997). Such in-stream fish movements may be related to a need to improve their fitness that, in turn, causes them to select a habitat that maximises their expected probability of survival over a specified time horizon considering both starvation and other risks (Railsback *et al.* 1999). The small numbers of stocked juveniles sampled at the NJU site may therefore have been related to a general movement downstream for survival purposes due probably to a lack of suitable habitat and food availability. Upstream movement from that location (NJU) would have been unlikely as it would involve shifting into steep-gradient sections of the river with fast-flowing runs and glides and eventually encountering impassable barriers on the coastal escarpment.

Both stocked (2009 year class) and wild *L. calcarifer* were relatively uncommon in high-flow habitats, particularly the middle reaches of the South Johnstone River and its tributary streams. Unlike in the North Johnstone River, where several hundred stocked fish were recaptured, very few ($n = 3$) of the more than 2000 fish stocked into this stream in late 2009 were ever recaptured. This suggests poor stocking survival and there was no evidence of stocked fish either occupying or moving upstream into tributary streams. One of these feeder streams, Utchee Creek, was of specific interest because it is the type location of the recently (2001) described and relatively uncommon *Melanotaenia utcheensis*. Whilst *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. These results support the contention by Dunstan (1959) and others of a general paucity of *L. calcarifer* in fast-flowing, typically high-gradient systems like much of the Johnstone River system and other Queensland Wet Tropics streams that do not have extensive inland freshwater lagoons or billabongs. Under such conditions, it would appear unlikely that either stocked or wild fish in any large numbers would move considerable distances upstream, including into small tributary streams.

In the current study, the diet of *L. calcarifer* appeared to be variable and was closely related to both its geographic location 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. The exotic cichlids *Tilapia mariae* and *Oreochromis mossambicus* were also found in the stomach contents of *L. calcarifer* in Tinaroo Falls Dam. In the lower reaches of the Johnstone River, *Caridina* spp. and *Macrobrachium* spp. were the dominant prey items. Further upstream, the dietary composition changed to include mostly fish such as members of the Gobidae and Eleotridae families, many of which are common in faster-flowing stream habitats, preferring areas of fine sand and gravel (Pusey *et al.* 2004; Thuesen *et al.* 2011). Unidentified, partially digested fish were also major components in the diets of *L. calcarifer* from these upper riverine sites. Aytid shrimp species, which were observed to be associated with macrophytes (e.g. *Vallisneria* sp.) and littoral grasses (e.g. *Urochloa mutica*) and were present in the lower freshwater reaches, were less common in the stomach contents of *L. calcarifer* from the upstream (NJU, NJM and SJM) zones 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* is an opportunistic predator. In northern Australia, Davis (1985) found the diet of *L. calcarifer* to show an ontogenetic progression from microcrustaceans to macrocrustaceans to fish while Morgan *et al.* (2004) noted that in the freshwater reaches of the lower Ord River and the Fitzroy River larger fish preyed primarily on teleosts (72%) and decapods (26%).

Cannibalism was recorded only once during this current study, although it has been reported at various levels in *L. calcarifer* elsewhere in Australia (Davis 1985), Papua New Guinea (Moore 1980, 1982) and also in Asia (De 1971). The lack of evidence of significant levels of cannibalism (at least in juvenile fish) at all sampling zones in this current study would suggest that stocking (at current densities) is having minimal direct predation impact on the abundances of either conspecific wild stocks or on other stocked *L. calcarifer* cohorts. This is supported by Davis (1985), who proposed that the reason why cannibalism was so rare in the Gulf of Carpentaria population was a reflection of prey availability rather than because of food preference. Consequently, a higher stocking rate (than that used in the current study) of *L. calcarifer* in the Johnstone River and even in Tinaroo Falls Dam may result in increased instances of cannibalism, particularly in areas where higher densities of both wild and stocked fish are likely to be found. Finally, given the long-standing presence of other self-sustaining populations of stocked predatory species in Tinaroo Falls Dam, including *Glossamia aprion aprion*, *Oxyleotris lineolatus* and *Toxotes chatareus* (Russell 1987; Russell *et al.* 2003) that were released into this impoundment some time after its completion in 1958, it is likely that the extra impacts of stocking greater numbers of *L. calcarifer* in the impoundment would be relatively minor.

The spatiotemporal variability in the availability of some prey and their subsequent consumption by predators highlights the inherent difficulties in using pooled diet data to infer the importance of predators on fish prey populations (Blaber 1980; Baker and Sheaves 2009). Various prey, perhaps including some that are rare or threatened, may be particularly vulnerable at certain times, such as during recruitment or spawning. Baker and Sheaves (2009) noted the difficulty of establishing relationships between the abundance of spatially patchy new recruits and their consumption by minor piscivores in tropical Queensland estuaries. They suggested that, during a recruitment ‘pulse’ of an uncommon prey species, predators may switch food preferences to heavily consume these new recruits that are only periodically abundant during, and shortly after, the event. This makes their detection in the stomach contents of predators problematic (Baker and Sheaves 2009) and the short length of time, generally only a few hours, that the predator takes to consume and digest the prey further decreases the probability of it being found (Baker and Sheaves 2009).

Some amphibian larval stages may be particularly difficult to detect from gut content analyses. Gillespie (2001) noted that the soft-bodied cartilaginous skeletons of tadpoles are easily digestible and were unlikely to be detected by examination of gut contents. In a study of the predatory impact of trout on *Litoria spenceri*, he suggested that the trout were most likely to exert their greatest influence on frog populations by preying upon larval amphibian stages. To design a general sampling program to overcome these types of issues is problematic, but for specific prey species it may be feasible to incorporate known aspects of their life history (i.e. lunar periodicity in spawning or known recruitment habitats) into the sampling design to improve the chances of detection in the stomach contents of predators. In some studies it may also be advantageous to consider the use of other tools such as DNA identification methodologies when examining stomach contents for amphibians and other species.

A comparison of dietary data from same-age-class juvenile stocked and wild *L. calcarifer* from the lower North Johnstone River identified no significant differences. At this location, the diet of both these groups was not particularly diverse, with Aytidae being the dominant prey item. Given that the prey of these two groups were similar, it is likely that any impacts of stocking juvenile *L. calcarifer*, in this area at least, will be density related.

Of the potential ecological effects that were examined in the current study, we found no significant demonstrable impacts that could be attributed directly to the stocking of *L. calcarifer*. However, this outcome may very well change with variations in stocking densities or in locations where there are special considerations such as parts of the Wet Tropics bioregion of northern Australia 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 extreme caution.

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