Spawning, egg development and recruitment of diadromous galaxiids in Taranaki, New Zealand.

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Ecology at Massey University, Palmerston North, New Zealand.

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ABSTRACT

Spawning biology of *Galaxias postvectis* Clarke (shortjaw kokopu) was investigated in streams in northwest Taranaki, New Zealand. Most *G. postvectis* were found to spawn near their adult habitat on the river bank during spate flows between May 9 and 17, 2001. Other galaxiids captured appeared to begin spawning earlier in late April. Sixteen galaxiid nests were discovered amongst flooded areas, lying on or amongst the substrate, vegetation and debris on the banks of the Katikara Stream. Mitochondrial (mt) DNA sequences were used to identify these morphologically similar eggs, with 12 being identified as *G. postvectis* and four as *G. fasciatus* Gray (banded kokopu). *Galaxias fasciatus* nest sites had more vegetation and debris than *G. postvectis* nests. The presence of a backwater or pool, cover and gravel were found to be important characteristics associated with both species nests.

Egg development took around three to four weeks on the Katikara Stream margins. Under experimental conditions, most *G. postvectis* and *G. fasciatus* eggs were found to hatch within the first hour of re-immersion in water, and more hatching occurred in moving than still water at warmer temperatures. In the Katikara Stream, eggs hatched and moved downstream only at times of increased water levels. MtDNA sequences were used to identify *G. fasciatus, G. postvectis* and *G. brevipinnis* Günther (koaro) larvae caught drifting downstream in May and June, 2001. From reproductive assessment of adults captured, developmental stage of eggs in nests found, and timing of emigrating larvae, *G. fasciatus* appeared to spawn earlier than *G. brevipinnis,* and both species spawned earlier than *G. postvectis.*

Whitebait migrations were examined within the tidal influence and c. 40 km inland in two rivers in south Taranaki, one with a dam and one without. Whitebait catches were dominated by *G. maculatus* (Jenyns) (inanga) and catches appeared to be similar in both rivers. Large schools of whitebait were however seen below the Patea Dam from October onwards in the Patea River. No whitebait were found to surmount the dam using the elver pass. So the presence of a dam does not appear to affect whitebait recruitment within the tidal influence, however it does block whitebait migrating further upstream to adult habitat.

EXPLANATION OF TEXT

This thesis is a combination of five individual papers. This has resulted in some repetition in introductions, methods and site descriptions between chapters. Chapter 3 was co-written with P.A. Ritchie (Institute of Molecular BioSciences – Massey University) and has been submitted to the New Zealand Journal of Marine and Freshwater Research.

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General Introduction

The New Zealand freshwater fish fauna is dominated by diadromous species that require access to and from the sea to complete their lifecycles (McDowall 1990). Five of these diadromous species belong to the genus *Galaxias* and are *Galaxias maculatus* (inanga), G. *brevipinnis* (koaro), *G. fasciatus* (banded kokopu), *G. argenteus* (Gmelin) (giant kokopu), and *G. postvectis* (shortjaw kokopu) (McDowall 1965).

Diadromous galaxiids spawn in freshwater and the newly hatched larvae migrate to the sea. Subsequently, after four to six months at sea these species, at this stage known commonly as whitebait, return to freshwater in mixed species shoals to find habitat to mature in. However, during peak migration into freshwater in late winter and spring, many whitebait are caught by both recreational and commercial fisherman (McDowall 1990).

Galaxias maculatus dominates the whitebait catch of most rivers with the remaining galaxiid species forming a small but variable part of the fishery (McDowall & Eldon 1980). If they escape capture by whitebaiters or predators, they continue to migrate upstream into adult habitats (McDowall 1990). Some adult whitebait habitats are long distances upstream in small headwater tributaries (McDowall 1990), and it is vital that fish passage is not prevented if headwater populations are to persist. Dams are one barrier to migration that has been found to have a negative effect on fish communities (Joy & Death 2000). The mechanism or cues underlying the movement of whitebait from the sea back into river mouths is unknown. However, it is thought that the presence of whitebait adults in the headwaters may cue whitebait's river selection (Rowe et al. 1992; Baker & Montgomery 2000). Furthermore, it is unknown whether the presence of a dam and a lack of whitebait adults upstream of such a barrier affects whitebait recruitment into a river.

The spawning biology and ecology of *G. maculatus* is relatively well known (McDowall 1968), however little is known about the other species. *Galaxias maculatus* spawn amongst terrestrial vegetation on full or new moons in estuarine areas when spring tides cover marginal vegetation (McDowall 1968). Spawning of *G. fasciatus* has been observed in autumn over a relatively brief period from April to mid June, among flooded terrestrial vegetation and nests have been found on stream margins among vegetation (Hopkins 1979; Mitchell 1991; Mitchell & Penlington 1982). Downstream movement of emigrating larvae have also been found in June during a flood (Ots & Eldon 1975). *Galaxias brevipinnis* nests have been discovered in autumn around adult habitat in New Zealand (Allibone & Caskey 2000) and southeastern Australia (O'Conner & Koehn 1998). McDowall & Suren (1995) have also recorded *G. brevipinnis* larvae emigrating downstream in March in a South Island stream. However no observations of spawning, emigrating larvae or egg deposition of *G. argenteus* or *G. postvectis* have been documented. McDowall & Kelly (1999) have suggested spawning occurs for *G. argenteus* between early June and early August, and downstream migrations of ripe male *G. argenteus* have also been seen during late autumn to winter (McDowall 1990). Ripe *G. postvectis* have been found in March and spent fish in May around their adult habitat, suggesting spawning occurs, like *G. fasciatus* and *G. brevipinnis*, during autumn and early winter (Caskey 1999; McDowall 1990; McDowall et al. 1996). However, much of the reproductive biology of *G. postvectis* and *G. argenteus* is currently unknown.

Terrestrial deposition of eggs at spawning is found in *G. maculatus, G. brevipinnis* and *G. fasciatus* (Benzie 1968a; Mitchell & Penlington 1982; O'Conner & Koehn 1998 respectively), but is rare in fish worldwide (Balon 1981). Closely related non-diadromous galaxiids however, have been found to spawn in the water (Benzie 1968b; Cadwallader 1976; Allibone & McDowall 1997; Allibone & Townsend 1997); therefore bankside spawning may be a diadromous galaxiid trait.

Little is known about hatching of galaxiid eggs, however re-immersion in water has been found to cue hatching of some galaxiid eggs laid in terrestrial environments (Mitchell 1989; O'Conner & Koehn 1998). Temperature affects hatching of *G. maculatus,* with hatching slow and poor below 10°C (Mitchell 1989). R.M. Allibone (pers. comm.) has found hatching of *G. maculatus* improves dramatically in the dark verses daylight. Australian *G. brevipinnis* eggs have been found to only hatch when shaken in water (O'Conner & Koehn 1998). Little is known about the hatching of *G. postvectis* and *G. fasciatus* eggs, and the effect water temperature, light, water movement and time since immersion have on egg hatching.

Early life stages of diadromous galaxiids, including egg, larval and whitebait stages, lack the diagnostic features of their adults, and consequently identification is difficult.

Galaxias maculatus whitebait can be identified when migrating into freshwater without difficulty (McDowall 1964), however the remaining whitebait species are very similar and thus harder to distinguish (McDowall & Eldon 1980). Morphological characteristics have been used to separate whitebait species in the past, except *G. postvectis* and *G. brevipinnis* whitebait that have no known morphological differences (McDowall 1990; McDowall et al. 1996). Identification of larval galaxiids (<35 mm) caught in the sea after emigrating from freshwater have been found to be incorrect using these taxonomic features (McDowall & Robertson 1975). A key to understanding the species lifecycles is going to be the ability to distinguish these species. One method used previously on other fish species is the use of molecular markers, like mitochondrial DNA sequences (Lindstrom 1999; Tringali et al. 1999). Problems have been found with hatching and sequencing whole galaxiid eggs (R.M. Allibone pers. comm.), but a method of identification for whitebait species is needed for all life stages.

This study focuses on the reproductive biology of *G. postvectis, G. fasciatus, G. brevipinnis, G. argenteus,* especially *G. postvectis,* and examines whitebait migration in a dammed and undammed river.

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2

Spawning of *Galaxias postvectis* (shortjaw kokopu) in northwest Taranaki, New Zealand.

ABSTRACT Spawning of *G. postvectis* was investigated in two streams in northwest Taranaki, New Zealand. Most *G. postvectis* were found to spawn in the riparian zone near their adult habitat at increased water levels between May 9 and 17, 2001. Other galaxiids were also captured within the study areas, with *G. fasciatus* (banded kokopu), *G. brevipinnis* (koaro) and *G. argenteus* (giant kokopu) appearing to begin spawning earlier in late April. Female *G. postvectis* were larger and ripe for less time than males. A small number of mature *G. postvectis* were found with bite marks during late March to mid June, probably from other fish present.

Keywords: Spawning; diadromous; *Galaxias*; *G, postvectis*; *G. brevipinnis*; *G. fasciatus*; *G. argenteus*.

INTRODUCTION

New Zealand has 18 recognized *Galaxias* species, 16 described (McDowall 2000), plus two as yet unnamed (R.M. Allibone pers. comm.). Of these, five are diadromous normally requiring access to the sea to complete their lifecycles. Known as whitebait, these species are found throughout New Zealand. The most common of these, *G. maculatus* (inanga) is catadromous and most often found at low altitude in highly modified habitat close to the sea (McDowall 1990). The remaining larger bodied species are *G. postvectis, G. fasciatus, G. brevipinnis* and *G. argenteus*. In contrast these species are amphidromous and are found in lower densities in more pristine environments (McDowall 1990). The spawning biology of *G. maculatus* is well known (e.g., McDowall 1968), but little is known about spawning in the remaining diadromous species.

Galaxias fasciatus has been reported breeding in autumn, over a relatively brief period from late April to mid June, with adults remaining in their usual habitat to spawn (Hopkins 1979; Ots & Eldon 1982; Mitchell & Penlington 1982; Mitchell 1991). O'Conner & Koehn (1998) have described the spawning of *G. brevipinnis* in southeastern Australia in mid autumn. While McDowall & Suren (1995), Duffy (1996) and Allibone & Caskey (2000) have all made observations of *G. brevipinnis* in New Zealand streams that suggest spawning occurs, like *G. fasciatus*, in autumn to early winter.

However, little is known about the breeding biology of the two remaining species, *G. postvectis* and *G. argenteus*. *Galaxias argenteus* have not been observed spawning, although downstream migrations of significant numbers of ripe males have been seen during late autumn to winter (McDowall 1990). Ripe *G. postvectis* have been collected as early as March and seem, like *G. fasciatus* and *G. brevipinnis,* ready to spawn around their adult habitat during autumn and early winter (McDowall 1990; McDowall et al. 1996; Caskey 1999).

This paper describes spawning of *G. postvectis* and other large bodied galaxiids present in two streams in northwest Taranaki, reporting on the timing of spawning, and characteristics and behaviour of *G. postvectis* around this time.

MATERIALS AND METHODS

Study area

Evidence of spawning was sort between March and June 2001 in two streams on the western slopes of Mt. Taranaki. Four sites of approximately 100 m were examined, three in the Katikara Stream and one in an unnamed tributary of the Stony River (Fig. 1). All sites had riparian vegetation and substrate dominated by boulders and cobbles with numerous emergent boulders. Individual site details at base flow are shown in Table 1.

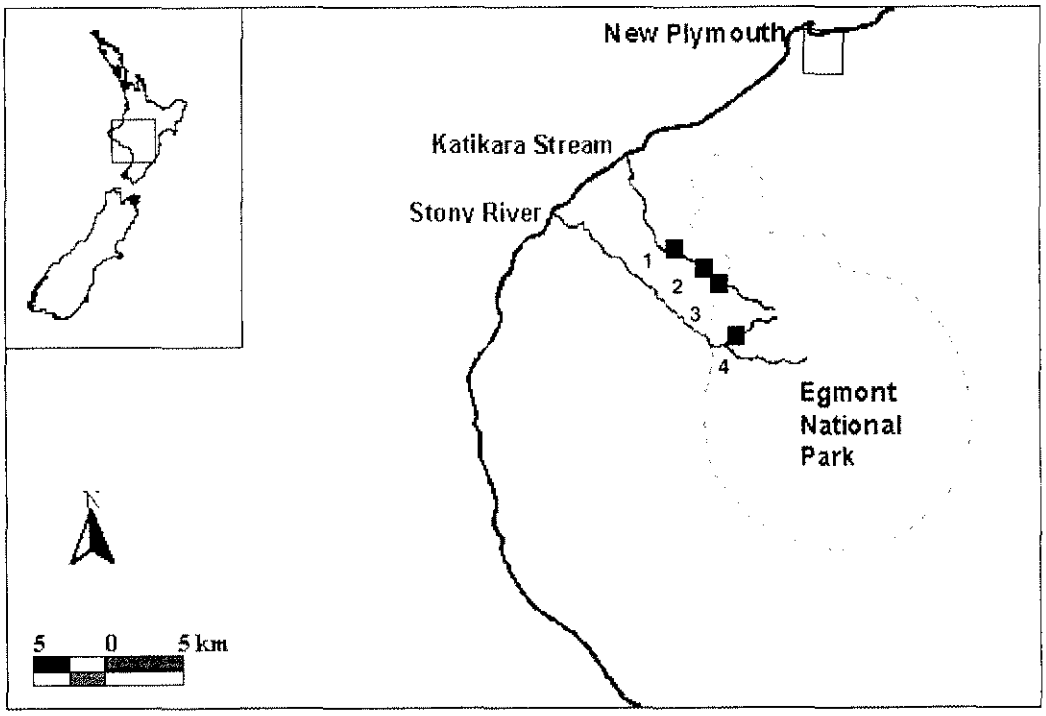

Fig. 1 A map of northwest Taranaki showing the location of the four study sites used for spawning investigations. 1 = lower Katikara site; 2 = mid Katikara site; 3 = upper Katikara site; 4 = unnamed Stony River tributary site.

Table 1. Physical characteristics of the four study sites in northwest Taranaki.

Study Sites	Lower Katikara	Mid Katikara	Upper Katikara	Unnamed tributary of the Stony River
NZMS Grid reference[1]	25924E 62206N	25933E 61197N	25937E 61195N	25934E 61162N
Altitude (m)[1]	300	420	440	400
Mean gradient (mm)[2]	257	184	311	-
Mean wet width (mm)[3]	635	396	416	782
Mean bankfull (mm)[3]	1328	770	900	1042
Mean water depth (mm)[4]	200	217	196	139
Embededness[5]	4	4	4	4-5
Median substrate size index[6]	6.44	6.56	6.22	6.26
Pfankuch stability score[7]	49	56	49	45
Overhead cover (%)[8]	20	35	81	22
Land use (%)[8]	50 native 50 farmland	60 native 40 modified	100 native	100 native
Debris jams (%)[8]	7	13	4	18
Habitat type[8]				
Pool	45	53	30	38
Run	23	30	55	18
Riffle	27	12	3	34
Fall	1	2	10	2
Backwater	5	3	2	3

1 Obtained from 1:50,000 NZMS topographic maps.
2 Mean of all measures every 5 m using a Theodolite (Berger Instruments, 3H6649).
3 Mean of five measures over the study reach.
4 Mean of five depth measurements across the width of a transect at the five points above.
5 Subjectively assessed at site after moving substrate (1 = loosely packed; 4 = tightly packed).
6 From 150 stones collected using the Wolman walk method, the index was calculated using Wolman (1954).
7 Visually assessed at site following Pfankuch (1975).
8 Visually estimated at site.

Sampling methodology

Monitoring of fish spawning condition was conducted on March 30, April 9, 23, May 1, 9, 16, 30 and June 4, 2001. Within sites every pool, run, riffle, cascade and backwater was coded and identified. Within each study area all *G. postvectis* observed were caught using spotlights and dip nets, and placed in holding buckets close to where they were caught. Fish in holding buckets were anaesthetised using a 25 mg/L Benzocaine solution prior to handling. Each fish was individually tagged with a 2.5 x 0.9 mm soft VIalpha tag, using a "Visual Implant Kit" syringe style injector (Northwest Marine Technology Inc.).

The habitat area each fish was captured in was recorded. Fish were weighed in grams using digital scales (Excel BH-3™) and length to caudal fork was measured in millimetres (mm). The reproductive status of the fish was determined visually and categorised as one of: stage 1, indeterminate; stage 2, ripe; stage 3, running ripe and stage 4, spent. Fish were identified as stage 1 when no eggs or milt was produced upon applying pressure to the abdomen and the sex could not be determined. Ripe fish (stage 2) were identified by their buldging abdomen, especially in the female, and by the presence of eggs from a female, or milt from a male when the abdomen was firmly stroked. Running ripe fish (stage 3) also produced eggs or milt, however they also had an obvious loss in condition and weight. Spent fish (stage 4) were recognized by the presence of a swollen genital opening that did not exude eggs or milt when pressure was applied, and an obvious hollow abdomen. Any other characteristics of the fish like bite marks were also noted. After examination, fish were placed back in the holding buckets until normal swimming behaviour was observed, and then all fish were released into the stream within 5 m of the point of capture. Other galaxiid species encountered were captured when possible and their weight, length and spawning status was assessed to allow comparison of spawning timing among the species.

Humidity and ambient air temperature was recorded every 10 minutes throughout this study, using Onset – Hobo pro series™ data loggers at two sites on the Katikara Stream, one under complete canopy cover (upper Katikara site) and one in open canopy (mid Katikara site). Onset tibet™ data loggers were also placed in pools of the lower Katikara, upper Katikara and the unnamed Stony tributary sites recording instream temperature every 10 minutes. Rainfall at each site was recorded every 24 hours. Maximum water level at the lower and upper Katikara sites was recorded every 24 hours by means of Manning's pegs, with classic school paste™ dissolved in water with red Chromos Tempera Powder™ to colour it.

RESULTS

A total of 119 fish were captured within the four study reaches from March 30 to June 4, 2001. *Galaxias postvectis* dominated all sites, with the greatest number (29) captured at the mid Katikara site (Fig. 2). Other galaxiid species caught were *G. fasciatus, G. brevipinnis* and *G. argenteus* but in much lower numbers (Fig. 2). Non-galaxiid species observed were *Gobiomorphus huttoni* (Ogilby) (redfin bullies) and *Anguilla dieffenbachii* Gray (longfin eels). No introduced fish were found at any sites. Generally similar numbers of galaxiids were caught at each site on the different dates (Fig. 3). However, the numbers of *G. postvectis* appeared to decline at all sites from May 9 onwards. From this time the fish were very flighty and appeared to be hiding deeper in rock crevices. Usually 1-4 fish per habitat area were seen with up to 6 recorded in one pool on one night.

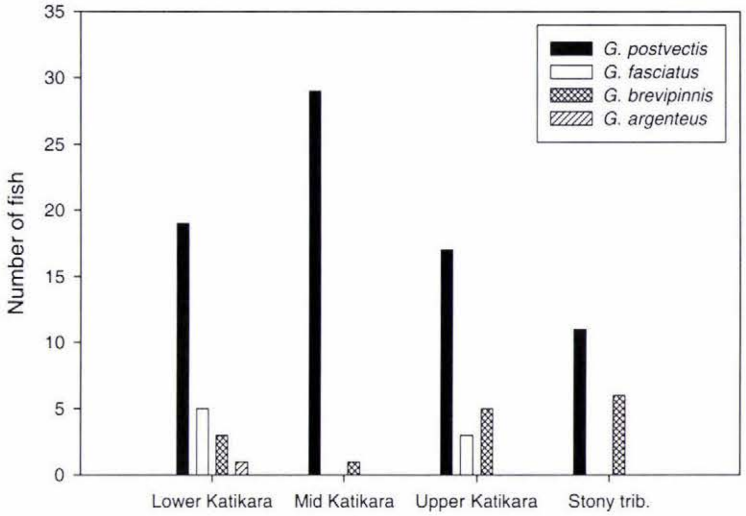

Fig. 2 The total number of fish caught at each site between March 30 and June 4, 2001.

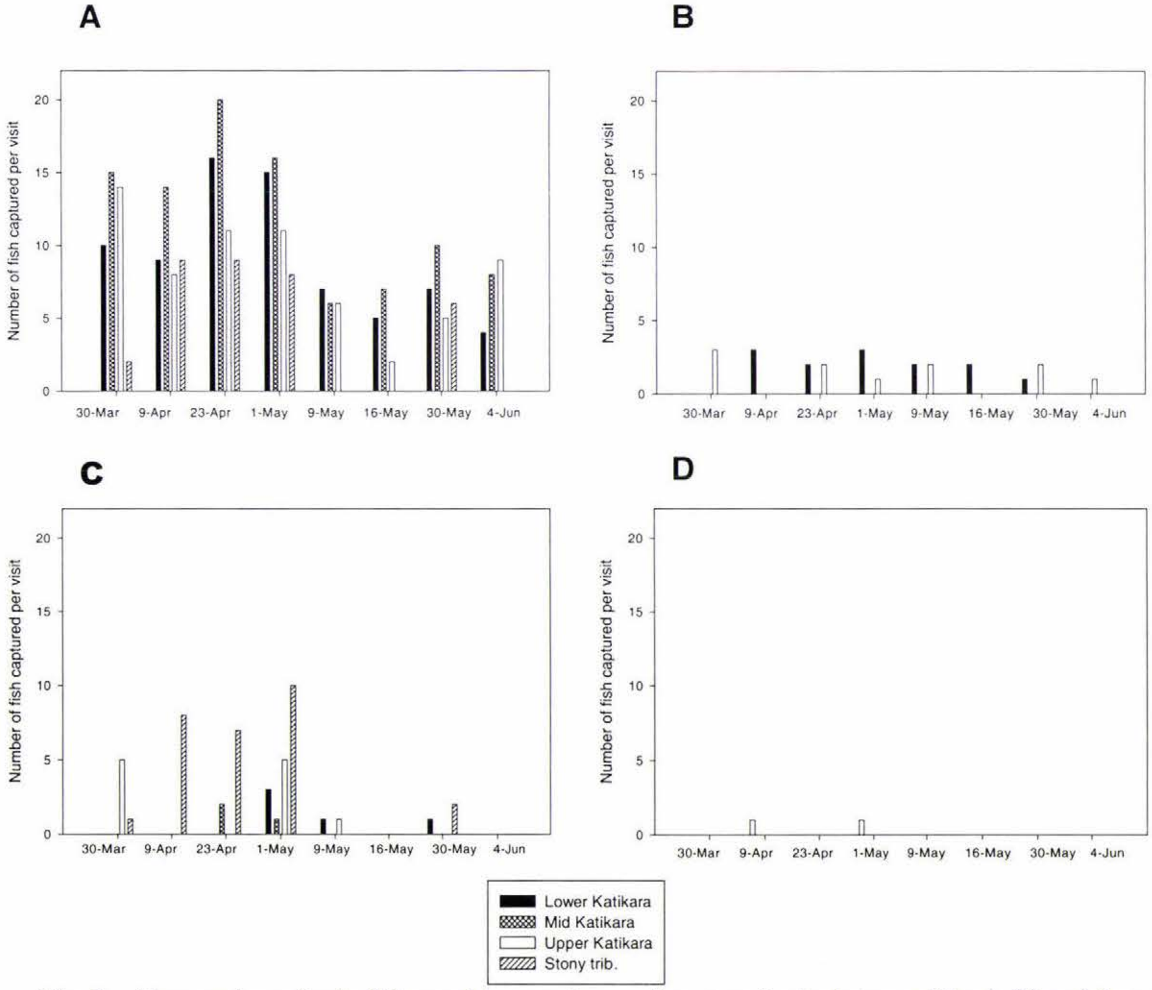

Fig. 3 The number of galaxiids caught on each occasion at each site between March 30 and June 4, 2001. A = *G. postvectis*; B = *G. fasciatus*; C = *G. brevipinnis*; D = *G. argenteus*.

Seventy-three *G. postvectis* were tagged, and 64 were recaptured on at least one occasion. Furthermore, most were recaptured multiple times, with one individual being caught on every survey date. Most *G. postvectis* were recaptured in the same habitat area over the study period (Fig. 4). Of the fish that did move, most only moved to an adjacent habitat area, generally moving back to the original habitat area by the next survey date. Some fish did move longer distances and the maximum distance moved was by one *G. postvectis* that moved c. 200 m between the mid and upper Katikara site. There was no difference between males and females in propensity for movement. The other galaxiid species also appeared to stay in their resident habitat areas.

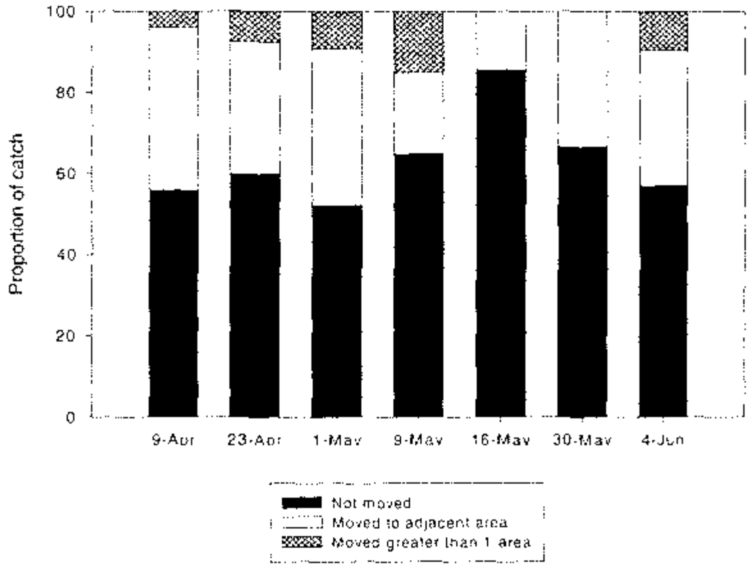

Fig. 4 The movement of all tagged *G. postvectis* from habitat areas between March 30 and June 4, 2001.

From March 30 to June 4, 2001 all fish were seen most often in pool habitat, especially *G. postvectis*. *Galaxias brevipinnis* was also in pool habitat areas but only where *G. postvectis* were less common, otherwise *G. brevipinnis* was found in riffles. *Galaxias argenteus* and *G. fasciatus* were never found to co-occur in the lower Katikara site.

Most *G. postvectis* were ripe when the study began and the proportion of spent fish increased from May 1 until May 30 when all were spent (Fig. 5). The lower Katikara site, closest to the sea, showed the first evidence of spawning with a few spent *G. postvectis* occurring earlier than at the upstream sites (Fig. 5). Furthermore some female *G. postvectis* took longer to become ripe with 100% of the catch not appearing ripe or running ripe until May 9, compared with males that were all ripe at the first visit on March 30 (Fig. 6). Generally males were also ripe for a few days after most females were spent (Fig. 6). A few males were spent earlier, between May 1-9, and the first spent females were captured on May 16. In addition the occurrence of partially spent females, with eggs still apparent near the vent, indicated that not all eggs were released at one time. The other galaxiids species all appeared to spawn in late April before *G. postvectis*. The first spent *G. fasciatus* appeared on April 23, with both *G. fasciatus* and *G. brevipinnis* all spent by May 30 and the one *G. argenteus* was spent on May 1. Some *G. fasciatus* and *G. brevipinnis* were still ripe on May 16.

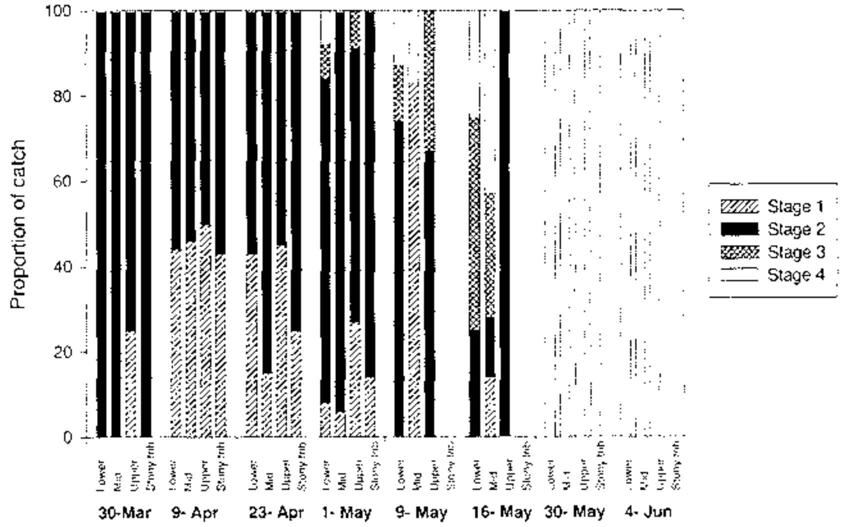

Fig. 5 The proportion of the four reproductive stages for each catch at all sites for tagged *G. postvectis* from March 30 to June 4, 2001 for sexes combined.

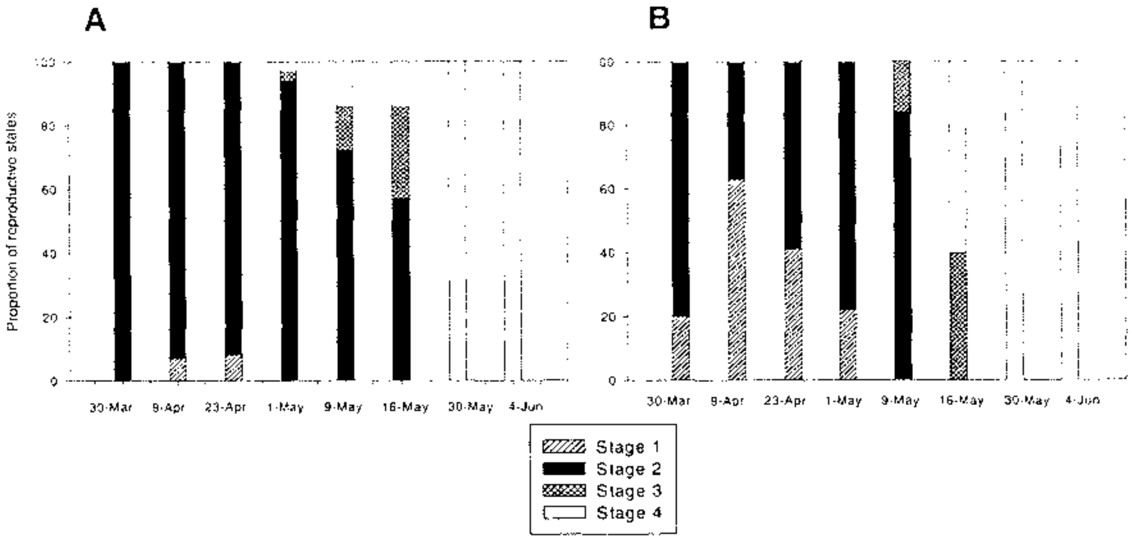

Fig. 6 The proportion of each reproductive stage of male (A) and female (B) *G. postvectis* from March 30 to June 4, 2001.

It appeared that spawning events were timed to flood flows (Fig. 7). After every significant increase in water level and significant rainfall, the proportion of spent *G. postvectis* increased. At times of increased water level during May, pairs of *G. postvectis* were often seen in shallow backwater habitats possibly waiting to spawn. Spawning also coincided with a drop in water and ambient air temperature. Humidity at the open and closed canopy areas generally stayed around 100% throughout the study, however, there was a notable drop just prior to the beginning of spawning in early May.

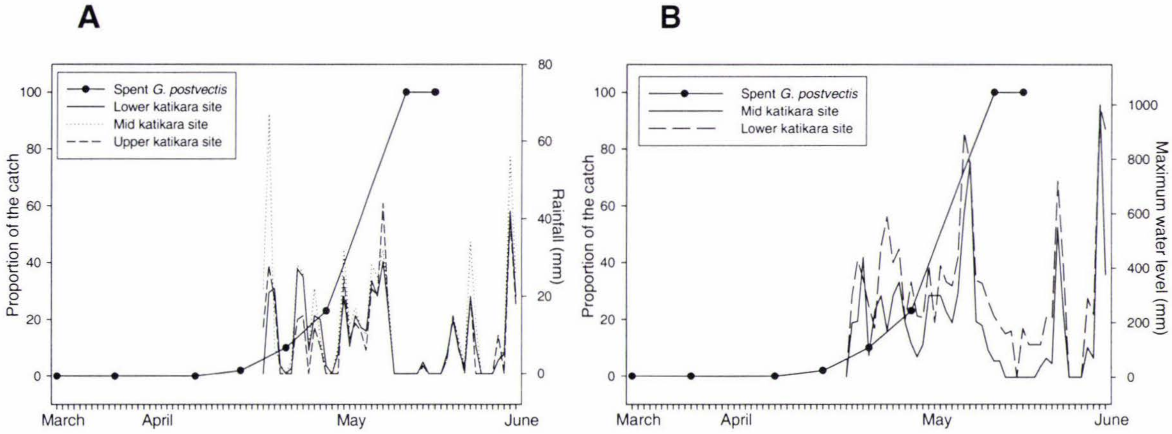

Fig. 7 Plots of daily rainfall (A) and maximum water level (B), and the proportion of spent *G. postvectis* from March 30 to June 4, 2001.

Sexes were morphologically identical, but from reproductive assessment the sex of most *G. postvectis* was determined, with all sites showing c. 1:1 ratio of males to females (Fig. 8). Examining the sex ratio per habitat area, c. 1:1 ratio was also found, although there were times when a 3:2 ratio of males to females was observed. *Galaxias fasciatus* and *G. brevipinnis* caught appeared to have a male biased sex ratio, however a number of fish of undetermined sex were also present. The sex of the one *G. argenteus* was not determined.

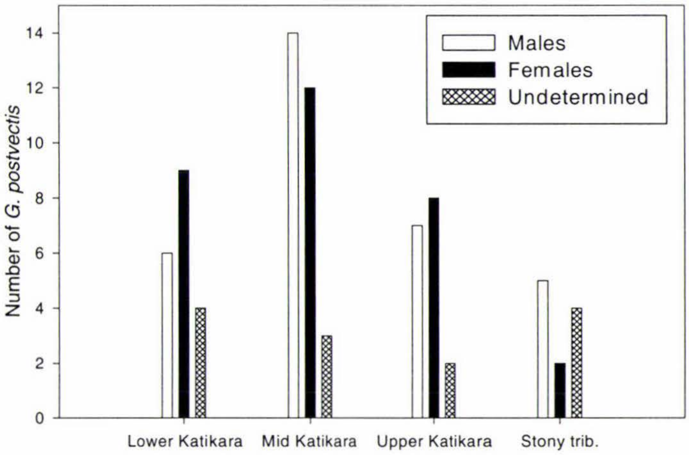

Fig. 8 The sex ratio of *G. postvectis* found between March 30 and June 4, 2001 for all study sites.

Some *G. postvectis* in all Katikara Stream sites had bite marks appear between March 30 and June 4, 2001. More females than males appeared to get bitten with a mean of two males and four females receiving bites per site from a mean of 19 *G. postvectis* per site. No fish appeared to have bites in the unnamed Stony tributary site. Most bites were distinct triangle bite marks on the fins especially the caudal fin; while a few had big grazes usually over the abdominal area (Plate 1). All fish with bites were sexually mature and occurred in habitat areas with other female and male *G. postvectis*. Some *G. postvectis* with bite marks co-occurred with *G. fasciatus, G. brevipinnis, G. argenteus* and *A. dieffenbachii*. All fish, except possibly one large male *G. postvectis* that became sluggish and was covered in fungi over extensive bite marks, were thought to have survived spawning.

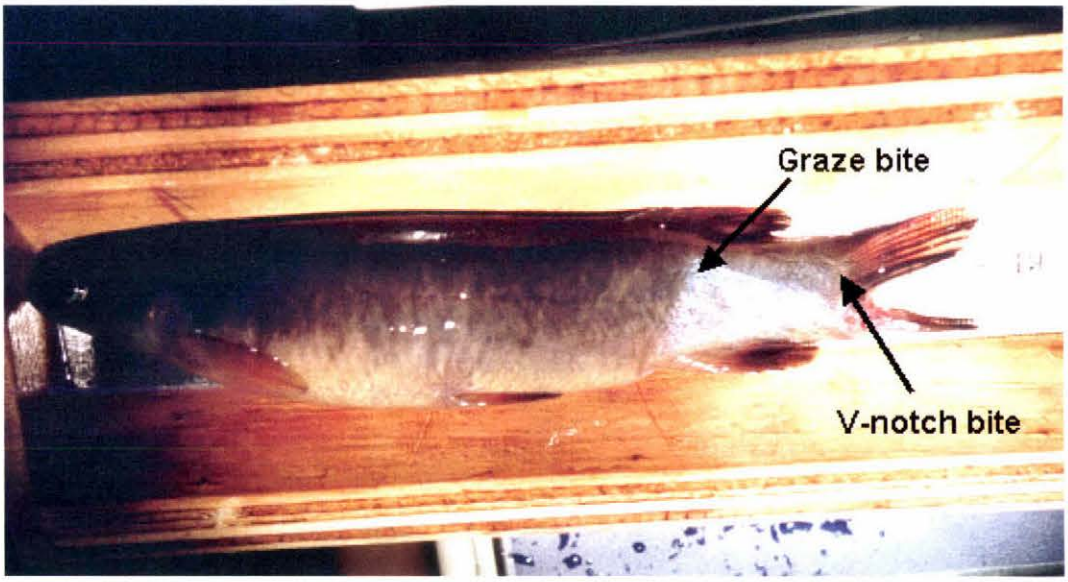

Plate 1 Female *G. postvectis* with both a v-notch bite in the caudal fin and graze bites behind the vent.

The length and median weight ranges of *G. postvectis*, *G. fasciatus* and *G. brevipinnis* appeared to be smaller and lighter in males than females (Table 2). A number of fish could not be sexed and these fish encompassed a large range of size and weights (Table 2). Examining only *G. postvectis*, the males were significantly smaller ($P < 0.0001$) and lighter, both ripe ($P < 0.0001$) and spent ($P = 0.01$), than the females. The differences between the ripe and spent weights of the females were significant ($P = 0.03$) with females loosing between 6 and 25 g. However for males, it was not significant ($P = 0.44$) only losing 4-10 g after spawning.

Table 2. Lengths and median weights of fish caught between March 30 and June 4, 2001.

	N	Lengths (mm)	Weights (gms)	
			Ripe	Spent
G. postvectis				
Males	33	116–215	15-115	33-105
Females	33	165-220	54-153	47-128
Indeterminate	18	51-225	6-128	
G. fasciatus				
Males	4	155-161	43-105	
Females	4	191-213	109-141	
Indeterminate	7	160-270	74-120	
G. brevipinnis				
Males	8	125-167	16-46	
Females	2	127-145	18-27	
Indeterminate	9	115-150	12-31	
G. argenteus				
Males	0	-	-	
Females	0	-	-	
Indeterminate	1	253	225-229	

DISCUSSION

All sites in these two streams were dominated by *G. postvectis* and one to four *G. postvectis* were usually found per habitat area with a range of 5-20 per site per visit. These densities are greater than most recorded populations where only one or two individuals are found per site (McDowall 1990; McDowall et al. 1996). *Galaxias postvectis* has a patchy distribution throughout New Zealand, however even compared to high population densities documented; those in the Katikara Stream sites are considerably higher (McDowall et al. 1996; Studholme et al. 1999; Jack & Barrier 2000).

Like D. Caskey & R.M. Allibone (unpublished data) have recently found, most galaxiids had resident habitat areas, even around spawning. Of the few fish that did move, most only moved small distances. This movement was not thought to be a direct result of spawning, as it was not limited to spawning time. The distribution of the galaxiids was similar to that found by Chadderton & Allibone (2000), with most found in pool habitats and only *G. brevipinnis* occupying riffles when other galaxiids were present. However when other galaxiids became rare, *G. brevipinnis* occupied all habitat areas including pools. Similarly *G. fasciatus* appeared to avoid habitats occupied by *G. argenteus* and *A. dieffenbachii* (Chadderton & Allibone 2000).

Spent *G. postvectis* appeared from May 1 with most adults spawning between May 9 and 30. The first spent fish were males with spent females only appearing after May 9 several days later. The reason no ripe females were caught with the ripe males maybe a result of the fish becoming flighty after spawning, such that spent females escaped capture. Newly laid *G. postvectis* nests were also discovered between May 11 and 17 (Chapter 4), and so peak spawning occurred between May 9 and 17 at the Katikara Stream. Caskey (1999) has also observed ripe and spent fish for the last two years in the Katikara Stream and estimated spawning to be between April 21 and May 15. Therefore a late autumn spawning for *G. postvectis* can be confirmed at the Katikara Stream and the unnamed tributary of the Stony River. Furthermore spent fish were found at the lower and mid Katikara sites earlier than the Upper Katikara site further inland. Thus spawning may occur earlier at lower elevations and distances inland, but this needs more thorough investigation.

Peaks in rainfall and water level clearly cued spawning for *G. postvectis,* with spawning occurring during or after an increase in water level and significant rainfall. As with other galaxiids (Mitchell & Penlington 1982; O'Conner & Koehn 1998), it seems *G. postvectis* spawning is associated with elevated water levels. So although *G. postvectis* spawning occurred within a short and sharply defined time period of c. three weeks, timing and length of the annual spawning event is dependent on the occurrence and timing of floods.

Similarly to *G. fasciatus* (Hopkins 1979), *G. postvectis* were found to survive spawning. Furthermore like some other galaxiids (Allibone & Townsend 1997), female *G. postvectis* were occasionally found partially spent indicating that all the eggs are not released at once. The other galaxiids, *G. fasciatus, G. brevipinnis* and *G. argenteus,* were thought to have started spawning from late April, earlier than *G. postvectis*. *Galaxias fasciatus'* earlier spawning was confirmed by the discovery of eyed nests earlier than those of *G. postvectis* (Chapter 4). Whether timing of large bodied diadromous galaxiids spawning in other areas of New Zealand is consistent with this pattern remains to be seen.

A small proportion of mature *G. postvectis,* especially females, were found bitten, but only in the Katikara Stream sites where there were much higher densities of fish. These bites were probably inflicted by other *G. postvectis* during spawning or by other fish species attempting to prey on spawning fish.

Female galaxiids were generally bigger than males with a buldging abdomen when ripe. The sex ratios in habitat areas, and sites were generally found to be 1:1. This also occurs in *G. vulgaris* (Benzie 1968), whereas *G. maculatus* are generally female biased (McDowall 1968). Similar to *G. fasciatus* and *G. maculatus* (McDowall 1968; Hopkins 1979), all female galaxiids found in this study were generally bigger than males. *Galaxias postvectis* females were found to be larger and heavier, both ripe and spent, and lost more weight after spawning than the males. The greater size of the females could be attributed to differences in the size and weight of the eggs compared to milt, or because females are generally older and larger than males, as in *G. maculatus* (McDowall 1968).

In conclusion, an increased water level cued the onset of spawning for *G. postvectis* near their resident habitat areas, in the Katikara Stream and the unnamed Stony River tributary in mid to late May. Whereas the other resident galaxiids appeared to spawn earlier from late April. The spawning period was short and there was high survival of adults.

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3

Identification of galaxiid nests, emigrating larvae and whitebait, using mitochondrial DNA control region sequences.

ABSTRACT We used mitochondrial (mt) DNA sequences to identify nests, emigrating larvae and whitebait of three species of diadromous galaxiids, collected from eight locations in Taranaki and Manawatu, New Zealand. DNA was extracted from whole larvae and fin clips, and a 416 base pair (bp) sequence of mtDNA control region was determined. Four *Galaxias fasciatus* (banded kokopu) and 12 *G. postvectis* (shortjaw kokopu) nests were identified. *Galaxias postvectis, G. fasciatus* and *G. brevipinnis* (koaro) individuals were detected migrating downstream from drift samples collected between May and June 2001. Furthermore, we identified three species from samples of whitebait revealing three *G. postvectis,* six *G. fasciatus* and three *G. brevipinnis*. Species of larval eggs, larvae and whitebait can be accurately identified by molecular methods. These methods provide an opportunity to help in the understanding of nest habitat choice, hatching time, migration and choice of rivers by whitebait species.

Keywords: PCR; *Galaxias*; mitochondrial DNA; control region.

INTRODUCTION

The New Zealand whitebait fishery is made up of five diadromous Galaxiidae taxa: *G. maculatus* (inanga), *G. brevipinnis*, *G. fasciatus*, *G. postvectis* and *G. argenteus* (giant kokopu) (McDowall 1965). *Galaxias maculatus* dominates the catch of most rivers with the remaining galaxiid species forming a small but variable part of the fishery (McDowall & Eldon 1980). Diadromous galaxiids lay their eggs in a riverine or estuarine environment and the newly hatched larvae migrate to the sea for about 4-6 months, then return to freshwater in mixed species shoals, commonly known as whitebait (McDowall 1990). The life history and ecology of *G. maculatus* is well known (McDowall 1968), but little is known about the remaining species.

Juvenile diadromous galaxiids lack the diagnostic characters of their respective adults (McDowall 1970). *Galaxias maculatus* whitebait (c. 50 mm) are easily identified on entry into freshwater, having the anal fin inserted directly below the dorsal fin (McDowall 1964). The subtle differences among the remaining galaxiid whitebait species make their identification more difficult (McDowall & Eldon 1980). McDowall & Eldon (1980) suggested morphological characteristics like fin placement, colouration and size to separate them, except *G. postvectis* that cannot be distinguished and may be misidentified as *G. brevipinnis* (McDowall 1990; McDowall et al. 1996). Nevertheless, these taxonomic characters have been problematic and studies often conclude that the identification of species is difficult (McDowall 1990; Bonnett 2000; McDowall 2000) and prone to error (Dijkstra & McDowall 1997). Furthermore, McDowall et al. (1975) found that identification of species of larval galaxiids (<35 mm) caught in the sea was inaccurate with standard taxonomic characters. Therefore the indistinguishable egg or larval forms have hindered studies of nest sites and hatching times for species. The accurate identification of species at each life stage is critical to the future management and understanding of this important fishery.

Molecular markers, like mtDNA sequences, have been used successfully to distinguish species of larval fish (e.g., Lindstrom 1999 & Tringali et al. 1999). MtDNA is a small covalently closed extranuclear genome present in all animal cells. This genome is haploid and only inherited through the maternal line (Birky 1995). Mitochondrial genes typically have higher mutation rates than nuclear genes; due mainly to the long transient single-stranded phase mtDNA has during replication (Reyes et al. 1998). The mtDNA control region, which is responsible for transcription and replication of the molecule, appears to be the most variable mt gene.

MtDNA has provided insight into galaxiid biogeography and revealed a high level of genetic structure within some species (Waters et al. 2000; Waters & Wallis 2001). DNA sequences provide an opportunity for species specific markers for this cryptic freshwater fauna, in addition to estimates of population level diversity and structure. This study aimed to develop mtDNA markers to distinguish diadromous galaxiid's nests, emigrating larvae and whitebait. This method of identification will help the understanding of nest habitat choice, hatching time, migration and choice of rivers by whitebait species.

METHODS

Specimens were obtained from eight locations in Taranaki and Manawatu in the lower North Island of New Zealand (Fig. 1). Nests were located at three sites along the Katikara Stream and a random collection of c. 50 eggs was taken from each nest and transported to Massey University in May 2001 (Chapter 4). Live eggs were kept on damp moss, at a constant 14°C and under a 12 hour light: dark regime. After three weeks the eggs were hatched and immediately preserved in 70% ethanol.

Recently hatched larvae were collected from the field in May and June 2001 by daily drift sampling from nets set over 24 hours at the downstream margin of sites 1 and 2 (Chapter 4). Samples were immediately placed in 70% ethanol and stored at 4°C until DNA extraction. Whitebait were collected from the Patea and Waitotara Rivers in Taranaki, and the Turitea and Kahuterawa Streams, two tributaries of the Manawatu River in Manawatu (Fig. 1). Whitebait specimens were collected with Southland Sock whitebait nets from October 2000 to January 2001. Samples were sorted, *G. maculatus* were returned to the rivers and some of the remaining whitebait were transported to Massey University where they were kept in tanks at 15°C (Chapter 6). Fish were anaesthetised using a 25 mg/L solution of benzocaine and a c. 5 mm^2 square section of the dorsal fin was taken. Fish were returned to their tank and their recovery monitored.

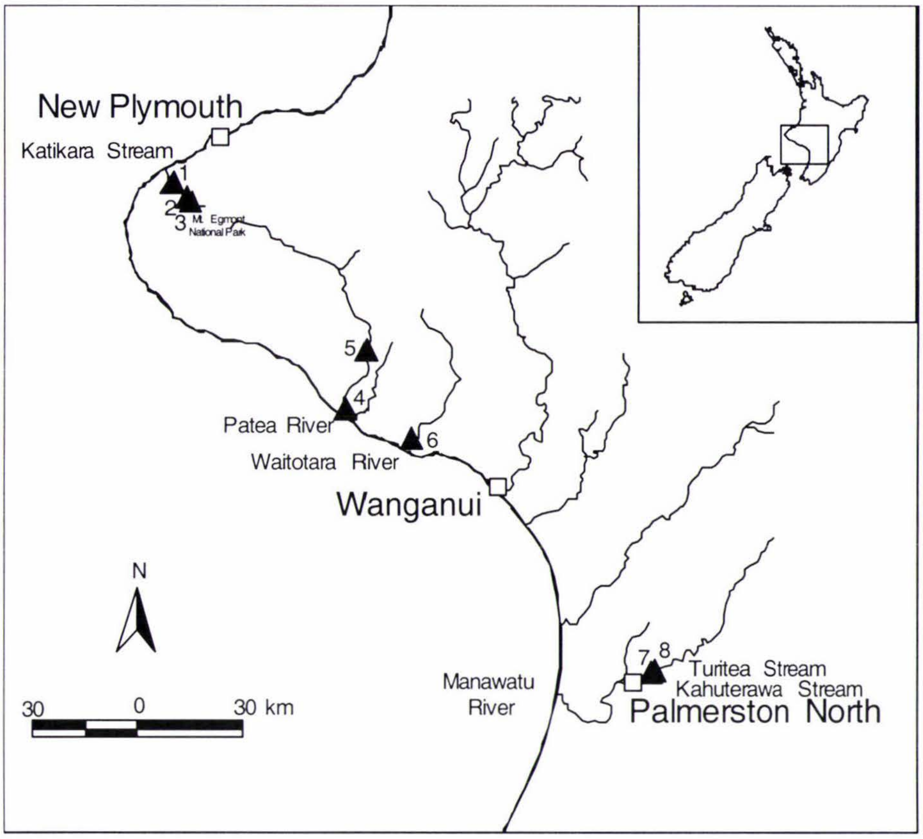

Fig 1. Map of the Taranaki and Manawatu Regions, showing collection sites of galaxiid specimens. N = nest larvae, E = emigrating larvae and W = whitebait. The locations (▲), followed by the sample size and type in brackets, are: 1 = lower Katikara site (5 N, 8 E), 2 = mid Katikara site (7 N, 7 E), 3 = upper Katikara site (4 N), 4 = lower Patea River (1 W), 5 = upper Patea River (5 W), 6 = lower Waitotara River (4 W), 7 = Turitea Stream (2 W), and 8 = Kahuterawa Stream (1 W).

DNA was extracted from 14 preserved nest larvae, two live nest larvae (including one unhatched larval egg), 14 preserved emigrating larvae and 12 fresh fin clip samples. All samples were placed in 400 μL of buffer (10 mM Tris pH 8.0, 50 mM NaCl, 10 mM EDTA), and dissolved with 0.2% SDS and 0.5 μg/μL proteinase-K while gently rotating at 50°C for 1-2 hours. The digested tissue was then purified by phenol-chloroform extraction. DNA was precipitated with 2.5 volumes of 100% ethanol and 85 mM sodium acetate, pH 5.2, followed by a 70% ethanol wash. The resulting precipitate was pelleted in a microfuge, dried and resuspended in 200 μL of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Mock extractions were also performed at the same time to control for cross contamination of samples. The DNA yields from different tissue types (i.e., eggs, larvae and fin clips) and preservation techniques (i.e., fresh and ethanol preserved specimens) were quantified using a Hoefer DyNA Quant 200 fluorometer.

A 416-bp region of the 5' end of the mtDNA control region (hypervariable region I, HVRI) was amplified using the polymerase chain reaction (PCR) with the primers L-P4β (5'-TAAACTACCCTCTGBSCCCG-3', modified from Waters & Wallis, 2001) and H-CSBd (5'-TGAAGAAGGAACCAAATGCCAG-3', this study). The heavy strand primer (H-CSBd) was designed from conserved sequence block D (Waters & Wallis 2001) of the control region of 13 galaxiid taxa. PCR amplifications containing 1 μL of extracted DNA were carried out in 25 μL volumes with 10 mM Tris pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.4 μg/μL bovine serum albumin, 0.8 μM L-P4β, 0.4 μM H-CSBd, 200 μM of each dNTP and one unit of *AmpliTaq*® DNA polymerase (Applied Biosystems). Thermal cycling was carried out in a Hybaid Omn-E at 94°C 10 s, 60°C 10 s and 72°C 25 s for 27 cycles. Three microlitres of each PCR was electrophoresed in 2% agarose at 12.5 V/cm for 30 mins, stained with ethidium bromide and visualised under 302 nm UV light. The remaining PCR product was purified using the High Pure PCR Product Purification kit (Roche), sequenced with the PRISM® BigDye™ Terminator v3.0 Cycle Sequencing kit (Applied Biosystems) and analysed on a 377A automated sequencer (Applied Biosystems).

DNA sequences were aligned by eye with nine published sequences representing standards for *G. maculatus*, *G. brevipinnis*, *G. fasciatus*, *G. postvectis* and *G. argenteus* (Waters et al. 2000; Waters & Wallis 2001). From this combined data set, base frequencies and a nucleotide substitution model were estimated using PAUP*4.0b8 (Swofford 2001). Haplotypic diversity was calculated as $h = (n/(n-1))(1-\sum f_i^2)$ where f_i is the frequency of the *i*th haplotype in a sample size of *n* individuals (Avise 1994). Nucleotide diversity was calculated as $\pi = (n/(n-1))(\sum f_i f_j p_{ij})$, where f_i and f_j are the frequencies of the *i*th and *j*th haplotypes in a sample of size *n*, and p_{ij} is the estimated sequence divergence between the *i*th and *j*th sequence (Nei 1987). Nucleotide diversity within species (π) and levels of sequence divergence between species (*d*) were calculated in MEGA version 2.1 (Kumar et al. 2001), with standard errors estimated from 500 bootstrap replicates. A two-parameter distance (Kimura 1980) matrix of all sequences was used to construct a neighbour-joining tree (Saitou & Nei 1987) in PAUP*4.0b8. DNA sequences can be retrieved from GenBank or Appendix 1.

RESULTS

DNA was successfully extracted and amplified from all samples regardless of their type or method of preservation. Live larvae that weighed 4.6 mg (SE = 0.11) yielded a mean of 4.8 μg of DNA. Emigrating larvae preserved in 70% ethanol weighed 2.6 mg (SE = 0.33) and yielded on average 2.7 μg of DNA. Fin clips that weighed 5.6 mg (SE = 0.49), yielded a mean of 2.8 μg of DNA. There was an obvious relationship between tissue weight and DNA recovery. For the PCR 400 ng of genomic DNA was used to amplify HVRI sequences. Replicate larval samples from the same nest all produced identical sequences, and HVRI sequences could be consistently amplified from single unfertilised *G. postvectis* eggs either frozen or preserved in 70% ethanol.

The aligned data set comprised 42 new sequences (340 bp, primers excluded) and the nine reference sequences. Together these data represent taxa sampled from the most southern location of the Maori River on Stewart Island (Waters & Wallis 2001) to the northern location of the Katikara Stream in Taranaki (this study). The 340-bp region of the HVRI (light strand sequence) showed asymmetric base frequencies ($\pi_A = 0.37$, $\pi_T = 0.27$, $\pi_C = 0.18$, and $\pi_G = 0.18$) and a bias towards transition substitutions (C↔T and A↔G) over 88 segregating sites. The relative rates for each substitution type (A↔C = 1.09, A↔G = 4.38, A↔T = 1.19, C↔G = 1.66, C↔T = 4.01, relative to G↔T = 1.0), was estimated under a general time-reversible model (Rodríquez et al. 1990) with maximum likelihood on a neighbour-joining tree.

The identity of all larvae was unambiguously assigned to one of three *Galaxias* taxa. Eight *G. brevipinnis*, 18 *G. postvectis* and 16 *G. fasciatus* were detected, but no *G. maculatus* or *G. argenteus*. Combining the 42 sequences with published data (Waters et al. 2000; Waters & Wallis 2001) gave a total of 13 *G. brevipinnis* sequences (all with unique haplotypes; $h = 1.0$), 19 *G. postvectis* (13 haplotypes; $h = 0.94$), and 17 *G. fasciatus* (9 haplotypes; $h = 0.71$). Populations of *G. brevipinnis* showed the highest level of nucleotide diversity ($\pi = 0.0124$ (SE = 0.0034)), whereas for *G. postvectis* $\pi = 0.0103$ (SE = 0.0031) and *G. fasciatus* $\pi = 0.0051$ (SE = 0.0018). Only having the reference sequence from *G. argenteus* prohibited an estimate of their population level diversity.

The level of sequence divergence between species (*d*) was estimated using all pair-wise comparisons with a two-parameter correction model, and considering each haplotype only once. The difference between *G. maculatus* and each of the other species was significantly greater (overall d = 0.4008 (SE = 0.0516)), than the difference found in any of the other comparisons of the four galaxiids. When *G. brevipinnis* was compared with *G. postvectis* (d = 0.1375 (SE = 0.0207)), *G. fasciatus* (d = 0.1654 (SE = 0.0244)) and *G. argenteus* (d = 0.1463 (SE = 0.0236)) there was no overlap between interspecific (*d*) and intraspecific (π) levels of variation. Similarly, *d* for *G. postvectis* with *G. fasciatus* was 0.1712 (SE = 0.0249) and with *G. argenteus* was 0.1412 (SE = 0.0222), and lastly for *G. fasciatus* with *G. argenteus* was 0.1343 (SE = 0.0218). An unrooted neighbour-joining tree, constructed from the distance matrix of all pair-wise sequence comparisons, is presented in Fig. 2.

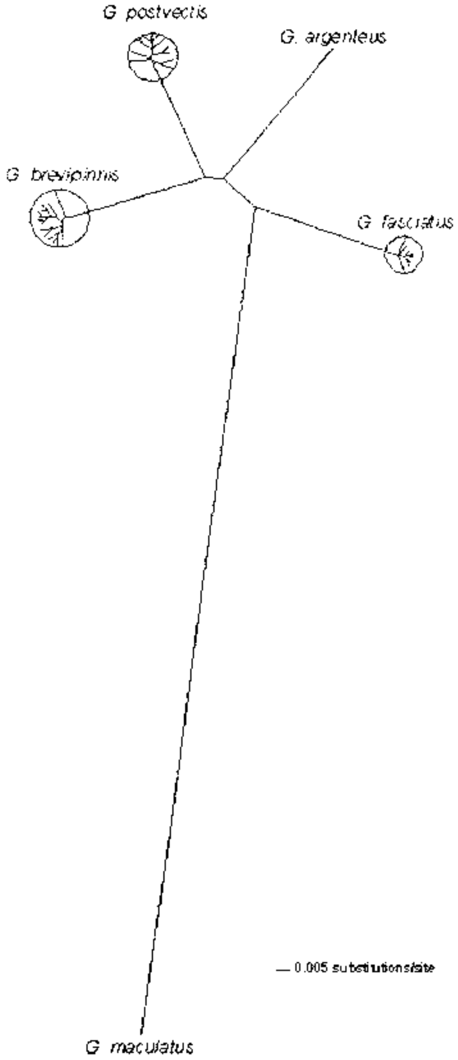

Fig 2. An unrooted neighbour-joining tree constructed from a pair-wise distance matrix of the five diadromous galaxiid species.

Twelve of the 16 nests found in the Katikara Stream were identified as *G. postvectis*, and four were *G. fasciatus*. Three *G. postvectis* and two *G. fasciatus* nests were found at site 1, seven *G. postvectis* at site 2, and two *G. postvectis* and two *G. fasciatus* nests at site 3 (Fig. 1). In the daily drift samples six *G. fasciatus* and five *G. brevipinnis* larvae were identified moving downstream at sites 1 & 2, whereas three *G. postvectis* were recorded at site 1 (Fig. 1; Chapter 3). Finally, 12 whitebait from Taranaki and Manawatu Streams were identified to be three *G. brevipinnis* (sites 5 & 7), six *G. fasciatus* (sites 5 & 6) and three *G. postvectis* (sites 4, 6 & 7) (Fig. 1). *Galaxias brevipinnis* and *G. postvectis* whitebait were found migrating from the sea in October and c. 40 km inland in December and January. While *G. fasciatus* whitebait was caught in October near the sea.

DISCUSSION

The problem of species identification at early life stages has hindered study of the basic factors affecting juvenile fishes and in particular those that can determine recruitment (Leary & Booke 1990). There is a need for a robust method of identification of the whitebait species and HVRI-sequences provide a good approach to this previously intractable problem. Previously problems have been found in hatching and sequencing whole eggs (R.M. Allibone pers. comm.). In this study tissue type or preservation did not affect the overall success of DNA extraction, amplification or sequencing, and multiple samples showed consistent and repeatable results. Moreover, the HVRI was routinely amplified from DNA extracted from single unfertilised *G. postvectis* eggs. An oocyte contains about 10^4 copies of a mt genome, whereas somatic cells only possess between 10 and 10^2 copies (Pikó & Taylor 1987). Hence an unfertilised egg that only has two copies of most nuclear genes represents an abundant source of mtDNA. Therefore any form of galaxiid egg or larvae found can be identified using this method.

A comparison of the level of genetic diversity among all the migratory galaxiids (excluding *G. argenteus*) revealed that *G. fasciatus* is the least diverse. The widespread and very common *G. maculatus* shows the highest level of haplotypic diversity (h = 0.999) with only five shared haplotypes among 139 individuals (Waters et al. 2000). In *G. fasciatus* the same haplotype was found in 10 individuals, with a distribution ranging from the Katikara Stream (the present study) to the Deborah Bay Creek, Otago (Waters & Wallis 2001). Low haplotypic diversity can be indicative of a small or declining effective population size, but a larger sample is needed for this type of inference (Grant & Bowen 1998).

Twelve *G. postvectis* and four *G. fasciatus* nests were identified within the Katikara Stream sites and there was no evidence for mixed species nests. Larvae of *G. postvectis*, *G. fasciatus* and *G. brevipinnis* were also identified in drift samples collected from nets set between May and June 2001. This is the first time *G. postvectis* nests and emigrating larvae have been found, and confirms that these species spawn in autumn (Chapter 2; McDowall 2000). Previously only *G. fasciatus* and *G. brevipinnis* larvae have been recorded moving downstream in June and March respectively (Ots & Eldon 1975; McDowall & Suren 1995). We identified *G. fasciatus* nests and larvae during May and June, which is earlier than Ots & Eldon (1975) (Chapter 4). No *G. brevipinnis* nests were detected in the present study, but larvae were shown to be moving downstream, hence nests must have been present. One *G. brevipinnis* nest has been previously documented in New Zealand, found in the Katikara Stream in late May 2000 (Allibone & Caskey 2000). Therefore spawning and subsequent hatching of eggs of these three species occurs in autumn (Chapter 4). This identification has allowed timing and nest site selection of whitebait species to be studied (Chapter 4).

Since *G. postvectis* and *G. brevipinnis* are morphologically indistinguishable at the whitebait stage (McDowall 1990; McDowall et al. 1996), it has never been possible to determine when and to which rivers *G. postvectis* whitebait migrate. Only a few individuals were sampled and identified, but from these data migration times of the diadromous galaxiid species back into freshwater can be suggested. Three *G. postvectis* were identified from the 12 whitebait sampled. In the two Taranaki rivers sampled, *G. postvectis*, *G. fasciatus* and *G. brevipinnis* were found entering river mouths during October, while *G. fasciatus* and *G. brevipinnis* were also present 40 km inland in October. Samples from the two tributaries of the Manawatu River showed that *G. postvectis* and *G. brevipinnis* were inland a significant distance by December and January. Therefore, these three species migrate from the sea into freshwater in October in Taranaki (Chapter 6). A more detailed analysis of whole catch compositions and timing of the species upstream migration are detailed in Chapter 6.

This study has shown that the HVRI can be used to distinguish diadromous galaxiid species at all life stages from unfertilised eggs to the adult fish. These methods are amenable to non-destructive sampling techniques since they only require a small amount of tissue. However, the identification of species from fixed mtDNA sequence differences assumes there has been no recent introgression of haplotypes among the taxa. This assumption can be tested as more mtDNA sequences are collected from the adults of each whitebait species. In the future, HVRI-identification of whitebait species will allow for more accurate measures of the taxonomic composition, their nesting habitat and timing of migration in this important New Zealand fishery.

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4

Nest site selection, egg development and emigration of diadromous galaxiids in northwest Taranaki.

ABSTRACT Twelve *Galaxias postvectis* (shortjaw kokopu) and four *G. fasciatus* (banded kokopu) nests were found on the margins of the Katikara Stream, Taranaki. Nests were found above base flow and near bankfull amongst substrate, vegetation and debris. Most *G. fasciatus* appeared to lay their eggs and subsequently hatch around three weeks earlier than *G. postvectis,* which spawned from May 9 to 17, 2001. *Galaxias brevipinnis* (koaro) larvae were also identified emigrating downstream in late May and mid June, 2001. Deposition of eggs and subsequent hatching was found to be associated with elevated flows.

Keywords: Spawning; nests; eggs; *Galaxias; G. postvectis; G. fasciatus; G. brevipinnis*, emigrating larvae.

INTRODUCTION

The New Zealand freshwater fish fauna is dominated by diadromous species (McDowall 1990). Five of these diadromous species, that require access to and from the sea to complete their lifecycles, belong to the genus *Galaxias* and are commonly known as whitebait species. The most common is *G. maculatus* (inanga), which are schooling fish that are often found in modified habitat throughout New Zealand. The other species, *G. postvectis, G. fasciatus, G. brevipinnis,* and *G. argenteus* (giant kokopu), are also found throughout New Zealand, however in lower densities and are most often found in streams lined with native bush (McDowall 1990). The breeding biology of *G. maculatus* has been well studied (Benzie 1968a; 1968b; 1968c; McDowall 1968; Mitchell 1991), however limited information is known about the remaining galaxiid species.

Galaxias brevipinnis nests have been found in New Zealand (Allibone & Caskey 2000) and southeastern Australia (O'Conner & Koehn 1998) in autumn around adult habitat; and McDowall & Suren (1995) have recorded *G. brevipinnis* larvae emigrating downstream in March in a South Island stream. Spawning of *G. fasciatus* has been observed in June among flooded terrestrial vegetation, and nests have been found on stream margins among vegetation (Mitchell & Penlington 1982; Mitchell 1991). Downstream movement of emigrating larvae have also been recorded during a flood in June (Ots & Eldon 1975). No observations of spawning, emigrating larvae or egg deposition have been documented for *G. argenteus* or *G. postvectis.* McDowall & Kelly (1999) have suggested from an otolith study of whitebait that spawning occurs for *G. argenteus* between early June and early August. Ripe *G. postvectis* have been found as early as March and spent fish have been found in May, suggesting spawning during autumn and early winter (McDowall 1990; McDowall et al. 1996; Caskey 1999).

Understanding where and when these species spawn is vital for future freshwater management. Therefore this paper documents the occurrence and nest site selection of *G. postvectis* and *G. fasciatus* in northwest Taranaki. Egg development at the Katikara Stream and within a controlled temperature is also documented, and the occurrence of emigrating larvae is recorded.

MATERIALS AND METHODS

Study area

The study was conducted in three sites of c. 100 m in the Katikara Stream on the northwestern slopes of Mt. Taranaki (Fig. 1). The Katikara Stream is a stable stream with native forest riparian margins and substrate dominated by boulders and cobbles. Three km of the stream is within the Egmont National Park, and the remaining 15 km flows through a mix of dry stock and dairy farming areas. *Galaxias postvectis* dominated the fish fauna of all sites (Chapter 2). Individual site details at base flow are shown in Table 1.

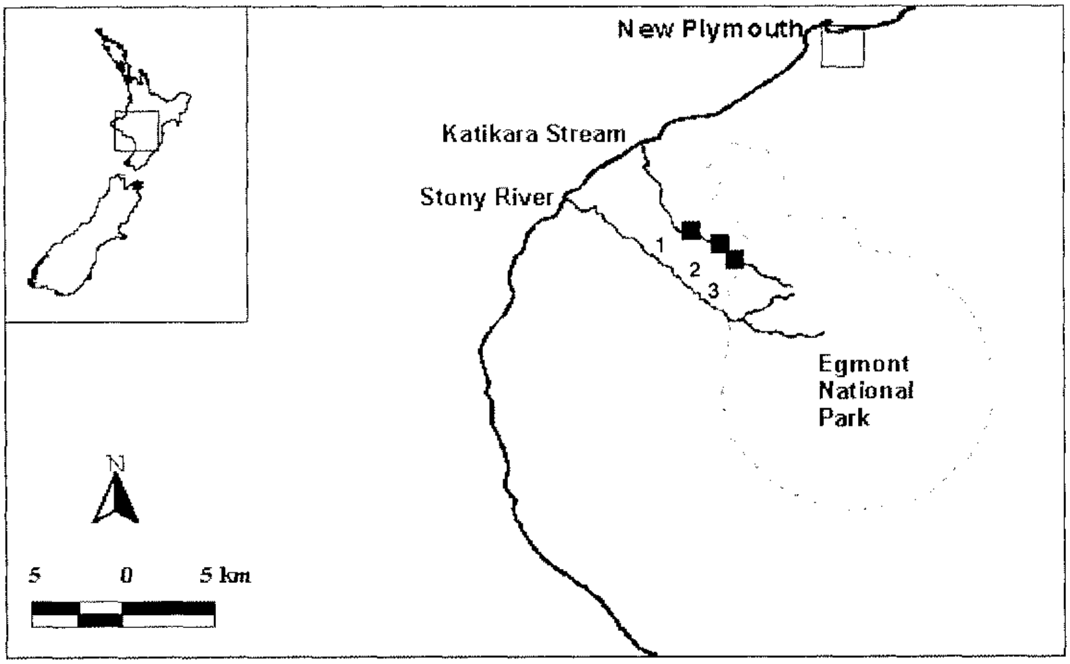

Fig. 1 A map of northwest Taranaki showing the location of the three study sites. 1 = lower Katikara site; 2 = mid Katikara site; 3 = upper Katikara site.

Table 1. Physical characteristics and fish densities of the three study sites in the Katikara Stream, Taranaki.

Study Sites	Lower Katikara	Mid Katikara	Upper Katikara
NZMS Grid reference[1]	25924E 62206N	25933E 61197N	25937E 61195N
Altitude (m)[1]	300	420	440
Mean gradient (mm)[2]	257	184	311
Mean wet width (mm)[3]	635	396	416
Mean bankfull (mm)[3]	1328	770	900
Mean water depth (mm)[4]	200	217	196
Embededness[5]	4	4	4
Median substrate size index[6]	6.44	6.56	6.22
Pfankuch stability score[7]	49	56	49
Overhead cover (%)[8]	20	35	81
Land use (%)[8]	50 native 50 farmland	60 native 40 modified	100 native
Debris jams (%)[8]	7	13	4
Habitat type[8]			
Pool	45	53	30
Run	23	30	55
Riffle	27	12	3
Fall	1	2	10
Backwater	5	3	2
G. postvectis[9]	19	29	17
G. fasciatus[9]	5	0	3
G. brevipinnis[9]	3	1	5
G. argenteus[9]	1	0	0
Gobiomorphus huttoni[10]	P	A	A
Anguilla dieffenbachii[10]	P	P	P

1 Obtained from 1:50,000 NZMS topographic maps.
2 Mean of all measures every 5 m using a Theodolite (Berger Instruments, 3H6649).
3 Mean of five measures over the study reach.
4 Mean of five depth measurements across the width of a transect at the five points above.
5 Subjectively assessed at site after moving substrate (1 = loosely packed; 4 = tightly packed).
6 150 stones were collected using the Woman walk method and the index calculated (Wolman 1954).
7 Visually assessed at site (Pfankuch 1975).
8 Visually estimated at site.
9 Number of individually tagged fish caught spotlighting between March 30 and June 4, 2001 (Chapter 2).
10 Species seen between March 30 and June 4, 2001 (P = present; A = absent).

Habitat assessment

Prior to spawning, habitat measurements were made between March 30 and April 2, 2001 at base flow conditions within each site. Habitat was recorded in 100 randomly located 20 x 20 cm quadrats within each site. Each quadrat was characterised as a pool, backwater, run, riffle or fall habitat area in the water, or if the quadrat was on the bank the adjacent habitat area was recorded. A pool was defined as a body of slowly moving water; a backwater as still water connected to but not part of the main channel or of a channel only active during a flood; a riffle as shallow, usually broken and fast moving water; a fall as fast flowing water over a short steep gradient; and a run as slow moving water, with a calm or rippled surface. Maximum water depth and distance to the closest waters edge was recorded, this value was positive if the quadrat was in the water and negative if on the bank. Percentage of substrate and vegetation present was visually assessed using the categories of boulders (>256 mm), large cobbles (128-256 mm), cobbles (64-128 mm), pebbles (16-64 mm), gravel (4-16 mm), fines (<4 mm), small vegetation/debris, and logs. Embededness was subjectively assessed after moving substrate by hand (1 = loosely packed; 4 = tightly packed). Slope at each quadrat was measured in degrees using an abney level and cover visually assessed as absent or present.

The same characteristics were also recorded at all nest sites, however slope from nest sites to the waters edge was measured with a Theodolite (Berger Instruments, Massachussets, 3H6649). Date, number, approximate development (eyed or not eyed), area covered and spread of eggs were noted. The vertical height above base flow was also measured with a Theodolite.

Spawning sites

Once spent *G. postvectis* were observed, intensive searches for nest sites were carried out from May 2 to 17, 2001. Searches were conducted during daylight hours in the shallow water and along the margins of the Katikara Stream, throughout and near all sites up to bankfull. Searching consisted of using a 30-watt handheld spotlight and examining all crevices, available substrate, woody material, debris deposits and other vegetation. All material seen was moved where possible and examined for egg deposits on, amongst, or under them.

Eggs and larvae

Diameters of 50 randomly chosen eggs per nest, all eggs in drift samples and all larvae were measured using Mitutoyo^{TM} (CD 8"CS) digital callipers. Approximately 50 eggs were also taken to Massey University, Palmerston North, where they were kept in sealed plastic containers with damp moss, at 14ºC and under a 12 hour light: dark regime. After three weeks 5-12 eggs per nest were hatched by immersion in moving water, and the newly hatched larvae were measured. A selection of larvae were then immediately preserved in 70% ethanol for identification using mitochondrial (mt) DNA sequences (Chapter 3).

Triplicate drift nets were set on both sides of the stream in the main current, at the downstream margins of the lower and mid Katikara sites. Drift samplers (mouth opening 55 x 105 mm, 250 µm mesh, length 800 mm) were secured to steel waratahs driven into the substrate. They were set from May 6 to June 17, 2001 and were checked and sorted every 24 hours. Eggs and larvae caught were measured and preserved in 70% ethanol, for identification using mtDNA sequences (Chapter 3).

Observations of eggs from each nest were made every 1-2 days on the banks of the Katikara Stream and in a controlled temperature room at Massey University. The development of eggs was compared to published data on *G. maculatus* by Benzie (1968c). Hatching of eggs at sites was also recorded.

Environmental variables

Rainfall at each site was recorded every 24 hours. Maximum water level at the lower and upper Katikara Stream sites was measured every 24 hours using Manning's pegs, with classic school paste™ dissolved in water with red Chromos Tempera Powder™ for colour. Humidity and ambient air temperature was recorded every 10 minutes, using Onset – Hobo pro series™ data loggers at two sites on the Katikara Stream, one under complete (upper Katikara site) and one in open (mid Katikara site) canopy. Onset tibet™ data loggers were also placed in pools of the lower and upper Katikara sites recording instream temperature every 10 minutes.

Data analysis

T-tests were carried out using Excel to determine size differences between species and over time. All other analysis was conducted using SAS (2000). ANOVAs (Analysis of Variance) were used to examine differences in egg and larvae size between nests. Discriminate and stepwise discriminate analyses were performed on all habitat variables to differentiate nest or non-nest sites for both *G. postvectis* and *G. fasciatus*.

RESULTS

Spawning sites

Sixteen nests were found between May 9 and 17, 2001. Three *G. fasciatus* nests were found on May 9 with eyed eggs, whereas all other nests (1 *G. fasciatus* & 12 *G. postvectis*) were found after floods between May 11 and 17, and had eggs with no eyes. All nests were found on the banks of the stream adjacent to pool and backwater habitats with resident *G. postvectis* or *G. fasciatus*. Nine nests were found within a few metres of another nest. Five nests, three *G. postvectis* and two *G. fasciatus,* were found around one pool in the upper Katikara site. Two male and four female *G. postvectis* and three *G. fasciatus* were recorded in this area between March and June. The number of female and males per site did not reflect the number of nests found. One nest was found at the same site (nest 13) as a nest found the previous year, and nest 5 was found close by to another nest discovered previously (R.M. Allibone & D. Caskey, unpublished data).

Nest site selection

Nest sites are quite variable in appearance (Plate 1). All were found on the banks of the Katikara Stream, near or just above bankfull, from 155 to 700 mm above the water, and 300 to 4300 mm from the wetted stream at base flow (Table 2). Most nests were generally downstream facing on sloping ground among tightly packed substrate and had at least a small amount of vegetation and debris. Nests covered an area from 0.1 to 2.8 m^2 and layers of eggs 1-2 deep were found scattered throughout, with a few higher density clumps. *Galaxias fasciatus* nests found on May 9 appeared to have fewer eggs per nest (Table 2).

A greater abundance of vegetation / debris ($P < 0.0001$), gravel ($P < 0.0001$), cover ($P = 0.003$), backwater ($P = 0.005$) and pool habitats ($P = 0.003$) was found to differentiate nest sites from non-nest sites. However, crossvalidation yielded four (Nests 4, 6, 13, 16) of the 16 nests reclassified as non-nest and 18 of the 300 quadrats as potential nest sites. Analysing only *G. postvectis* nests, greater vegetation / debris ($P < 0.0001$), gravel ($P < 0.0001$), cover ($P = 0.004$) and backwater ($P = 0.03$) were again important in differentiating nest sites, with greater distance to water ($P = 0.003$) also important. Once more crossvalidation yielded four (Nests 4, 6, 12, 16) of the 12 *G. postvectis* nests misclassified and 18 quadrats as nests. Analysing *G. fasciatus* nests only, a greater presence of vegetation / debris ($P < 0.0001$) and cover ($P < 0.0001$) was found to be important, with no nests misclassified as quadrats and three quadrats as nests. Using only the habitat factors important for discriminating nests, vegetation / debris ($P = 0.02$) was the greatest difference between *G. fasciatus* and *G. postvectis* nests, with *G. fasciatus* having more vegetation and debris than *G. postvectis* nests (Table 2). One *G. fasciatus* nest was misclassified as *G. postvectis* and five *G. postvectis* as *G. fasciatus.*

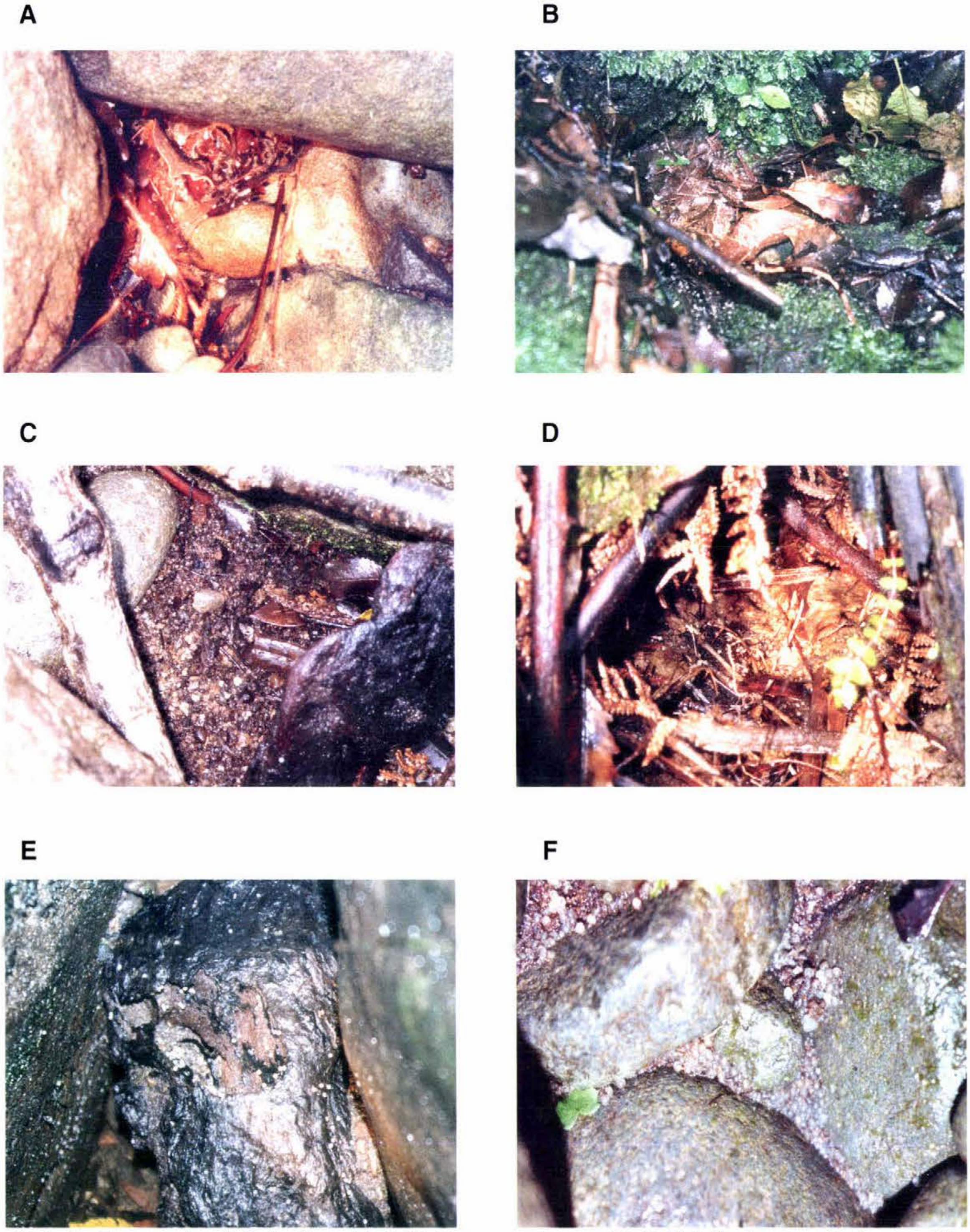

Plate 1 Photographs of *G. fasciatus* (A & B) *G. postvectis* nests (C, D, E & F). A = nest 1; B = nest 10; C = nest 7; D = nest 11; E = nest 8; F = nest 6.

Table 2 Characteristics of the 16 galaxiids nests found along the banks of the Katikara Stream, Taranaki in May 2001.

Nest	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Date found	9/05	9/05	9/05	11/05	11/05	11/05	11/05	12/05	12/05	12/05	15/05	15/05	15/05	17/05	17/05	17/05
Species[1]	B	B	B	S	S	S	S	S	S	B	S	S	S	S	S	S
Site found[2]	U	U	L	U	BM	M	MBU	U	L	L	M	MBU	M	AU	AU	U
Distance to waters edge (mm)	1600	1500	300	1500	2500	600	600	2000	2000	1700	2500	1100	300	1800	2000	4300
Distance to bankfull (mm)	400	0	100	0	1000	400	0	0	60	250	0	300	150	200	200	0
Adjacent habitat type[3]	P	P	BW	P	BW	P	P	P	P	BW	BW	P	P	BW	BW	P
Cover[4]	P	P	P	P	P	A	A	A	P	P	P	P	P	A	P	P
% substrate & vegetation[5]																
Boulders (>256 mm)	0	18	5	40	0	25	5	50	0	35	20	30	50	0	20	35
Large cobbles (128-256 mm)	40	0	0	20	0	50	0	0	10	10	0	10	0	0	15	35
Cobbles (64-128 mm)	5	0	0	0	0	10	0	0	20	0	0	5	0	0	5	5
Pebbles (16-64 mm)	0	0	0	0	0	0	0	0	5	0	0	5	0	0	0	0
Gravel (4-16 mm)	2	2	0	10	0	0	90	0	15	0	0	0	15	0	35	0
Fines (<4 mm)	0	0	0	10	20	10	0	5	25	10	20	10	30	10	15	20
Small vegetation/debris	60	80	95	20	80	5	5	20	25	45	60	35	5	70	5	5
Logs	0	0	0	0	0	0	0	45	0	0	0	5	0	20	5	0
Embededness[6]	4	5	5	3	5	4	2	5	4	4	3	4	5	5	5	3
Vertical height (mm)	340	486	196	663	155	248	358	208	343	290	484	318	399	265	320	700
Slope (°)	30	30	30	20	20	20	10	10	20	20	30	25	40	10	5	20
Direction[7]	D	D	N	D	D	N	D	D	D	N	D	D	D	D	D	N
Approximate no. of eggs	100	200	10	600+	200	1000	700+	500+	400+	1000	600+	150	300	200	800	500
Area covered (m^2)	0.1	1	0.9	1.2	0.3	2.8	1.5	0.6	1.8	1	1.25	0.6	0.8	0.3	1.2	2
Spread of eggs[8]	SC	SC	SS	SC	S	SC	SM	S	S	SC	SC	S	S	S	SC	SC
Eggs appearance[9]	E	E	E	N	N	N	N	N	N	N	N	N	N	N	N	N

1. Identified using mitochondrial (mt) DNA sequences (Chapter 3).
2. L = lower Katikara site; M = mid Katikara site; U = upper Katikara site; BM = downstream of M; MBU = between M & U; AU = upstream of U.
3. P = pool; BW = backwater.
4. P = present; A = absent.
5. Visually assessed using categories from Wolman (1954).
6. 1 = loosely packed; 4 = tightly packed.
7. D = downstream facing; N = no direction.
8. S = scattered; C = clumped; M = continuous mat.
9. E= eyed eggs; N= early stage of development, no eyes.

Eggs, larvae and development

All fertilised eggs found were clear, smooth surfaced and spherical. Mean egg diameter of *G. postvectis* was 2.05 mm (*n* = 593, range 1.16 to 2.40 mm) and *G. fasciatus* was 2.06 mm (*n* = 156, range 1.75 to 2.97 mm). There was also a difference in egg diameters found in the different nests ($F_{14, 733} = 5.54$; $P < 0.001$), but not between species ($F_{1, 733} = 0.47$; $P = 0.49$).

Eggs kept in the lab at 14°C, which appeared undeveloped on collection took between 10 to 18 days to fully develop. One egg kept in stream water from May 13, remained viable until July 2, when the larvae tried to hatch but died half hatched. Five *G. postvectis* eggs kept on damp moss at the lab from mid May until mid July all hatched successfully when re-immersed in water and shaken. Thus *G. postvectis* eggs could remain viable damp but out of water for at least 1½ months.

On the stream bank eggs appeared to take around three to four weeks to develop with all eggs fully developed by early June. At this time, instream temperature ranged from 6-14°C and ambient air temperature from 0-19°C. Nest sites appeared to be damp and moist at all times and relative humidity stayed fairly constant at around 100% (range = 51–103). Eggs in all nests were completely submerged many times throughout May, and although egg numbers declined most remained. Not all nests disappeared at the same time, but all eggs were completely gone by June 17. The three *G. fasciatus* nests found on May 9 were gone by May 15. Nest distance to the waters edge ($P = 0.42$) and height above base flow ($P = 0.29$) were not related to the timing of the disappearance of eggs.

Eggs were caught drifting downstream from May 7 to June 17, and newly hatched larvae (Fig. 2) were recorded from May 11 to June 17, on elevated flows (Fig. 3). All collected larvae had prominent yolk sacs. Twenty larvae and four eggs was the maximum caught in one 24 hour period. Six *G. fasciatus*, five *G. brevipinnis* and three *G. postvectis* larvae were identified moving downstream (Chapter 3). *Galaxias fasciatus* were recorded on May 11, 12, 13, 14, 26 and June 17. *Galaxias brevipinnis* was identified on May 26 & 27 and June 10, and *G. postvectis* was found on June 10, 11 & 17. It appeared that hatching of galaxiid eggs occurred during flood flows (Fig. 3).

Emigrating larvae and eggs were not caught drifting downstream during normal flows. Hatching also coincided with a drop in water and air temperature by several degrees during May and June.

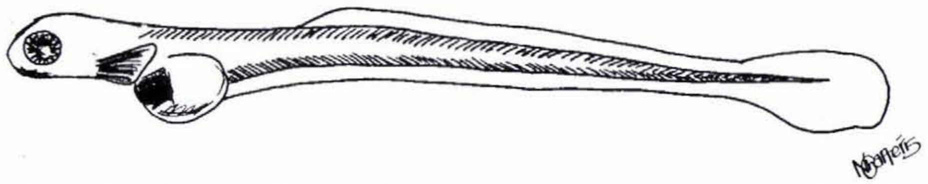

Fig. 2 Newly hatched G. *postvectis* larvae (length = 8.9 mm) from an egg collected at the Katikara Stream in May 2001.

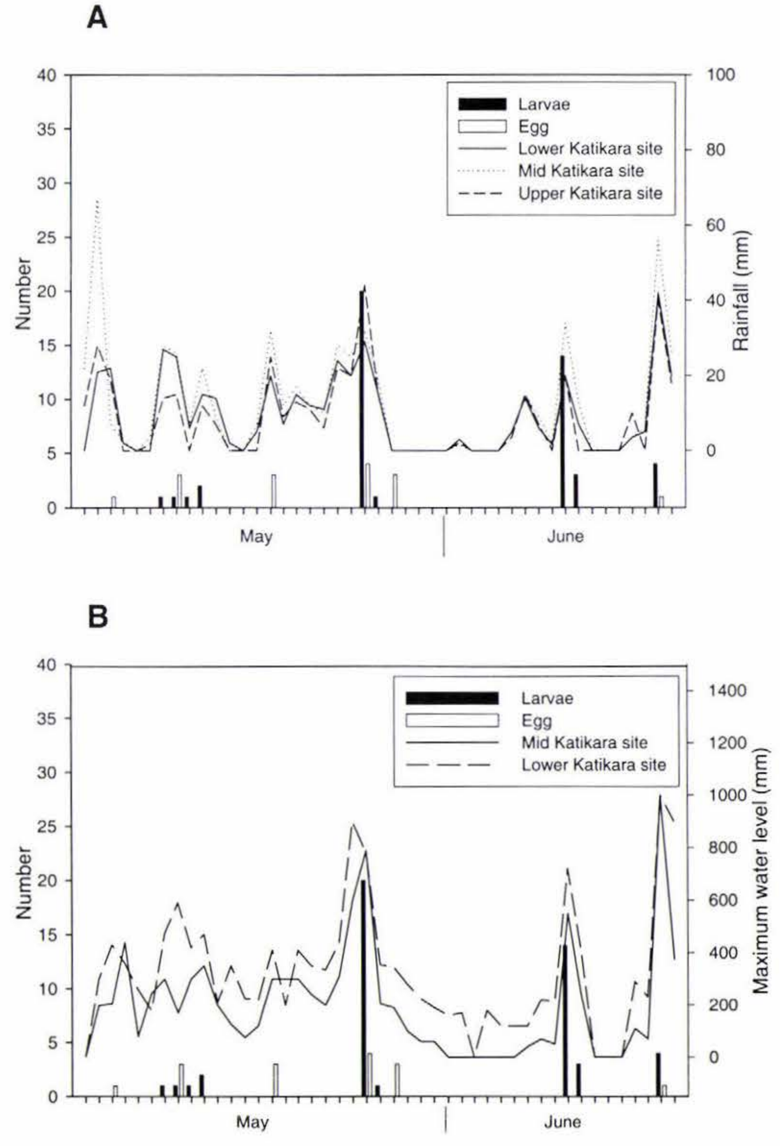

Fig. 3 The number of larvae and eggs caught in drift samples between May 7 and June 17, 2001 as a function of daily rainfall (A) and maximum water level (B).

Larvae collected from drift samples had a mean length of 8.9 mm (n = 36, range 6.9-10.5 mm) and the eggs were 1.9 mm in diameter (n = 14, range 1.5-2.2 mm). Larvae caught did not differ in size between capture dates (P = 0.41) and nor did eggs (P = 0.37). The mean size of larvae hatched from all nests kept at Massey University was 8.8 mm (n = 146, range 5.4-10.4 mm). The mean length of *G. postvectis* larvae was 8.8 mm (n = 110, range 5.4-10.4 mm) and mean length for *G. fasciatus* larvae was 9 mm (n = 36, range 8.2-9.8 mm). The mean length differed between species ($F_{1,\ 129}$ = 4.91; P = 0.03) and nests ($F_{14,\ 129}$ = 3.76; P <0.001), with *G. fasciatus* having slightly longer larvae than *G. postvectis*. Drift larvae and nest larvae were not different (P = 0.53), however drift eggs were smaller than nest eggs (P = 0.02).

DISCUSSION

Sixteen galaxiid nests were discovered along the banks of the Katikara Stream, Taranaki between May 9 and 17, 2001 after increased flows. Twelve nests were identified as *G. postvectis* and four as *G. fasciatus* (Chapter 3). No *G. argenteus* and *G. brevipinnis* nests were found even though adults were present at the sites. *Galaxias fasciatus, G. brevipinnis* and *G. postvectis* larvae were also identified moving downstream between May and June (Chapter 3), with all eggs at nests disappearing by June 17. This is the first time *G. postvectis* nests have been identified, and coincides with my observations of spent fish in May at these sites (Chapter 2).

Galaxias fasciatus nests found with small numbers of eyed eggs suggested that these nests had been laid several days prior to being found. Later in May another *G. fasciatus* nest was discovered with high numbers of early development stage eggs. Therefore similar to Hopkins (1979) and my observations (Chapter 2), *G.* fasciatus spawning occurs from late April to mid June. Most *G. fasciatus* hatching was recorded in May and mid June in the Katikara Stream, similar to Ots & Eldon (1975). McDowall et al. (1994) also found that hatching for *G. fasciatus* is synchronous with a major peak followed by a secondary hatching pulse around 30 days later. This suggests there are two peaks in spawning and subsequent hatching as found in this study, with most *G. fasciatus* spawning in late April and another lot in mid May. *Galaxias brevipinnis* larvae were identified moving downstream in late May and mid June. Therefore *G. brevipinnis* eggs must have been deposited but not found, and hatched with emigrating larvae moving downstream around two months later in the Katikara Stream, than previously found in New Zealand (McDowall & Suren 1995). However timing was similar to O'Conner & Koehn (1998) that recorded Australian *G. brevipinnis* larvae emigrating in mid June. This suggests *G. brevipinnis* begin spawning after *G. fasciatus* but before *G. postvectis* in the Katikara Stream, although all spawning did overlap.

As with other galaxiids, all *G. postvectis* and *G. fasciatus* egg masses were found near adult habitats in stream margins at the limit of increased flows (Mitchell & Penlington 1982; Mitchell 1991; O'Conner & Koehn 1998; Allibone & Caskey 2000). Like Mitchell & Penlington (1982) and Mitchell (1991), *G. fasciatus* eggs were deposited amongst flooded terrestrial twigs and leaves at the Katikara Stream. The terrestrial deposition of eggs is quite an unusual reproductive strategy for fish, with only a few species worldwide recorded to spawn on the river bank, one being *G. maculatus,* the most common whitebait species (Balon 1981). However, bankside spawning has not been reported in non-diadromous galaxiids, which are known to spawn in the water (Benzie 1968a; Cadwallader 1976; Allibone & McDowall 1997; Allibone & Townsend 1997). This major difference in life history strategies between these closely related species may be a result of a diadromous galaxiid mechanism to avoid instream predation. Therefore bankside spawning seems to be a diadromous galaxiid trait.

Riparian margins of streams especially near backwater and pool habitats with resident adult galaxiids, with obvious cover, gravel, small vegetation and debris need to be protected from stock trampling and other forms of modification to promote future survival of *G. postvectis* and *G. fasciatus*. These habitat factors were found to be important at all nests for both species. Nest site selection was similar between *G. postvectis* and *G. fasciatus* with the former having a slight tendency to have more vegetation and debris in their nests. A limited number of possible nest sites appear to be available within sites. In addition it appears that *G. postvectis* may select the same or close by nest sites each year for spawning, so protection of such areas is vital.

Fertilised *G. postvectis* and *G. fasciatus* eggs were around 2 mm in diameter, similar to what Hopkins (1979) and Mitchell & Penlington (1982) have found for *G. fasciatus*. Interestingly egg size was different among nests. In other species of fish, egg size has been found to differ with fish size (Kamler 1992), and a range of *G. postvectis* and *G. fasciatus* were captured around nests in the Katikara Stream (Chapter 2). Eggs collected in drift samples were slightly smaller than nest eggs. This may be because eggs caught in the drifts were dead or unfertilised; and unfertilised *G. fasciatus* eggs have been found to be smaller than spawned eggs (Hopkins 1979). Similarly to Ots & Eldon (1975) and Hopkins (1979), *G. fasciatus* newly hatched larvae were found to be around 9 mm, and were slightly longer than *G. postvectis*. The larger size of *G. postvectis, G. fasciatus* and *G. brevipinnis* larvae and eggs compared with *G. maculatus,* maybe a result of the distance they have to travel to get to the sea where they get their first meal (McDowall 1990). Adults of *G. postvectis, G. fasciatus* and *G. brevipinnis* are found long distances inland, while *G. maculatus* is found usually at low elevations and nearest the sea, and therefore need less reserves to survive (McDowall 1990).

Egg development took around a month on the banks of the Katikara Stream. This is similar to what Mitchell & Penlington (1982), O'Conner & Koehn (1998) and Allibone & Caskey (2000) have found for other galaxiids. In a controlled 14°C, development took approximately two weeks, again similar to other galaxiids in controlled environments (Benzie 1968b; Mitchell 1989). Development was longer at the Katikara Stream than in the controlled environment, as egg development is dependant on temperature (Kamler 1992), and the Katikara Stream has generally lower temperatures. Humidity has been found to be important for successful development and hatching of *G. maculatus* eggs (Taylor 1996), and all nests in this study stayed relatively damp with high humidity.

All hatching and subsequent emigration of larvae occurred on flooded flows, when nests were immersed by water. Spawning also occurred at times of floods (this study), thus timing of spawning and subsequent hatching depends on the timing of flood events during the late autumn and early winter. Floods that cue hatching also assist the larvae with a faster passage to the sea than base flow.

Therefore this diadromous galaxiid reproductive strategy seems very risky with spawning and survival of the young dependant on flooding events at the right time and ideal ambient conditions around nest areas. It seems that galaxiid eggs on the bankside are very hardy and can survive a significant time without a flood. However, the changes in timing frequency and magnitude hypothesised with global warming may be a serious threat to the reproductive cues for these species.

In conclusion the first *G. postvectis* nests were found in May along the margins of the Katikara Stream, and it appears that most *G. fasciatus* spawn and hatch earlier than *G. postvectis*. The requirement for cover and suitable riparian habitat are essential for nest site selection in these species, and increased flows in late autumn for spawning and hatching. Thus it is essential that flows are managed and riparian margins protected along streams to preserve these species.

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5

Determinates of hatching in *Galaxias postvectis* (shortjaw kokopu) and *G. fasciatus* (banded kokopu) eggs.

ABSTRACT An experiment was carried out to investigate if water temperature, light, water movement and time following immersion into water affects the hatching of *G. postvectis* and *G. fasciatus* eggs. The majority of eggs hatched in the first hour after immersion, with only a few hatching in the remaining four hours of the experiment. More hatching occurred in moving than still water. However, the presence of light was not found to affect hatching. There was greater hatching at 15 and 20°C after five but not one hour, and additionally hatching was more apparent in moving water after one hour than overall.

Keywords: *Galaxias*; larvae; experiment; water movement; bubbler; light; temperature.

INTRODUCTION

New Zealand diadromous galaxiids deposit their eggs in a riverine or estuarine environment. The newly hatched larvae migrate to the sea for about four to six months, before returning to freshwater in mixed species shoals, commonly known as whitebait where they mature (McDowall 1990). Whitebait commonly comprises *G. maculatus* (inanga), *G. brevipinnis* (koaro), *G. fasciatus*, *G. postvectis* and *G. argenteus* (giant kokopu) (McDowall 1965). Eggs of *G. maculatus* are laid on vegetated estuarine areas covered by water only at spring tides in early autumn (McDowall 1968). Whereas G. *brevipinnis*, *G. fasciatus* and *G. postvectis* eggs are deposited along the banks of the stream near adult habitat during increased flow events in April and May (Hopkins 1979; O'Conner & Koehn 1998; Allibone & Caskey 2000; Chapter 4). Nothing is known of egg deposition by *G. argenteus*.

Fully developed *G. maculatus* eggs do not hatch when exposed to light rain or condensation (Benzie 1968), but most hatch within minutes at 12-16°C when fully re-immersed in water (Mitchell 1989). Temperature affects the hatching of *G. maculatus,* with Mitchell (1989) recording slow and poor hatching below 10°C. R.M. Allibone (pers. comm.) has found that hatching rate of *G. maculatus* improved dramatically in the dark verses daylight. Furthermore fully developed eggs of the Australian *G. brevipinnis* do not hatch unless they are manually shaken in water, hatching within 30 minutes if treated in such a way (O'Conner & Koehn 1998).

Nothing is known about hatching stimuli of *G. postvectis* and *G. fasciatus* eggs. Although immersion in water has been found to be one obvious cue for other diadromous galaxiids. In this study, we investigate the effect of water temperature, light, water movement and time since immersion on the hatching of *G. postvectis* and G. *fasciatus* eggs.

MATERIALS AND METHODS

Egg collection

Eggs were collected from 12 *G. postvectis* and four *G. fasciatus* nests found between May 9-17, 2001 along the margins of the Katikara Stream, Taranaki (Chapter 4). Approximately 50 eggs per nest were transported back to Massey University, Palmerston North. Eggs were kept in sealed plastic containers on damp moss at a constant 14°C under a 12 hour light: dark regime until June 12, 2001, when the experiment was conducted. All eggs appeared viable and at a stage of development when hatching was highly probable.

Experimental protocol

Four temperature control rooms set at 5, 10, 15 and 20°C were used. Within each temperature there was four 80 ml plastic test containers, 40 mm in diameter and 50 mm high. All eggs collected were pooled and ten eggs assigned at random to each container, no eggs were used more than once. Nests were genetically identified subsequent to the experiment (Chapter 3) and no species difference was expected. Water movement and light treatments were randomly assigned among the four containers. Light was provided with a 60-watt standard white light bulb positioned directly above the test containers, while no light was created by surrounding the test containers with black paper. Water movement was either still (no bubbler) or created with a Super Airmaster™ 2000 bubbler unit.

Forty millilitres of water from the collection stream was added to each test container to signal the start of the experiment and ensure all eggs were completely submerged. The experiment was run for five hours with all containers checked hourly and the number of hatched larvae recorded. Three trials were carried out for all treatments.

Data analysis

The experiment had a split plot design with repeated measures over time. Temperature was the main plot factor, and water movement and light were the spilt plot factors. The number of larvae hatched in each treatment after one and five hours was examined with analysis of variance (ANOVA) using SAS (2000). Lsmeans tests were calculated to assess which treatments were significantly different. Normality and homogeneity assumptions were assessed and met for both data sets.

RESULTS

Most hatching occurred in the first hour following immersion in water at all four temperatures investigated (Fig. 1). Twice as many larvae hatched from eggs in the first hour at 15 and 20°C with a bubbler (Fig. 1). Although there was no significant difference found between the four temperatures at hour one, there was after five hours (Table 1). Water movement produced approximately four times more hatching after one and five hours (Table 1; Fig. 1). Only one trial had 100% hatching, which occurred in the dark at 20°C with a bubbler. Several trials, without a bubbler at warmer temperatures and all treatments at 5°C, had no eggs hatch after five hours. A number of eggs died after five hours in several treatments with the 5 and 10°C treatments generally having the highest mortality (Table 2). All other factors investigated were found to have no effect on hatching (Table 2).

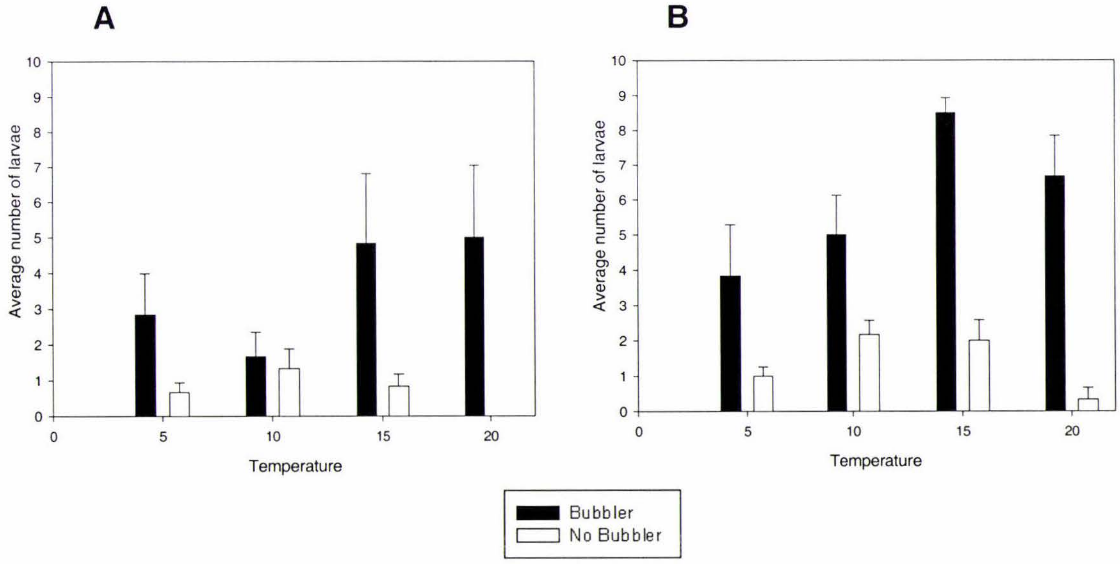

Fig. 1 The average number of *G. postvectis* and *G. fasciatus* eggs hatched per hour for each treatment at four temperatures.

Table 1 ANOVA results from hour one and five data on the effect of temperature, light and water movement on hatching of galaxiid eggs. * = interaction

Source	DF	Error DF	F Value	Pr > F
Hour one				
Temperature	3	8	0.68	0.59
Light	1	24	0.01	0.94
Water movement	1	24	32.10	**<0.0001**
Light*water movement	1	24	0.33	0.57
Temperature*light	3	24	1.01	0.40
Temperature*water movement	3	24	4.12	**0.02**
Temperature*light*water movement	3	24	0.94	0.44
Hour five				
Temperature	3	8	3.72	**0.06**
Light	1	24	0.00	0.95
Water movement	1	24	55.00	**<0.0001**
Light*water movement	1	24	1.29	0.27
Temperature*Light	3	24	0.75	0.53
Temperature*water movement	3	24	2.75	**0.06**
Temperature*light*water movement	3	24	0.28	0.84

Table 2 The number of hatched larvae and unhatched dead and live eggs from all trials for each treatment in all temperatures after five hours.

°C	Treatment	N	Number hatched	Number of dead eggs	Number of live eggs
20	Dark / bubbler	30	16	1	13
	Light / bubbler	30	24	1	5
	Dark / no bubbler	30	2	1	27
	Light / no bubbler	30	0	1	29
15	Dark / bubbler	30	27	0	3
	Light / bubbler	30	24	0	6
	Dark / no bubbler	30	9	0	21
	Light / no bubbler	30	3	2	25
10	Dark / bubbler	30	14	2	15
	Light / bubbler	30	16	3	11
	Dark / no bubbler	30	6	3	21
	Light / no bubbler	30	7	3	20
5	Dark / bubbler	30	11	3	16
	Light / bubbler	30	12	3	15
	Dark / no bubbler	30	4	5	21
	Light / no bubbler	30	2	4	24

Temperature and water movement interacted to alter hatching success (Table 1). After one hour with a bubbler, 5 out of 10 eggs on average hatched at 15 and 20°C, while only 0-3 eggs hatched at 5 and 10°C (Fig. 2). While after five hours most of the eggs had hatched at 15 and 20°C, and around 5 out of 10 eggs had hatched at 5 and 10°C (Fig. 2). After one hour, treatments 15 and 20°C with a bubbler had a greater number hatched than treatments with no bubbler ($P = 0.01$; $P = <0.01$ respectively).

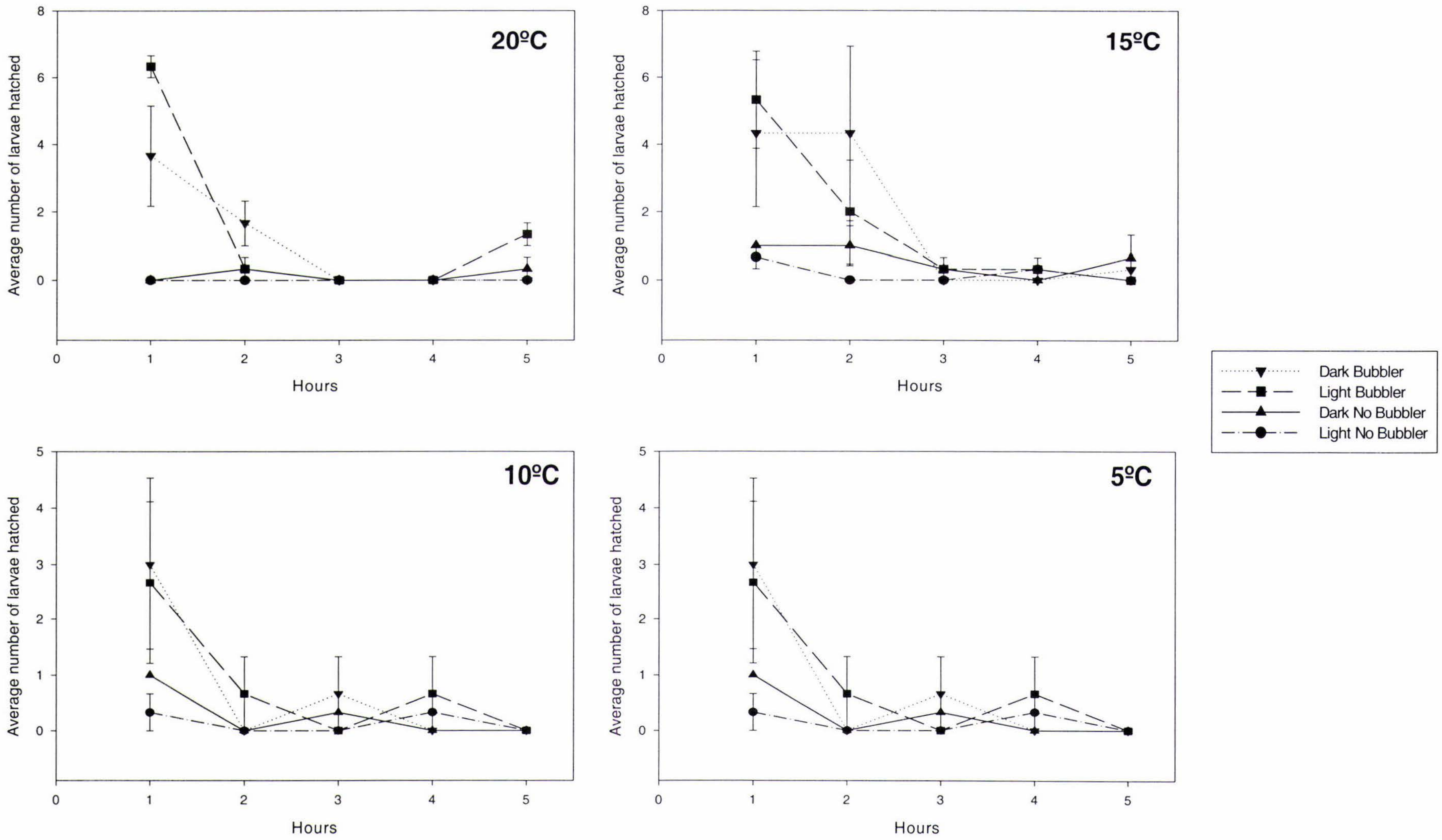

Fig. 2 The average number of eggs hatched after one (A) and five (B) hours with and without a bubbler at 5, 10, 15 and 20°C.

Furthermore both 15 and 20°C with a bubbler was significantly different to most other temperatures with and without a bubbler (*P* = 0.08-0.001; *P* = 0.03-0.001 respectively). Additionally hatching at 15°C with a bubbler after five hours was greater than at 10 and 20°C with a bubbler (*P* = 0.02 - < 0.0001).

DISCUSSION

The majority of *G. postvectis* and *G. fasciatus* eggs hatched within the first hour of submersion in water. This is similar to hatching of *G. maculatus* and *G. brevipinnis* eggs that occurs within 20-30 minutes of flooding with water (Mitchell 1989; O'Conner & Koehn 1998 respectively).

Overall a number of the eggs were found not to hatch with only one trial having 100% hatching success. Mortalities occurred in several treatments and so these galaxiid eggs may not be very hardy and a high mortality might occur in nature. Another explanation may be that eggs were not ready to hatch. This was seen in the Katikara Stream nests when most eggs were at a stage when hatching was likely, but all eggs did not hatch when the subsequent flood occurred.

Water movement was the most important factor that affected galaxiid hatching. O'Conner & Koehn (1998), similarly found that the majority of eggs did not hatch in still water but did if the water was moved. Eggs are clearly stimulated to hatch from the water turbulence of a flood and not by rain or condensation alone (Benzie 1968). *Galaxias postvectis* and *G. fasciatus* nests are found in areas only covered by flowing water during increased flows and generally on sloping ground so water does not form still puddles of water (Hopkins 1979; Chapter 4). Therefore eggs are only exposed to river flows when the stream is in flood, and can only hatch and emigrate to sea at these times.

The number of eggs hatched did not change with or without light. Differences in hatching occurred however between the four temperatures investigated, but only after five hours. Temperature and water movement together affected the hatching of *G. postvectis* and *G. fasciatus* eggs. Approximately double the number of eggs hatched in treatments with a bubbler especially at 15 and 20°C. Thus as in *G. maculatus,* hatching below 10°C was poor (Mitchell 1989). However, we found hatching was not slower in colder temperatures as was found by Mitchell (1989). The warmer temperatures, 15 and 20°C, were the most favourable temperatures at which the eggs hatched in the experiment. In the Katikara Stream, where these eggs were collected, larvae were recorded emigrating downstream in water temperature from 5-15°C and ambient air temperature from 0-14°C (Chapter 4). So successful hatching evidently occurs at a range of temperatures. The failure of experimental eggs to hatch at lower temperatures, as experienced at the Katikara Stream, and the higher rate at warmer temperatures could be due to the constant 14°C pre-experiment conditions. Benzie (1968) has found that *G. maculatus* egg development is very responsive to temperature changes, developing faster in warmer temperatures. Larvae also seem to be more energetic in warmer temperatures. Thus putting eggs previously developing in 14°C into colder temperatures, i.e., 5 and 10°C, may slow down their response and hatching. The higher mortality seen in the lower temperatures could be a result of reduced viability from the sudden change in temperature. Furthermore air temperature on flood days at the Katikara Stream was generally colder by a few degrees, than the instream temperature at the same time. The stimuli for hatching may therefore be an increase in temperature, resulting in the larvae becoming more active as it is warmer and subsequently hatching.

In conclusion over a five hour period, immersion into moving water, and the subsequent temperature change was found to stimulate the hatching of *G. postvectis* and *G. fasciatus* eggs, whereas light did not. Over this five hour period, most hatching occurred in the first hour in turbulent water, with minimal hatching occurring in the remaining four hours especially in still water.

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Spatial and seasonal patterns in whitebait recruitment, and the effect of river impoundment, in south Taranaki.

ABSTRACT Whitebait migrations were investigated within the tidal influence and c. 40 km inland in two rivers in south Taranaki, one with a dam and one without. Peak whitebait migration occurred in September and October 2000, within the tidal influence and in October 2000, 40 km inland. Whitebait catches were dominated by *Galaxias maculatus* (inanga) at all sites and appeared to be similar in both rivers. Large schools of whitebait were seen below the dam from October onwards in the Patea River. But no whitebait were found above the dam. The presence of a dam does not appear to affect whitebait recruitment within the tidal influence, however it does block whitebait migrating further upstream.

Keywords: *Galaxias;* dam; migration; *G. maculatus; G. brevipinnis; G. fasciatus; G. postvectis; G. argenteus.*

INTRODUCTION

Many New Zealand native freshwater fish are diadromous, requiring access to and from the sea to complete their lifecycles (McDowall 1990). Five of these diadromous species are commonly known as whitebait and are *G. maculatus*, G. *brevipinnis* (koaro), *G. fasciatus* (banded kokopu), *G. argenteus* (giant kokopu), and *G. postvectis* (shortjaw kokopu) (McDowall 1965). Whitebait species spawn in freshwater and the newly hatched larvae migrate to the sea. Subsequently four to six months later they return to freshwater, and during this migration in late winter and spring, many whitebait are caught by both recreational and commercial fisherman (McDowall 1990).

Some adult whitebait habitats are long distances upstream in small headwater tributaries (McDowall 1990), and unimpeded access is therefore very important. The large numbers of dams found in the Taranaki region have been found to have a discernable negative effect on freshwater fish communities (Joy & Death 2000). The presence of some structures in rivers can therefore cause problems for fish passage, both up and downstream.

The mechanism underlying the directed movement back into river mouths is unknown, however it has been hypothesised that whitebait generally enter rivers that lead to habitats where adults are present (Rowe et al. 1992; Baker & Montgomery 2000). A variety of variables have been suggested to act as river selection cues, including water temperature, water quality, suitable catchment vegetation and presence of adult whitebait (McDowall & Eldon 1980; Rowe et al. 1992). Rowe et al. (1992) found that the presence of an established population of conspecifics was more important than vegetation type, with *G. brevipinnis* whitebait selecting the river with a higher number of adult *G. brevipinnis* in the upper catchment than other surrounding rivers with few adult *G. brevipinnis*. Furthermore, Baker & Montgomery (2000) found in a laboratory choice study that *G. fasciatus* whitebait showed a species-specific attraction to chemicals produced by adult conspecifics. They demonstrated that *G. fasciatus* whitebait have the ability to discriminate species-specific chemicals during their migratory phase, possibly the basis for river selection.

Despite the fact that whitebaiting is a common past time of many New Zealander's, little has been documented about whitebait migrations in North Island rivers (McDowall 1965; Stancliff et al. 1988; Rowe et al. 1992). If the presence of adult whitebait species in the headwaters is a requirement for juvenile recruitment, then barriers that prevent adult fish from reaching headwater streams should subsequently affect whitebait migration. In this paper I investigate whether the presence of a dam prevents whitebait migrating upstream and influences the recruitment potential of a river. Also the time at sea for whitebait is suggested given spawning of *G. postvectis*, *G. fasciatus* and *G. brevipinnis* during late April/ May 2000 and 2001 in north Taranaki (Allibone & Caskey, 2000; Chapter 2 & 4).

MATERIALS AND METHODS

Study area

The study was carried out at four sites, one within the tidal influence c. 2-3 km from the coastline, and one c. 40 km inland in the Waitotara and Patea Rivers, Taranaki (Fig. 1). The Patea River has a 63 m high earth filled dam c. 42 km inland from the river

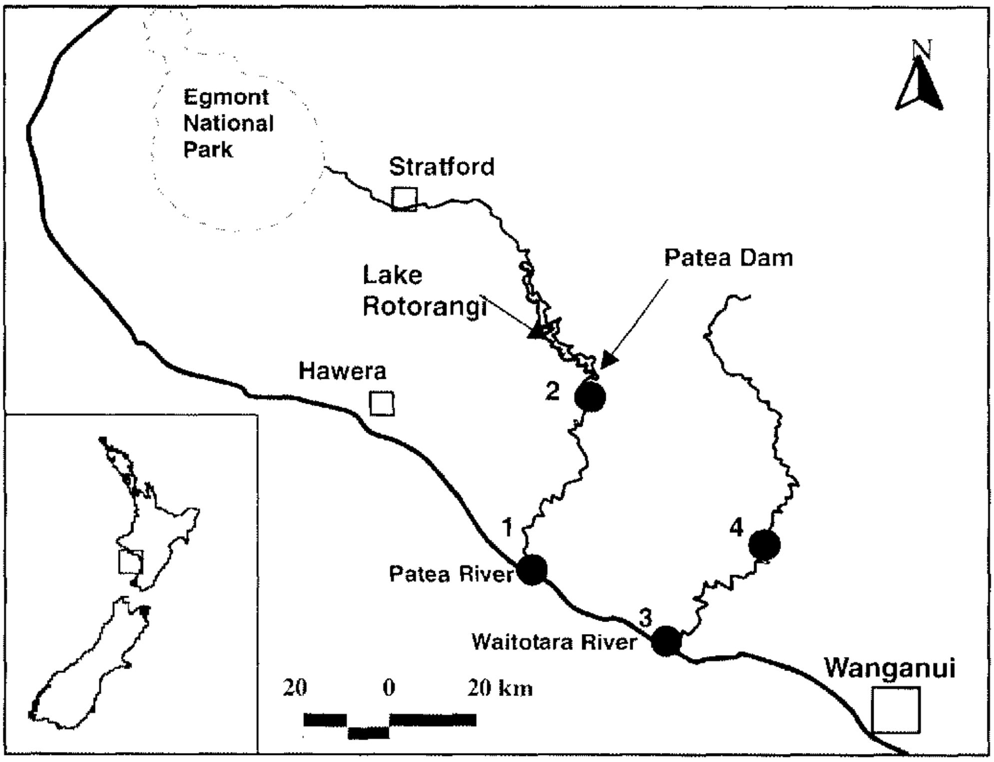

Fig. 1 A map of south Taranaki showing the whitebait collection sites. 1 = lower Patea site; 2 = upper Patea site; 3 = lower Waitotara site; 4 = upper Waitotara site.

mouth (Voice 1987). The dam forms a 45 km long lake upstream and has an elver pass that was installed in 1984 (Jowett 1987). Very few adult whitebait species have been recorded in the upper catchment of this river (New Zealand Freshwater Fish Database (NZFFD); McDowall & Richardson 1983), and from spotlighting tributaries below and just above the dam in December 2000, the only galaxiid species found was *G. maculatus* downstream of the dam. In contrast, the Waitotara River has no dam and *G. maculatus, G. fasciatus* and *G. argenteus* have been recorded (NZFFD; McDowall & Richardson 1983), although only *G. maculatus* were found from spotlighting several tributaries in December 2000. The two rivers are within 20 km of each other and have similar physical and chemical characteristics (Table 1). They are comparatively long with similar discharge and silty substrate, have similar catchment vegetation, relatively large tidal influence, receive similar sea currents and are known whitebait fisheries (Taranaki Catchment Commission 1981).

Table 1. Habitat and environmental characteristics of the four study sites in south Taranaki.

Study Sites	**Lower Patea site**	**Upper Patea site**	**Lower Waitotara site**	**Upper Waitotara site**
NZMS Grid reference[1]	26368E 61603N	26444E 61798N	26560E 60612N	26658E 60612N
Altitude (m)[1]	0	21	0	20
Distance from the coast (m)[1]	2	40	2	39
Mean wet width (m)[2]	40	20	60	20
Embededness[3]	1	2	1	1
Overhead cover (%)[2]	5	10	10	10
Land use (%)[2]	30% farmland 70% modified	100% farmland	100% farmland	100% farmland
Substrate type[2]				
Boulders (>256 mm)	0	0	0	0
Large cobbles (128-256 mm)	0	20	0	0
Cobbles (64-128 mm)	0	0	0	0
Pebbles (16-64 mm)	5	5	0	0
Gravel (4-16 mm)	0	10	0	0
Fines (<4 mm)	95	75	100	0
Mean temperature (°C)[4]	15	15	14	15
range	(9-23)	(9-21)	(8-23)	(9-21)
Mean conductivity (mS/cm)[4]	232	121	62	126
range	(0.01-2460)	(63-180)	(0.01-606)	(23-241)
Mean pH[5]	8	9	8	9
range	(7-9)	(8-10)	(8-9)	(8-9)
Mean dissolved oxygen (%)[6]	54	64	52	57
range	(11-80)	(54-69)	(7-74)	(44-71)
Mean salinity (ppt)[6]	9	0.1	10	0.05
range	(0.1-35)	(0-0.2)	(0-35)	(0-0.1)

[1] Obtained from 1:50,000 NZMS topographic maps.
[2] Visually assessed at each site.
[3] Subjectively assessed at site after moving substrate (1 = loosely packed; 4 = tightly packed)
[4] Mean of measures taken at every sampling prior to nets being lifted using Orion 122 Conductivity Meter™.
[5] Mean of measures taken at every sampling prior to nets being lifted using Orion Ouickcheck model 106™.
[6] Mean of measures taken at every sampling prior to nets being lifted using YSI model 59™.

Fish collection

Sampling was conducted between July 15, 2000 and February 18, 2001, which includes the whitebaiting season in the North Island that runs from August 15 to November 30 (Whitebait Fishing Regulations 1991). Single trap Southland Sock whitebait nets were used at all study sites. Sites within the tidal influence were sampled every week from August to December, unless flooding prevented sampling. Additionally these sites were sampled once in January and February. Nets were in the water on average two hours before and one hour after high tide. Sites around 40 km inland were sampled approximately every four weeks and nets were left in the water for c. 20 hours. All nets were set in the same place each sampling. In addition, metal shields with 2 cm diameter holes were placed on the entrance of the Southland Sock traps at the upper sites in an attempt to exclude adult *Anguilla* sp. from December 2000 to February 2001.

All fish collected were measured, counted and identified. All non-whitebait species and *G. maculatus* were returned to the river, and some of the remaining whitebait were transported back to Massey University, Palmerston North. These whitebait were kept in aquaria until identification was certain. Nine whitebait kept from the catches on October 8 and 28, 2000 were anaesthetised using 25 mg/L Benzocaine, and a non-destructive dorsal fin clip taken for use in mitochondrial DNA sequencing (Chapter 3). For general catch compositions, *G. brevipinnis* and *G. postvectis* were treated as one group as they are morphologically indistinguishable as whitebait (McDowall 1990).

Every month from August 2000 to February 2001, a box trap was placed on the outlet of the elver pass at the top of the Patea Dam for c. 20 hours. All fish caught were identified and counted.

Data analysis

T-tests were used to determine differences in whitebait size using Excel. Analysis of variance (ANOVA) was preformed using SAS (2000), to assess if there were differences between sites, rivers, time and lengths of whitebait species. An lsmeans test was used *a posteriori* to assess which months were significantly different. Normality and homogeneity assumptions were assessed and met for all data sets.

RESULTS

Whitebait migration

The number of whitebait caught on each occasion at all sites was quite variable and generally low (<20) (Fig. 2). There was no difference in the number of whitebait caught between sites (lower $F_{1,45} = 0.61$, $P = 0.46$; upper $F_{1,2} = 2.26$, $P = 0.18$) or collection time (weekly $F_{7,45} = 1.76$, $P = 0.23$; monthly $F_{6,2} = 1.10$, $P = 0.45$). However, there were definite peaks in whitebait catches in September (520) and October (91) within the tidal influence at the lower Patea and Waitotara sites respectively, and in late October in the upper Patea site (1500). Large schools of whitebait were seen in the river below the dam

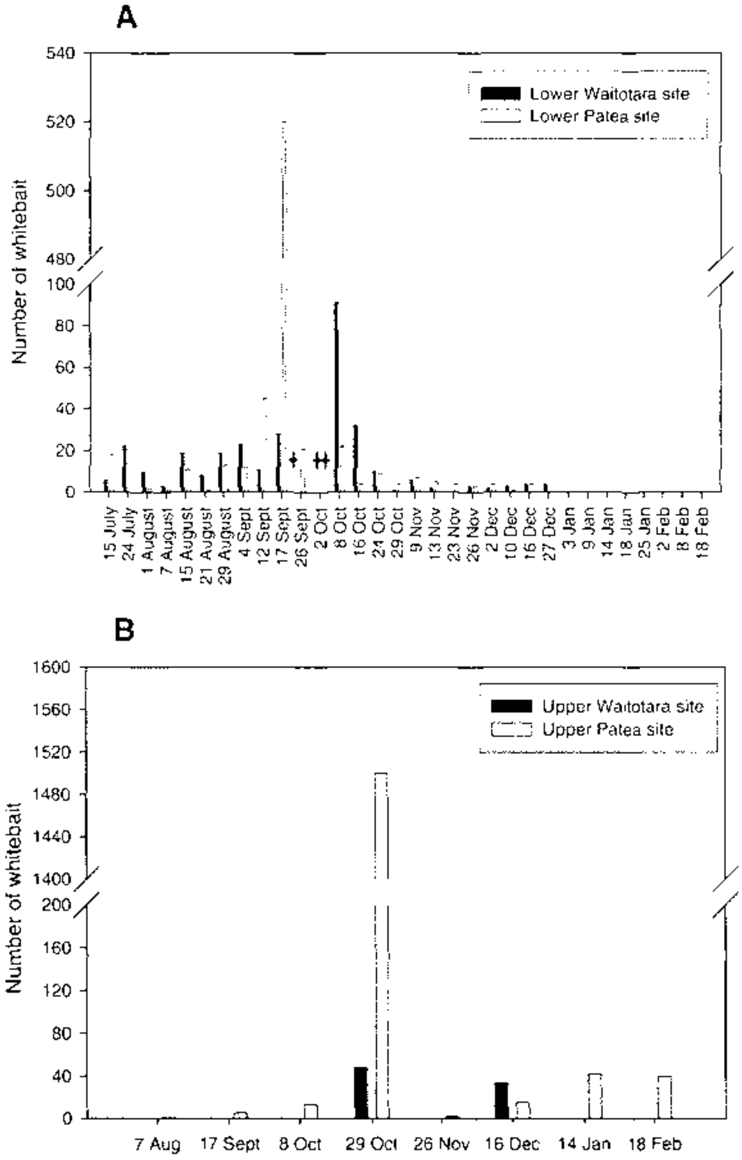

Fig. 2 Number of whitebait caught weekly within the tidal influence (A), and monthly (B) c. 40 km inland in Southland Sock samples in the Waitotara and Patea Rivers between July 15, 2000 and February 18, 2001. ✧ represents weeks when flooding prevented sampling.

from late October onwards. The upper Patea site appeared to have a greater overall presence of whitebait over time than the upper Waitotara site (Fig. 2B). Tide size, sampling time, time since last fresh, time of day, temperature, pH, conductivity, salinity and dissolved oxygen were not correlated with catch numbers (r^2 = 0.001-0.06). A mixture of freshrun and other whitebait were caught in most samples from July to December within the tidal influence of both study rivers, with around 70% freshrun whitebait caught each month.

Species composition

All whitebait catches were dominated by *G. maculatus* (Fig. 3). The other whitebait species were found irregularly from August to December comprising 0-50% of the catch (Fig. 3). Lower and upper site whitebait compositions were generally similar, although *G. maculatus* was replaced with other whitebait species more often in the upper sites (Fig. 3). The *G. brevipinnis/ G. postvectis* group appeared in the catch from mid August until early October within the tidal influence in both rivers, but not until early October in the upper Patea site and late October in the upper Waitotora site (Fig. 3). This whitebait group makes up a variable contribution of the catch from a minor contribution to 40% of the catch. Genetic identification (Chapter 3) identified *G. postvectis* whitebait at both lower Patea and Waitotara sites on October 8, and *G. brevipinnis* whitebait on October 28 at the upper Patea site (Table 2). *Galaxias fasciatus* were recorded in the Waitotara and Patea Rivers at all sites, from early September in the lower Waitotara site, mid September in the lower Patea site, and late October in the upper sites (Fig. 3; Table 2). *Galaxias argenteus* whitebait was only caught in mid December in both lower sites and the upper Waitotara site (Fig. 3). There appeared to be no distinctive pattern to the appearance of species at the lower and upper sites.

Table 2 Details of the whitebait genetically identified from the Waitotara and Patea Rivers (Chapter 3).

Species	Date caught	Site	Length (mm)
G. postvectis	8/10/2000	Lower Patea site	45
G. brevipinnis	28/10/2000	Upper Patea site	50
G. fasciatus	28/10/2000	Upper Patea site	40
G. fasciatus	28/10/2000	Upper Patea site	49
G. fasciatus	28/10/2000	Upper Patea site	45
G. fasciatus	28/10/2000	Upper Patea site	38
G. postvectis	8/10/2000	Lower Waitotara site	50
G. fasciatus	8/10/2000	Lower Waitotara site	40
G. fasciatus	8/10/2000	Lower Waitotara site	38

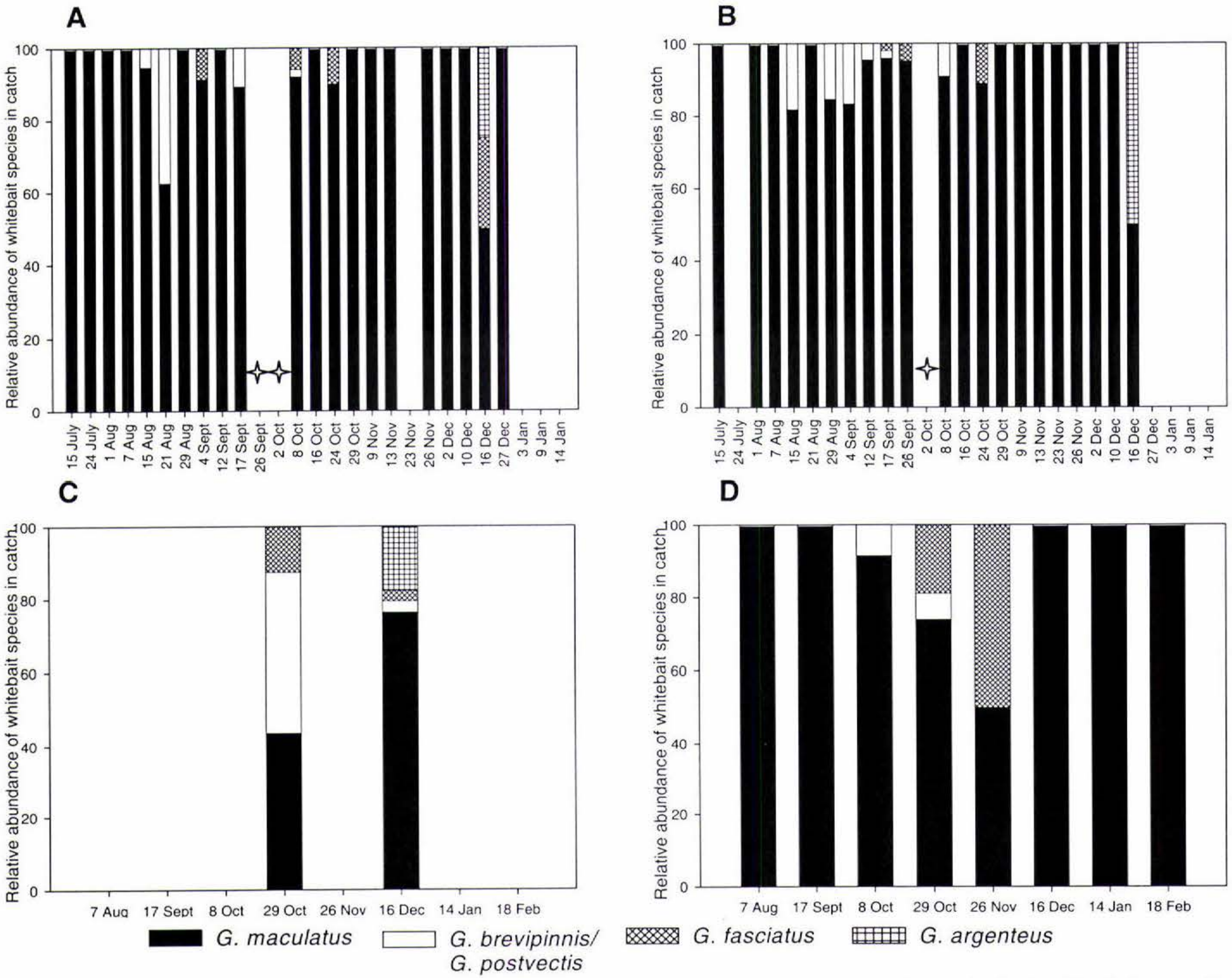

Fig. 3 Relative abundance of whitebait species caught in Southland Sock samples in the Waitotara and Patea Rivers within the tidal influence (A & B), and c. 40 km inland (C & D) between July 15, 2000 and February 18, 2001. A = lower Waitotara site; B = lower Patea site; C = upper Waitotara site; D = upper Patea site. ✧ Represents weeks when flooding prevented sampling.

Whitebait size

Mean *G. maculatus* whitebait caught were 50 mm ($n = 716$, range 36 to 70 mm). Upper Waitotara *G. maculatus* whitebait were longer than all other sites ($P = 0.01$-<0.001), and upper Patea *G. maculatus* were also larger than *G. maculatus* at both lower sites ($P = 0.01$-0.001) (Fig. 4B). *Galaxias fasciatus* whitebait were slightly shorter in the upper than lower sites ($P = 0.07$). Otherwise all other within species comparisons (lower site *G. maculatus* and *G. argenteus* and *G. postvectis/ G. brevipinnis* whitebait) did not differ between sites or rivers ($P = 0.12$-0.75).

Lengths of *G. maculatus* caught at lower sites declined from July to December, however no difference was found between sites ($F_{1,\ 44} = 0.93$, $P = 0.34$) (Fig 5). Lengths between months were significantly different ($F_{5,\ 494} = 7.84$, $P <0.001$), especially in September when longer *G. maculatus* whitebait were recorded than October and November ($P = 0.001$; $P = 0.09$ respectively).

Overall *G. maculatus* caught were quite variable in length compared to other whitebait species, although all whitebait species were different sizes to each other ($P = 0.01$-<0.001) (Fig. 4A). *Galaxias brevipinnis/ G. postvectis* whitebait caught were a mean of 51 mm ($n = 41$, range 45 to 55 mm), slightly longer than *G. maculatus* and longer than *G. fasciatus* whitebait that were a mean of 43 mm ($n = 29$, range 36 to 52 mm) (Fig. 4A). *Galaxias argenteus* were the longest whitebait with a mean length of 57 mm ($n = 9$, range 50 to 60 mm) (Fig. 4A).

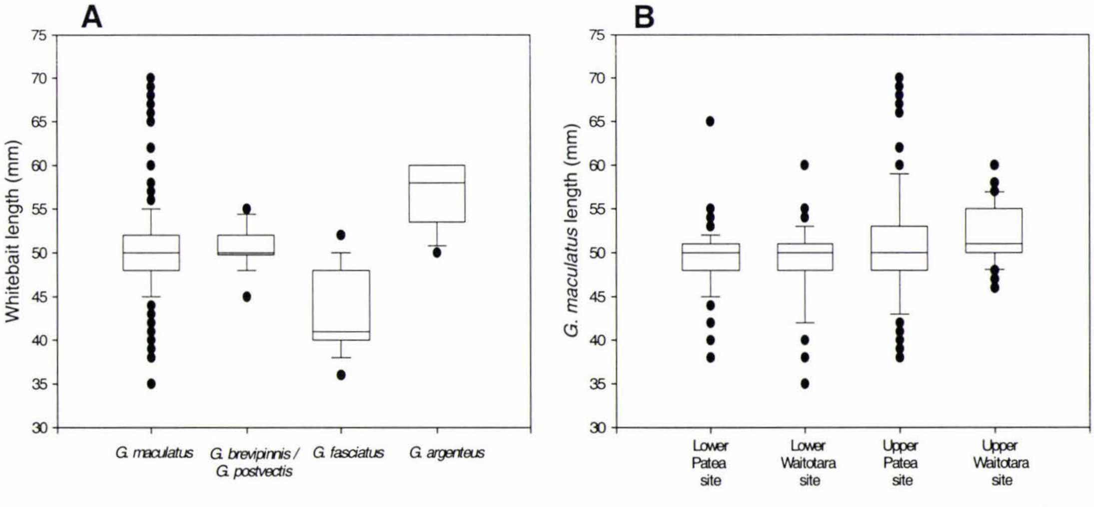

Fig. 4 Box and whisker plots showing the lengths of *G. maculatus* (A) caught at the lower sites and lengths of all whitebait species collected from all sites (B). The boundary of the box closest to zero shows the 25th percentile; the line in the box is the median; and the boundary of the box farthest from zero indicate the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles, and dots show the outliers.

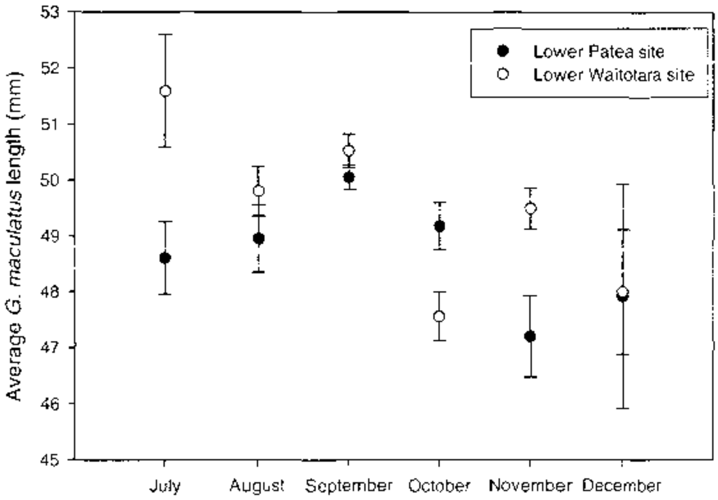

Fig. 5 Average length of all *G. maculatus* caught each month from July to December, 2000 at the lower Waitotara and Patea sites.

Other fish

A number of non-whitebait fish as well as shrimps, comb jellies and crabs were collected (Table 3). These included finding two marine species wandering into tidally influenced freshwater, *Chelidonichthys kumu* (gurnard) in the lower Patea site and a *Genyagnus monopterygius* (spotted stargazer) in the lower Waitotara site (Table 3). Species found at both lower sites were quite similar and many catches were dominated by *Retropinna retropinna* and *Aldrichetta forsteri* (Fig. 6). Juvenile bullies were found in the lower sites from July to September, and *Gobiomorphus cotidianus* were caught in small numbers throughout (Fig. 6). Upper site compositions of non-whitebait fish differed to lower sites with *Anguilla sp.* and *Gobiomorphus* sp. dominating (Fig. 6). *Gobiomorphis cotidianus* dominated many upper site catches from August to October in both rivers (Fig. 6).

Elver pass

At the Patea dam no whitebait species were found in the trap at the outlet of the elver pass between July 2000 and February 2001. However elvers were found in December, January and February ranging from 23 in December 2000 to 157 in February 2001 with a mean length of 72 mm (n =162, range 50-90 mm).

Table 3 Size of other fish species caught in the whitebait catches between July 2000 and February 2001.

Species	N	Mean length (mm)	Range
Adult *G. maculatus*	27	76	70-90
Retropinna retropinna (Richardson) (common smelt)	367	66	40-650
Aldrichetta forsteri (Valenciennes) (yelloweye mullet)	209	81	50-160
Anguilla dieffenbachii (longfin eel)	110	415	30-1000
Anguilla australis Richardson (shortfin eel)	1	750	-
Elver	12	84	50-105
Juvenile bully	25	26	8-40
Gobiomorphus huttoni (redfin bully)	1	40	-
Gobiomorphus cotidianus McDowall (common bully)	53	70	36-115
Gobiomorphus breviceps (Stokell) (upland bully)	4	71	38-100
Perca fluviatilis Linnaeus (perch)	2	118	95-140
Rhomobosolea retiaria Hutton (black flounder)	2	130	60-200
Juvenile Marine fish			
Chelidonichthys kumu (gurnard)	1	15	-
Genyagnus monopterygius (spotted stargazer)	1	27	-

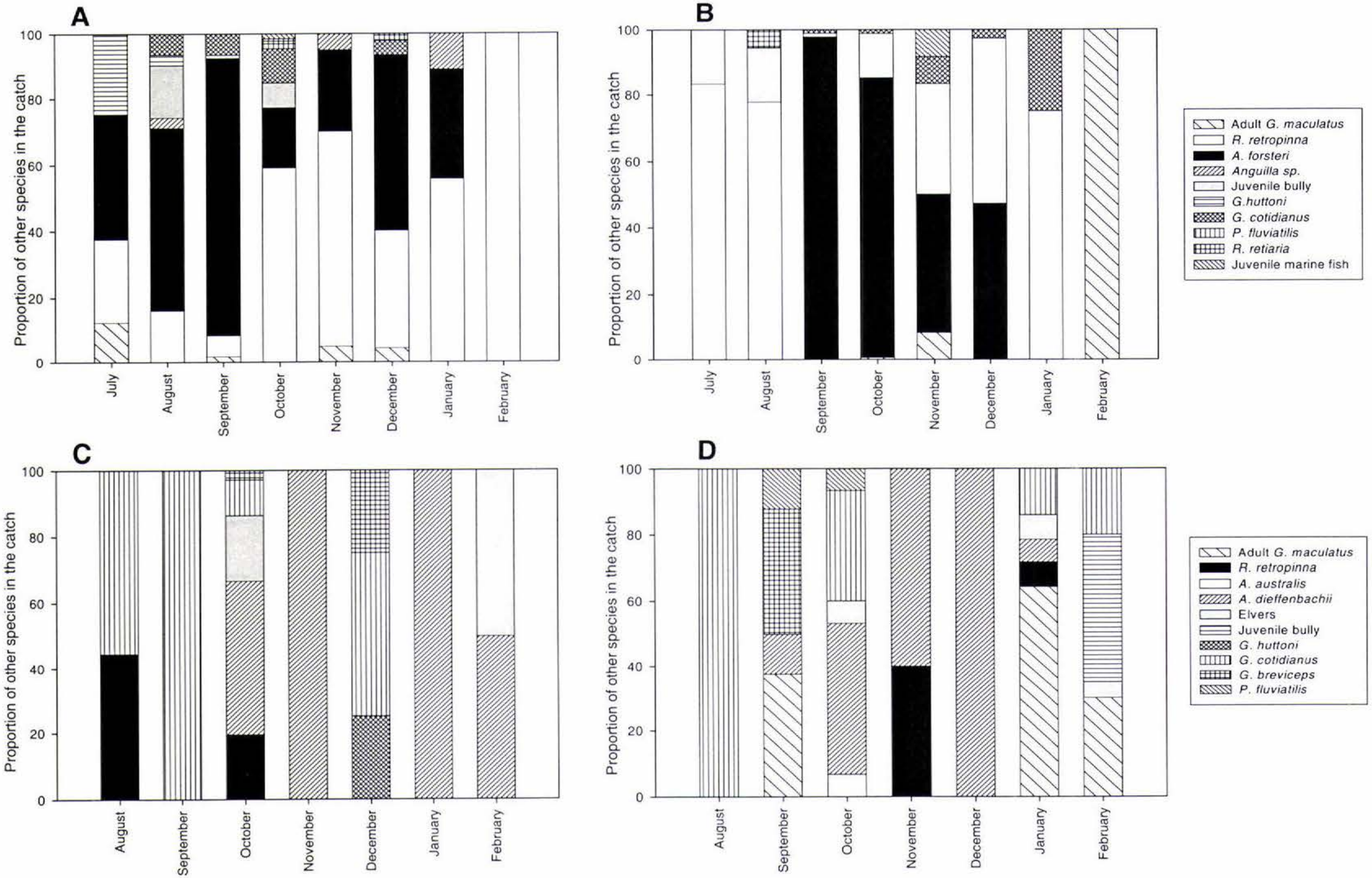

Fig. 6 Proportions of non-whitebait fish caught in Southland Sock samples in the Waitotara and Patea Rivers within the tidal influence (A & B), and c. 40km inland (C & D) between July 15, 2000 and February 18, 2001. A = lower Waitotara site; B = lower Patea site; C = upper Waitotara site; D = upper Patea site.

DISCUSSION

The whitebait catch within the tidal influence of the Waitotara and Patea Rivers were quite similar, however catch numbers were commonly low and highly variable. The upper Patea site appeared to have a greater presence of whitebait overall than the upper Waitotara site, with whitebait caught in all months and many whitebait also observed often just below the dam. Problems with fluctuating flows from power generation and predation by *Anguilla* sp. caused problems with evaluating numbers and identification of migrating whitebait in the upper sites, so the number of whitebait caught in some months may be underestimated.

Peaks in whitebait catches in the Waitotara and Patea Rivers occurred within the tidal influence in September and October respectively, while peak numbers were collected in late October around 40 km inland. This peak in migration is similar to seasonal patterns recorded in other New Zealand rivers (McDowall 1968; McDowall 1980).

As in most New Zealand rivers, Waitotara and Patea Rivers both have *G. maculatus* dominated whitebait catches, with other whitebait species making only minor contributions (McDowall 1965). The seasonal runs of *G. brevipinnis, G. fasciatus,* and *G. argenteus* though, differ from other documented timings, mostly from the South Island (McDowall & Eldon 1980). *Galaxias fasciatus* was found entering the river mouths from early to mid September in the Waitotara and Patea Rivers, similar to that recorded by Rowe et al. (1992) in North Island rivers, but a month earlier than migrations of *G. fasciatus* whitebait in South Island rivers (McDowall & Eldon 1980). *Galaxias brevipinnis/G. postvectis* whitebait were recorded during mid August to early October within the tidal influence of the Waitotara and Patea Rivers, slightly earlier than recorded in Bay of Plenty and South Island rivers (McDowall & Eldon 1980; Rowe et al. 1992). A few *G. postvectis* whitebait were identified migrating into freshwater in early October in the Waitotara and Patea Rivers (Chapter 3), which is a month earlier than *G. postvectis* whitebait collected from a South Island creek (McDowall et al. 1994). McDowall (1999) has recorded *G. argenteus* entering South Island rivers in early November and December, similar timing to the *G. argenteus* collected entering the Waitotara and Patea Rivers. Therefore similar to Rowe et al. (1992), it appears whitebait migrations are earlier in North Island than South Island rivers.

Length of whitebait migrations for each species corresponds to the timing and length of spawning. *Galaxias maculatus* whitebait dominated and occurred in whitebait catches from July to February, which corresponds with this species longer spawning season (McDowall 1968). The remaining species all have relatively short periods of spawning (Hopkins 1979; Mitchell & Penlington 1982; Chapter 2 & 4), and correspondingly short whitebait migrations. The earlier timing of the whitebait runs coincides with the earlier spawning of *G. fasciatus* and *G. brevipinnis* before *G. postvectis* (Chapter 2 & 4), although only two *G. postvectis* were identified in this study. In addition spawning of *G. postvectis, G. brevipinnis* and *G. fasciatus* occurred in late April to mid May in 2000 and 2001 (Allibone & Caskey 2000; Chapter 2 & 4), and emigrating larvae of these species were also recorded moving downstream in May and June 2001 (Chapter 4), all in the Katikara Stream, north Taranaki. Thus combining this timing with the timing of the whitebait runs of each species, it appears that *G. postvectis, G. brevipinnis* and *G. fasciatus* all spend approximately four to five months at sea, similar to timing suggested by McDowall (1990).

Galaxias maculatus, G. fasciatus and *G. brevipinnis/ G. postvectis* whitebait caught in the Waitotara and Patea Rivers were on average similar in size to others collected in New Zealand (McDowall 1968; McDowall & Eldon 1980; McDowall 1990). *Galaxias argenteus* whitebait were the largest caught in my study and were generally longer than previous *G. argenteus* records (McDowall 1990; McDowall 1999).

Galaxias maculatus were found to be longer 40 km inland than near the river mouth, while *G. fasciatus* whitebait were shorter. This difference in length for *G. fasciatus* is probably a result of shrinkage that occurs after a couple of days in freshwater (McDowall & Eldon 1980). *Galaxias maculatus* may show the reverse pattern because of a faster growth rate and collected whitebait may have been feeding and older than most relatively new whitebait (McDowall 1968). *Galaxias maculatus* lengths in this study were also found to vary between months, finding similar to McDowall & Eldon (1980) that maximum size occurred at times of peak migrations and size declined towards the end of the season.

There were similar patterns of occurrence for all whitebait species in both rivers. A lack of adult whitebait species in the Patea River headwaters does not therefore appear to influence river choice by juvenile whitebait. However the consistently low and highly variable nature of the Southland Sock samples make generalisations from these results very difficult. A study of whitebait migrations in several rivers with high densities of whitebait adults, and rivers lacking adults is needed to test this postulated mechanism of river mouth selection more thoroughly (Rowe et al. 1992; Baker & Montgomery 2000).

A few *G. brevipinnis* whitebait have been recorded at the outlet of the elver pass on the Patea Dam in the past (Taranaki Regional Council 2000), however no whitebait species were recorded in the present study. Thus it is unlikely that significant numbers of whitebait are getting above the dam, but large numbers are present below the dam from October onwards. Clearly whitebait are not getting above the Patea Dam and it may be worth trialing translocation of whitebait above the dam, or improving habitat and access to the tributaries of the main river below the dam. Therefore the presence of a dam, like the Patea Dam, does not affect whitebait recruitment within the tidal influence, however it does block whitebait migrating further upstream.

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SYNTHESIS

Most adult *Galaxias postvectis* (shortjaw kokopu) were found spent in their resident habitats in mid May at the Katikara Stream and an unnamed tributary of the Stony River, Taranaki. *Galaxias postvectis* spawning appeared to be associated with increased water levels and may occur earlier at lower elevations and distances inland. Female fish were larger than males. Some mature fish were bitten around this time, however these bites were not thought to be associated directly with spawning. The other galaxiids, *G. fasciatus* (banded kokopu), *G. brevipinnis* (koaro) and *G. argenteus* (giant kokopu), found were thought to have started spawning earlier probably from late April.

Sixteen galaxiid nests were discovered adjacent to adult habitat amongst the riparian margins of the Katikara Stream, between May 9 and 17, 2001 after increased flows. All eggs were round clear and approximately 2 mm in diameter, however they were unidentifiable to species level by visual assessment. Thus mtDNA sequences were used to identify eggs from 12 *G. postvectis* and four as *G. fasciatus* nests. No *G. argenteus* and *G. brevipinnis* nests were found even though adults were present. Three nests found on May 9 contained eyed eggs and were identified as *G. fasciatus*. Further on May 15, another *G. fasciatus* nest was discovered with all the eggs at an early developmental stage. All other nests found were *G. postvectis* and contained eggs at early developmental stages when located. All egg masses were found deposited amongst terrestrial vegetation, debris and substrate in areas flooded near bankfull. The nests were adjacent to backwater and pool habitats where galaxiids had regularly been caught. *Galaxias fasciatus* nests had more vegetation and debris than *G. postvectis* nests, and presence of a backwater or pool, cover and gravel were found to be important characteristics of all nest sites. The terrestrial deposition of eggs is quite unusual and this spawning behaviour highlights the importance of riparian management, not only for terrestrial species but also some New Zealand's native fish.

Egg development on the Katikara Stream banks took three to four weeks, while eggs keep constantly at 14°C took around two weeks. Under experimental conditions, most fully developed *G. postvectis* and *G. fasciatus* eggs were found to hatch within an hour of immersion in water, and more hatching occurred in moving than still water and at warmer than colder temperatures. In the Katikara Stream, eggs hatched and were caught moving downstream only at times of increased water levels. Larvae caught could not be identified from morphological features, but mtDNA sequences identified *G. fasciatus*, *G. brevipinnis* and *G. postvectis* larvae moving downstream in May and June. All eggs at nests had completed hatching by mid June 2001. Newly hatched larvae were around 9 mm long with *G. fasciatus* larvae slightly longer than *G. postvectis* larvae. Thus flow management and protection of these riparian margins along streams is essential to preserve these species.

Whitebait catches in the Waitotara and Patea Rivers in south Taranaki were dominated by *G. maculatus*, with other galaxiid species making only a small variable contribution. The presence of the Patea Dam c. 42 km inland on the Patea River does not appear to effect whitebait recruitment, with catch numbers being similar, and commonly low and highly variable within the tidal influence of both rivers. Using mtDNA sequences, two *G. postvectis* were identified amongst the morphologically similar *G. brevipinnis,* migrating into river mouths in early October. The Patea River, downstream of the dam, appeared to have a greater presence of whitebait overall compared with the Waitotara River, with whitebait recorded in all months and large numbers of whitebait also observed in the main river below the dam from October onwards. No whitebait were found to surmount the Patea Dam. Therefore it may be worth trialing translocation of whitebait above the dam, or improving habitat and access to the tributaries of the main river below the dam to mitigate the effect the dam is having on galaxiids in the Patea River.

So in conclusions a late autumn spawning is confirmed for *G. postvectis,* with spent fish, nests, and later emigrating larvae being recorded at this time. *Galaxias fasciatus* appeared to begin spawning earlier than *G. brevipinnis,* and both were thought to spawn earlier than *G. postvectis* in all study sites in northwest Taranaki. Riparian margins are important for these species as they lay their eggs in flooded terrestrial substrate, vegetation and debris. Additionally the presence of the Patea Dam does not affect whitebait recruitment below the dam, however it does block them from migrating upstream. Finally morphologically indistinguishable life stages of the whitebait species including eggs, larvae and whitebait can now be accurately identified using robust molecular methods.

APPENDIX 1: MITOCHONDRIAL DNA SEQUENCES

Key

GE01-GE52 = Eggs, nest larvae, emigrating larvae and fin clip samples.
* replicate sample.

All other sequences are reference sequences from Waters et al. (2000) and Waters & Wallis (2001).

k = *G. brevipinnis*
b = *G. fasciatus*
i = *G. maculatus*
s = *G. postvectis*
g = *G. argenteus*

```
[                                       10        20        30        40        50]

'GE30_{k}'                      CCGCGCGCCACAAGAAATGTACTTTTCGG-GTGGGGCAGCTCCTGGAAGG   [49]
'GE32_{k}'                      .............................-....................   [49]
'GE34_{k}'                      .............................-....................   [49]
'GE37_{k}'                      .............................-....................   [49]
'GE39_{k}'                      .............................-....................   [49]
'GE44_{k}'                      .............................-............?.......   [49]
'GE49_{k}'                      .............................-....................   [49]
'GE50_{k}'                      .............................-....................   [49]
'StIbrv22_{k}'                  .............................-....................   [49]
'StIbrv23_{k}'                  .........C...................-....................   [49]
'12Mile25_{k}'                  .............................-....................   [49]
'Chalbrev_{k}'                  .............................-....................   [49]
'GE01_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE02_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE03_{s*}'                     ..........GGTA.....AG.....TAA-A...................   [49]
'GE04_{s*}'                     ..........GGTA.....AG.....TAA-A...................   [49]
'GE05_{s*}'                     ..........GGTA.....AG.....TAA-A...................   [49]
'GE10_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE12_{s}'                      .........GGGTA.....AG.....TAA-A...................   [49]
'GE13_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE14_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE15_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE18_{s}'                      ?????????????????????????????-????????????????????   [49]
'GE19_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE20_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE21_{s}'                      ??........GGTA....?AG.....TAA-A........?..?..?....   [49]
'GE22_{s}'                      .........GGGTA.....AG.....TAA-A...................   [49]
'GE23_{s}'                      ..........GGTA.....AG.....TAA-A...................   [49]
'GE27_{s}'                      ?????????????????????????????-?........?..........   [49]
'GE33_{s}'                      .........GGGTA.....AG.....TAA-A...................   [49]
'GE35_{s}'                      .........GGGTA.....AG.....TAA-A...................   [49]
'GE38_{s}'                      .........GGGTA.....AG.....TAA-A...................   [49]
'GE45_{s}'                      ?????????????????????????????-?........T.......?..   [49]
'GE48_{s}'                      .........GGGTA.....AG.....TAA-A...................   [49]
'GE51_{s*}'                     ..........GGTA.....AG.....TAA-A...................   [49]
'GE52_{s*}'                     ..........GGTA.....AG.....TAA-A...................   [49]
'Postvect_{s}'                  .........GGGTA.....AG.....TAA-A...................   [49]
'Fasciat2_{b}'                  ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE07_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE08_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE09_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE17_{b}'                      ......A...T..A......C.G...T.A-.........TG.G...G..C   [49]
'GE24_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE25_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE28_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE29_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE31_{b}'                      ......A...T..A......C.G...T.A-.........TG.G...G..C   [49]
'GE36_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE40_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE41_{b}'                      ......A...T..A......C.G...T.A-.........TG.G...G..C   [49]
'GE42_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE43_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE46_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'GE47_{b}'                      ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'Fasciat3_{b}'                  ......A......A......C.G...T.A-.........TG.G...G..C   [49]
'Argenteu_{g}'                  ???????????GTA......C.C...TTA-.........TAT....GG.A   [49]
'Tukituki_{i}'                  .........CGG.A..T..CG.CC.GTA.C...T....C.AG......A-   [49]

[                                       60        70        80        90       100]

'GE30_{k}'                      -AGATGCATATTATGGTGTATATGATATATGCAGTAGGGATATATATGTA   [98]
'GE32_{k}'                      -.................................................   [98]
'GE34_{k}'                      -.....................................A...........   [98]
'GE37_{k}'                      -.................................................   [98]
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'GE39_{k}'            -.................................................  [98]
'GE44_{k}'            -.................................................  [98]
'GE49_{k}'            -.................................................  [98]
'GE50_{k}'            -...........................................C.....  [98]
'StIbrv22_{k}'        -.................................................  [98]
'StIbrv23_{k}'        -......?..........................................  [98]
'12Mile25_{k}'        -.................................................  [98]
'Chalbrev_{k}'        -.................................................  [98]
'GE01_{s}'            -..........A.....A.....A.C........................  [98]
'GE02_{s}'            -..........A.....A.....A.C........................  [98]
'GE03_{s*}           '-..........A.....A.....A.C........................   [98]
'GE04_{s*}'           -..........A.....A.....A.C........................  [98]
'GE05_{s*}'           -..........A.....A.....A.C........................  [98]
'GE10_{s}'            -..........A.....A.....A.C........................  [98]
'GE12_{s}'            -..........AG....A.....A.C........................  [98]
'GE13_{s}'            -..........A.....A.....A.C........................  [98]
'GE14_{s}'            -..........A.....A.....A.C........................  [98]
'GE15_{s}'            -..........A.....A.....A.C........................  [98]
'GE18_{s}'            ??????????????????????????????????????????????????  [99]
'GE19_{s}'            -..........AG....A.....A.C........................  [98]
'GE20_{s}'            -..........AG....A.....A.C........................  [98]
'GE21_{s}'            -..........A.....A.....ACC....................?...  [98]
'GE22_{s}'            -..........A.....A.....A.C........................  [98]
'GE23_{s}'            -..........A.....A.....A.C........................  [98]
'GE27_{s}'            -..........AG....A.....A.C........................  [98]
'GE33_{s}'            -..........AG....A.....A.C........................  [98]
'GE35_{s}'            -..........AG....A.....A.C........................  [98]
'GE38_{s}'            -..........AG....A.....A.C........................  [98]
'GE45_{s}'            -..........?GG...A.....A.C...............?........  [98]
'GE48_{s}'            -..........A.....A.....A.C........................  [98]
'GE51_{s*}'           -..........A.....A.....A.C........................  [98]
'GE52_{s*}'           -..........AG....A.....A.C........................  [98]
'Postvect_{s]'        -..........AG....A.....A.C........................  [98]
'Fasciat2_{b}'        -G.........AT....A.....A.C............A.C.........  [98]
'GE07_{b}'            -G.........AT....A.....A.C..............C.........  [98]
'GE08_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE09_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE17_{b}'            -G?........AT....A.....A.C............A.C.........  [98]
'GE24_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE25_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE28_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE29_{b}'            -G.........AT....A.....A.C..............C.........  [98]
'GE31_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE36_{b}'            -G.........AT....A.....A.C..............C.........  [98]
'GE40_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE41_{b}'            -G?........AT....A.....A.C............A.C.........  [98]
'GE42_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE43_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE46_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'GE47_{b}'            -G.........AT....A.....A.C............A.C.........  [98]
'Fasciat3_{b}'        -G.........AT....A.....A.C............A.C.........  [98]
'Argenteu_{g}'        G..G.......AT..........A.C........................  [99]
'Tukituki_{i}'        A..G...................A.C.....G........C.........  [99]

[                             110       120       130       140       150]

'GE30_{k}'            TAAACACCATTAATCTAATGAAAACACACAGGCTAAATGTATTTGGAGGG  [148]
'GE32_{k}'            ..............................A..C................  [148]
'GE34_{k}'            ..................................................  [148]
'GE37_{k}'            .................................C................  [148]
'GE39_{k}'            ..................................................  [148]
'GE44_{k}'            .C............................A...................  [148]
'GE49_{k}'            ..................................................  [148]
'GE50_{k}'            ..............................A..C................  [148]
'StIbrv22_{k}'        ..............................A.A.................  [148]
'StIbrv23_{k}'        ..............................?...................  [148]
'12Mile25_{k}'        ..............................A..C................  [148]
'Chalbrev_{k}'        ..............................A...................  [148]
'GE01_{s}'            ..............T...........A.......................  [148]
'GE02_{s}'            ..............T...........A...C...................  [148]
'GE03_{s*}'           ..............T...........A.......................  [148]
'GE04_{s*}'           ..............T...........A...C...................  [148]
'GE05_{s*}'           ..............T...........A.......................  [148]
'GE10_{s}'            ..............T...........A...A.A.................  [148]
'GE12_{s}'            ..............T...........A.......................  [148]
'GE13_{s}'            ..............T...........A.......................  [148]
'GE14_{s}'            ..............T...........A...A...................  [148]
'GE15_{s}'            ..............T...........A.......................  [148]
'GE18_{s}'            ????.C.....?..T..........?A.......................  [149]
'GE19_{s}'            ..............T...........A.......................  [148]
'GE20_{s}'            ..............T...........A.......................  [148]
'GE21_{s}'            ...C.?........T...........AC....?.................  [148]
'GE22_{s}'            ..............T...........A.......................  [148]
'GE23_{s}'            ..............T...........A...A...................  [148]
'GE27_{s}'            ..............T...........A.......................  [148]
'GE33_{s}'            ..............T...........A.......................  [148]
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'GE35_{s}'                   ..............T...........A.......................  [148]
'GE38_{s}'                   .........C....T...........A.......................  [148]
'GE45_{s}'                   ..............T...........A?...........?..........  [148]
'GE48_{s}'                   ..............T...........A...A...................  [148]
'GE51_{s*}'                  ..............T...........A...A...................  [148]
'GE52_{s*}'                  ..............T...........A.......................  [148]
'Postvect_{s}'               ..............T...........A.......................  [148]
'Fasciat2_{b}'               .........A....A...............A.A............A...A  [148]
'GE07_{b}'                   .........A....A...............A.A............A...A  [148]
'GE08_{b}'                   .........A....A...............A.A............A...A  [148]
'GE09_{b}'                   .........A....A...............A.A............A...A  [148]
'GE17_{b}'                   .........A....A...............A.A............A...A  [148]
'GE24_{b}'                   .........A....A...............A.A............A...A  [148]
'GE25_{b}'                   .........A....A...............A.A............A...A  [148]
'GE28_{b}'                   .........A....A...............A.A............A...A  [148]
'GE29_{b}'                   .........A....A...............A.A............A...A  [148]
'GE31_{b}'                   .........A....A...............A.A............A...A  [148]
'GE36_{b}'                   .........A....A...............?.A............A...A  [148]
'GE40_{b}'                   .........A....A...............A.A............A...A  [148]
'GE41_{b}'                   .........?....A......T........A.A............A...A  [148]
'GE42_{b}'                   .........A....A...............A.A............A...A  [148]
'GE43_{b}'                   .........A....A...............A..............A...A  [148]
'GE46_{b}'                   .........A....A...............A.A............A...A  [148]
'GE47_{b}'                   .........A....A...............A.A............A...A  [148]
'Fasciat3_{b}'               .........A....A...............A.A............A...A  [148]
'Argenteu_{g}'               ..............................A.A.................  [149]
'Tukituki_{i}'               .........A.C.AA.....T.....T...A.TC....ACC.GC.A...A  [149]


[                                 160       170       180       190       200]

'GE30_{k}'                   GAACATGTACAAAAAATTAAATATTTAACCGATCATTAAAAGGAGCCAAC  [198]
'GE32_{k}'                   ............G.G..........................A........  [198]
'GE34_{k}'                   ..................................................  [198]
'GE37_{k}'                   ..................................................  [198]
'GE39_{k}'                   .........................................A........  [198]
'GE44_{k}'                   .........................................A........  [198]
'GE49_{k}'                   .........................................A........  [198]
'GE50_{k}'                   .........................................A........  [198]
'StIbrv22_{k}'               .........................................A........  [198]
'StIbrv23_{k}'               ..............................A..........A........  [198]
'12Mile25_{k}'               .........................................A........  [198]
'Chalbrev_{k}'               .........................................A........  [198]
'GE01_{s}'                   ...T..A.G....G.........C......T.A...GG...TA.C.....  [198]
'GE02_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE03_{s*}'                  ...T..A.G....G.........C......T.A...GG...TA.C.....  [198]
'GE04_{s*}'                  ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE05_{s*}'                  ...T..A.G....G.........C......T.A...GG...TA.C.....  [198]
'GE10_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE12_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE13_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE14_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE15_{s}'                   ...T..A.G....G.........C......T.A...GG...TA.C.....  [198]
'GE18_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [199]
'GE19_{s}'                   ...T..A.G....GG........C......T.....GG...TA.C.....  [198]
'GE20_{s}'                   ...T..A.G....GGG.......C......T.A...GG...TA.C.....  [198]
'GE21_{s}'                   ...T..A.G....GGG.......C......T.A...GG...TA.C.....  [198]
'GE22_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE23_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE27_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE33_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE35_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE38_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE45_{s}'                   ...T..A.G....GG........C......T.A...GG..?T?.C...?.  [198]
'GE48_{s}'                   ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE51_{s*}'                  ...T..A.G....GG........C......T.A...GG...TA.C.....  [198]
'GE52_{s*}'                  ...T..A.G....GGG.......C......T.A...GG...TA.C.....  [198]
'Postvect_{s}'               ...T..A.G.....G........C......T.A...GG...TA.C.....  [198]
'Fasciat2_{b}'               .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE07_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE08_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE09_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE17_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE24_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE25_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE28_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE29_{b}'                   .......AG....T.T..C.CA........C.AA.CCC...C........  [198]
'GE31_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE36_{b}'                   .............T.T..C.CA........C.AA.CCC...C?.......  [198]
'GE40_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE41_{b}'                   ........?....T.T..CTCA.....?..C???????????????????  [198]
'GE42_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE43_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE46_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'GE47_{b}'                   .............T.T..C.CA........C.AA.CCC...C........  [198]
'Fasciat3_{b}'               .............T.T..C.CA........C.AA.CCC...C........  [198]
'Argenteu_{g}'               ....................TA......T.T..AT.G....AC.......  [199]
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```
'Tukituki_{i}'                 .......A.ACG...C.GT..G.CAC...AA.A..CCT...A.GA.A..T  [199]


[                                     210       220       230       240       250]

'GE30_{k}'                     TTAAACAGCTTTATTACCTAAACCGCAATAATG-ACCTTCATATTCCGAC  [247]
'GE32_{k}'                     .................................-................  [247]
'GE34_{k}'                     .................................-................  [247]
'GE37_{k}'                     .................................-................  [247]
'GE39_{k}'                     .................................-................  [247]
'GE44_{k}'                     .................................-.............???  [247]
'GE49_{k}'                     .................................-................  [247]
'GE50_{k}'                     .................................-G...............  [247]
'StIbrv22_{k}'                 .................................-G...............  [247]
'StIbrv23_{k}'                 .................................-................  [247]
'12Mile25_{k}'                 .................................-................  [247]
'Chalbrev_{k}'                 .................................-................  [247]
'GE01_{s}'                     ........A.......A.AC..T..........-................  [247]
'GE02_{s}'                     ........A....C..A.?C..T..........-?...............  [247]
'GE03_{s*}'                    ........A.......A.AC..T..........-................  [247]
'GE04_{s*}'                    ........A....C..A.AC..T..........-................  [247]
'GE05_{s*}'                    ........A.......A.AC..T..........-................  [247]
'GE10_{s}'                     ........A.......A.AC..T..........-................  [247]
'GE12_{s}'                     ........A....C..A.AC..T..........-................  [247]
'GE13_{s}'                     ........A....C..A.AC..T..........-................  [247]
'GE14_{s}'                     ........A.......A.AC..T..........-................  [247]
'GE15_{s}'                     ........A.......A.AC..T..........-................  [247]
'GE18_{s}'                     ........A....C..A.AC..T..........-................  [248]
'GE19_{s}'                     ........A.......A.AC..T..........-................  [247]
'GE20_{s}'                     ........A.......A.AC..T..........-................  [247]
'GE21_{s}'                     ........A....C..A.AC..T..........-................  [247]
'GE22_{s}'                     ........A....C..A.AC..T..........-................  [247]
'GE23_{s}'                     ........A.......A.AC..T..........-................  [247]
'GE27_{s}'                     ........A....C..A.AC..T..........-................  [247]
'GE33_{s}'                     ........A....C..A.AC..T..........-................  [247]
'GE35_{s}'                     ........A....C..A.AC..T..........-................  [247]
'GE38_{s}'                     ........A....C..A.AC..T..........-................  [247]
'GE45_{s}'                     ....?...A....C..A.AC.?T........?.-................  [247]
'GE48_{s}'                     ........A....C..A.AC..T..........-..............C.  [247]
'GE51_{s*}'                    ........A.......A.AC..T..........-................  [247]
'GE52_{s*}'                    ........A.......A.AC..T..........-................  [247]
'Postvect_{s}'                 ........A....C..A.AC..T..........-................  [247]
'Fasciat2_{b}'                 ........A..A....A.A...T....T.....-................  [247]
'GE07_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE08_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE09_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE17_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE24_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE25_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE28_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE29_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE31_{b}'                     ........A..A....A.A..GT....T.....-................  [247]
'GE36_{b}'                     ........A..?....A.AC..T....T.....-..............?.  [247]
'GE40_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE41_{b}'                     ????????????????????????...T.....-................  [247]
'GE42_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE43_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE46_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'GE47_{b}'                     ........A..A....A.A...T....T.....-................  [247]
'Fasciat3_{b}'                 ........A..A....A.A...T....T.....-................  [247]
'Argenteu_{g}'                 AA......A.......A.A...T.C..T.....-................  [248]
'Tukituki_{i}'                 CG...TT.A..AGCA.A.AG..TAC.....AATG...........?????  [249]


[                                     260       270       280       290       300]

'GE30_{k}'                     CCAACTTATTAACGGCCTCCAAATCTTAATGTAGTAAGAAACCACCAACC  [297]
'GE32_{k}'                     ..............A...................................  [297]
'GE34_{k}'                     ..............A............................???????  [297]
'GE37_{k}'                     ..............A.........T......................?..  [297]
'GE39_{k}'                     ..................................................  [297]
'GE44_{k}'                     ??????????????????????????????????????????????????  [297]
'GE49_{k}'                     ..............A...................................  [297]
'GE50_{k}'                     ..............A...................................  [297]
'StIbrv22_{k}'                 ..............A...................................  [297]
'StIbrv23_{k}'                 ..............A...................................  [297]
'12Mile25_{k}'                 ..............A.........T.........................  [297]
'Chalbrev_{k}'                 ..............A...................................  [297]
'GE01_{s}'                     ..............AA........T.........................  [297]
'GE02_{s}'                     ..............AA........T......................?..  [297]
'GE03_{s*}'                    ..............AA........T.........................  [297]
'GE04_{s*}'                    ..............AA........T.........................  [297]
'GE05_{s*}'                    ..............AA........T.........................  [297]
'GE10_{s}'                     ..............AA........T.........................  [297]
'GE12_{s}'                     ..............AA........T.........................  [297]
'GE13_{s}'                     ..............AA........T.........................  [297]
'GE14_{s}'                     ..............AA........T.........................  [297]
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'GE15_{s}'                  ..............AA........T.........................  [297]
'GE18_{s}'                  ..............AA........T.........................  [298]
'GE19_{s}'                  ..............AA........T.........................  [297]
'GE20_{s}'                  ..............AA........T.........................  [297]
'GE21_{s}'                  ..............AA........T.........................  [297]
'GE22_{s}'                  ..............AA........T.........................  [297]
'GE23_{s}'                  ..............AA........T.........................  [297]
'GE27_{s}'                  ..............AA........T.........................  [297]
'GE33_{s}'                  ..............AA........T.........................  [297]
'GE35_{s}'                  ..............AA........T.........................  [297]
'GE38_{s}'                  ..............AA........T.........................  [297]
'GE45_{s}'                  ..............A?........T...?......?.......?......  [297]
'GE48_{s}'                  ..............AA..????????????????????????????????  [297]
'GE51_{s*}'                 ..............AA........T.........................  [297]
'GE52_{s*}'                 ..............AA........T.........................  [297]
'Postvect_{s}'              ..............AA........T.........................  [297]
'Fasciat2_{b}'              ..............A.......G.T.........................  [297]
'GE07_{b}'                  ..............A.......G.T.........................  [297]
'GE08_{b}'                  ..............A.......G.T.........................  [297]
'GE09_{b}'                  ..............A.......G.T.........................  [297]
'GE17_{b}'                  ..............A.......G.T.........................  [297]
'GE24_{b}'                  ..............A.......G.T.........................  [297]
'GE25_{b}'                  ..............A.......G.T.........................  [297]
'GE28_{b}'                  ..............A.......G.T.........................  [297]
'GE29_{b}'                  ..............A.......G.T.........................  [297]
'GE31_{b}'                  ..............A.......G.T.........................  [297]
'GE36_{b}'                  ...?..........A.......G.T..???????????????????????  [297]
'GE40_{b}'                  ..............A.......G.T.........................  [297]
'GE41_{b}'                  ..............A.......G.T.........................  [297]
'GE42_{b}'                  ..............A.......G.T.........................  [297]
'GE43_{b}'                  ...........???????????????????????????????????????  [297]
'GE46_{b}'                  ..............A.......G.T.............????????????  [297]
'GE47_{b}'                  ..............C..?????????????????????????????????  [297]
'Fasciat3_{b}'              ..............A.......G.T.........................  [297]
'Argenteu_{g}'              T.............AT........T.........................  [298]
'Tukituki_{i}'              ??????????????????????????????????????????????????  [299]


[                                310       320       330       340]

'GE30_{k}'                  GGTGATGTCTATTTGTTTATTATTCGTGAGGGTCAGGTCCAT   [339]
'GE32_{k}'                  ..........................................   [339]
'GE34_{k}'                  ??????????????????????????????????????????   [339]
'GE37_{k}'                  .?????????????????????????????????????????   [339]
'GE39_{k}'                  ..........................................   [339]
'GE44_{k}'                  ??????????????????????????????????????????   [339]
'GE49_{k}'                  ..........................................   [339]
'GE50_{k}'                  .......C..................????????????????   [339]
'StIbrv22_{k}'              .......C..................................   [339]
'StIbrv23_{k}'              ..........................................   [339]
'12Mile25_{k}'              ..........................................   [339]
'Chalbrev_{k}'              ..........................................   [339]
'GE01_{s}'                  .......C..................................   [339]
'GE02_{s}'                  .......C...................?..............   [339]
'GE03_{s*}'                 .......C..................................   [339]
'GE04_{s*}'                 .......C..................................   [339]
'GE05_{s*}'                 .......C..................................   [339]
'GE10_{s}'                  .......C..................................   [339]
'GE12_{s}'                  .......C..................................   [339]
'GE13_{s}'                  .......C..................................   [339]
'GE14_{s}'                  .......C..................................   [339]
'GE15_{s}'                  .......C..................................   [339]
'GE18_{s}'                  .......C..................................   [340]
'GE19_{s}'                  .......C..................................   [339]
'GE20_{s}'                  .......C..................................   [339]
'GE21_{s}'                  .......C..................................   [339]
'GE22_{s}'                  .......C..................................   [339]
'GE23_{s}'                  .......C..................................   [339]
'GE27_{s}'                  .......C..................................   [339]
'GE33_{s}'                  .......C..................................   [339]
'GE35_{s}'                  .......C..................................   [339]
'GE38_{s}'                  .......C..................................   [339]
'GE45_{s}'                  .......C................?...?...??.?.??...   [339]
'GE48_{s}'                  ??????????????????????????????????????????   [339]
'GE51_{s*}'                 .......C..................................   [339]
'GE52_{s*}'                 .......C..................................   [339]
'Postvect_{s}'              .......C..................................   [339]
'Fasciat2_{b}'              .......C..................................   [339]
'GE07_{b}'                  .......C..................................   [339]
'GE08_{b}'                  .......C..................................   [339]
'GE09_{b}'                  .......C..................................   [339]
'GE17_{b}'                  .......C..................................   [339]
'GE24_{b}'                  .......C..................................   [339]
'GE25_{b}'                  .......C..................................   [339]
'GE28_{b}'                  .......C..................................   [339]
'GE29_{b}'                  .......C..................................   [339]
'GE31_{b}'                  .......C..................................   [339]
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'GE36_{b}'                ??????????????????????????????????????????    [339]
'GE40_{b}'                .......C............??????????????????????    [339]
'GE41_{b}'                .......C............??????????????????????    [339]
'GE42_{b}'                .......C..................................    [339]
'GE43_{b}'                ??????????????????????????????????????????    [339]
'GE46_{b}'                ??????????????????????????????????????????    [339]
'GE47_{b}'                ??????????????????????????????????????????    [339]
'Fasciat3_{b}'            .......C..................................    [339]
'Argenteu_{g}'            .......C..................................    [340]
'Tukituki_{i}'            ??????????????????????????????????????????    [341]
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