

# Assessment of Australian bass (*Percales novemaculeata*) restocking in the Snowy River

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Work reported in this document was undertaken in accordance with Animal Care and Ethics Permit (ACEC 00/06 and ACEC 09/05) and VIC Sampling Permits (RP497 and RP1031).

## NON-TECHNICAL SUMMARY

Assessment of Australian bass restocking in the Snowy River

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### OBJECTIVES:

- To determine the success of Australian bass stocking in the Snowy River.
- To assess how the Australian bass population interacts with wild fish communities.
- To determine if Australian bass disperse and migrate through the Snowy River.
- To develop a technique for differentiating wild from stocked fish.

### NON TECHNICAL SUMMARY:

There is a worldwide decline in freshwater fish stocks, particularly over recent decades, and many species are now at the risk of extinction. Numerous reasons for decline exist but in many instances substantial investment in rehabilitation programs often do little to restore wild recruitment. Often when there is a decline in fish stocks, stocking is used to replenish wild stocks and to assist in recovery. Stocked species may be either already native to the recipient water body, or are introduced. Stocking now occurs globally and is used to rehabilitate over 300 species with the annual cost of restocking activities exceeding billions of dollars.

The Snowy River is a major river in south-eastern Australia originating at Mount Kosciuszko, in the Snowy Mountains region of NSW. It flows into Bass Strait at Marlo, on the coast of Victoria. Flows in the system are largely regulated by releases from Jindabyne Dam, a large upland storage. After release, the river flows for 352 km and drains an area of 13,785 km<sup>2</sup>. The Snowy River is one of the great Australian natural icons having featured in many books, movies and poetry. Unfortunately, much of the attention surrounding the Snowy River today is that of environmental degradation and the consequences arising from the completion of the Snowy Mountains Hydro-electric Scheme (SMHS) in 1974. The diversion of flows has led to dramatic changes to riverine habitats, the magnitude and frequency of floods and the complete loss of seasonal flow variability. These changes have substantially affected the distribution and abundance of native fish in this system.

The Snowy River Native Fish Recovery Plan (Lugg *et al.*, 2006) notes that Australian bass were considered a 'rare' species in the Snowy catchment but an important species for the recreational fishery of the area. Prior research had suggested that there was poor natural recruitment and the majority of the population was quite old. These concerns about a declining Australian bass population in the Snowy River resulted in a large restocking program which aimed to assist the population in returning to previous levels. In total, 232,100 Australian bass fry were stocked into New South Wales waters of the Snowy River below Jindabyne Dam. This study was developed to determine the success of these restocking efforts. Specifically, this project aimed to determine the survival, growth and potential sources of mortality of the stocked Australian bass in the Snowy River. We also sought to determine the habitat used by stocked Australian bass, assess how the

stocked population interacts with wild fish communities, determine the rate at which stocked Australian bass disperse and migrate through the Snowy River and develop and trial a non-lethal marking technique for Australian bass fry and fingerlings.

### **Monitoring and growth of the stocked population**

Monitoring of the stocked Australian bass population and resident fish species of the Snowy River occurred for two years following the initial stocking in November 2007. A total of 2,850 fish from 10 species were collected during routine sampling in 2008 and 2009. Six species were considered native to the system and four were introduced. Australian bass were recaptured in every year following stocking activities. Initial growth rates of Australian bass were high, and most individuals doubled their length every three months. Calculated condition factors were excellent and most fish were in good to excellent condition. However, no Australian bass were collected in any spring sampling event suggesting that survival may have been limited or migration to lower altitude areas may have occurred between winter and spring.

### **Dietary overlap among fish species**

During regular monitoring activities, stomach samples were collected from six co-existing fish species to compare diets and determine dietary overlap. This study confirmed that each of the six fish species examined possesses an individual feeding strategy and this is a likely reason for the co-existence of these species. Four different feeding strategies were evident; carnivores, omnivores, piscivores or herbivores. Dietary overlap between Australian bass and the other five species examined was low and considered not biologically significant.

### **Minimum temperature tolerance of Australian bass**

This study showed that high mortality rates for Australian bass are likely to occur in the wild if water temperatures remain low for long periods, or continue to decrease below a threshold level of 6°C. During temperature tolerance experiments, upon loss of equilibrium, fish lost any capacity to recover and rapidly died. Unless fish can move to warmer areas, or avoid cold conditions, high mortality rates are likely. Stocking of hatchery-reared 0+ Australian bass into upland reaches of the Snowy River, particularly where low temperatures will persist below critical levels will most likely result in high mortality rates during June, July and August unless migration to lower altitudes and more favourable conditions is possible.

### **Marking method for Australian bass**

Two separate experiments were completed using calcein to mark hatchery-reared Australian bass of two sizes. Calcein-marking via osmotic induction had no negative impacts on the growth or survival of hatchery-reared fry and fingerlings. A hand-held GFP meter used to distinguish unmarked from calcein-marked fish correctly separated the two groups by detecting high levels of fluorescence in calcein-marked fish. These levels of fluorescence did not vary greatly among calcein-marked treatments suggesting that even the lowest concentrations and immersion times would result in a reliable detectable calcein-mark in fish.

### **Natal origin and movement patterns of Australian bass**

Examination of chemical ratios at the otolith core (early life) of Australian bass collected from NSW waters of the Snowy River, suspected of being stocked fish from the Narooma hatchery, were found to have significantly different Sr:Ca and Ba:Ca ratios compared to fish collected elsewhere. Examination of chemical ratios across the entire fish otolith (known as transitional analysis) confirmed that juvenile Australian bass collected from the upper Snowy River (NSW waters) originated from an environment much higher in salinity (likely representative of hatchery

conditions) prior to being introduced to the freshwater reaches of the upper Snowy River. Both mature and juvenile sized fish collected from the lower Snowy River were found to move frequently between freshwater and marine environments, as expected by this catadromous fish species.

## 1. GENERAL INTRODUCTION

### 1.1. Fish stocking

There is a worldwide decline in freshwater fish stocks, particularly over recent decades and many species are now at the risk of extinction (Myers and Worm, 2003). Numerous reasons for decline exist but in many instances substantial investment in rehabilitation programs often do little to restore wild recruitment. Efforts to arrest fishery declines frequently involve regulating harvest, habitat restoration, improving water quality, delivery of environmental flows and restocking, or a combination of these approaches.

Stocked species may be either already native to the recipient water body or exotic to it (Cowx, 1998). In the case where a species is exotic to an individual water body, success is usually defined by establishment of a self-sustaining population (Arahamian *et al.*, 2003). Freshwater fish stocking has been practised throughout the world for centuries and is believed to have first occurred in Roman Times when common carp (*Cyprinus carpio*) were introduced to Italy and Greece from Asia (Welcomme, 1998). Stocking now occurs globally and is used to rehabilitate over 300 species worldwide (Welcomme and Bartley, 1998). The annual cost of restocking activities exceeds billions of dollars (Brown and Day, 2002).

Most investment is directed towards stocking activities and considerably less effort to assessing the response and survival of the stocked individuals (Cowx, 1994). Generally, fish stocking is used to either enhance fish stocks in areas of decline, to create new fisheries, to enhance recreational opportunities for anglers or a combination of these purposes. If stocking is used as a surrogate for natural recruitment, it may not lead to a recovery unless the fundamental problems that cause the population to decline are also addressed (Jonsson *et al.*, 1999; Arahamian *et al.*, 2003). In this situation, restocking is regularly undertaken to maintain the population. Unless stocked fish are distinguishable from non-stocked individuals, it then becomes difficult to determine the overall contribution of stocking activities relative to wild recruitment. This scenario precludes effective management of subsequent fisheries because individuals are of undefined origin.

### 1.2. Fish stocking in Australia

Freshwater fisheries in Australia were initially extremely productive, but by the mid 1900's all freshwater fisheries were in decline. The number of professional licences reduced substantially, and by 2000, all freshwater commercial fisheries, excepting common carp (*Cyprinus carpio*) and yabbies (*Cherax destructor*) were closed in New South Wales (NSW).

A need for fish stocking was recognised early during the colonial period. Most initial activities, however, focused on the acclimatisation of exotic species to Australian waters from Europe and North America. The five most translocated species were brown trout (*Salmo trutta*) and rainbow trout (*Onchorhynchus mykiss*) in the late 1800's, common carp (*Cyprinus carpio*) in 1850-1860 and again during the 1960's, roach (*Rutilus rutilus*) between 1860 and 1880, and redfin perch (*Perca fluviatilis*) in the 1860's (McDowall, 1996). Acclimatisation was attempted throughout the colony but was most successful in temperate zones where climatic conditions were most suitable.

The artificial propagation of native species did not commence until the early to mid 1900's (Harris and Battaglione, 1989). Husbandry methods were poorly understood, and early work focused on determining the most appropriate manner to extrude eggs and sperm. There was little focus on rearing and development until the mid 1950's when a native fish hatchery was established within the Murray-Darling Basin. Early work largely focused on percichthyid species; Murray cod (*Maccullochella peelii peelii*) and golden perch (*Macquaria ambigua*), and was generally performed to create several 'put and take' fisheries in areas where wild populations had become extinct upstream of migration barriers. However, it was also used to enhance declining wild fisheries in some riverine reaches.

Improvements in hatchery techniques, combined with the detrimental effects of the construction of large reservoirs, provided the major driver to enhance native fish stocks in the latter half of the 20<sup>th</sup> century. As a result, the stocking of large numbers of native and exotic species into these newly constructed impoundments increased, primarily for recreational purposes. Stocking efforts were expanded to rivers and streams in the 1970's and 80's. Today, there are continued efforts to stock a large number of non-native fish annually. Many of these stockings occur in southern Australia with stockings for salmonids far exceeding those of native fish stockings in NSW.

### 1.3. Australian bass

The Australian bass (*Percales novemaculeata*) is a member of the family Percichthyidae known as the 'perch-like fishes'. The species is a catadromous, long lived (over 20 years), highly fecund, euryphagic carnivore fish species (Harris, 1987). Catadromous fish are defined as ones 'which spend most of their lives in freshwater and which migrate to the sea to breed' (McDowall, 1996). The geographic range of this species encompasses a considerable amount of Australia's most densely populated areas (Harris, 1985) and some of the most regulated rivers (Jerry and Cairns, 1998). The Australian bass is a widespread species native to coastal drainages of south-eastern Australia. Fish grow to 600 mm long and 3.8 kg in weight (Harris and Rowland, 1996).

The presence of barriers can disrupt fish passage and fragment populations (Ward and Stanford, 1979). Catadromous fish species like Australian bass are unable to make recolonising migrations from estuarine areas following the construction of a dam or weir (Jerry and Cairns, 1998, Gehrke and Harris, 2000). In addition, regulated flow from upland storages can have a profound impact on macroinvertebrate communities within a system (Gowns and Gowns, 2001), impacting in turn on fish which feed on these invertebrates. In many areas, migration barriers have facilitated local extinctions of Australian bass from upstream reaches.

Successful hatchery programs have provided an opportunity stock fish into areas of decline. Many land-locked populations have been established and these support several successful recreational fisheries. The inability of Australian bass to reproduce in impoundments reduces potential concerns over the establishment of new populations of inappropriate genetic origin. The situation is more complex in riverine environments. If access to estuarine areas is possible, stocked fish may be able to spawn and successfully recruit. However, the reintroduction of hatchery-reared fish introduces several variables that require control including management of stocking density, maintenance of genetic diversity and minimising impacts on wild populations.

Genetic studies suggest significant genetic diversity between populations of Australian bass in geographically isolated areas (Jerry, 1997; Jerry and Cairns, 1998). So while Australian bass in neighbouring catchments may share common or very similar genetic characteristics, fish

from further away are more genetically diverse. Any stocking programs for Australian bass in NSW must now conform to strict genetic guidelines to ensure diversity is maintained (NSW Fisheries, 2003). By managing restocking programs in an appropriate manner, that is informed by research, successful recovery of wild Australian bass populations may be achieved.

#### **1.4. Snowy River and flow regulation**

The Snowy River is a major river in south-eastern Australia originating at Mount Kosciuszko, in the Snowy Mountains region of NSW. It flows into Bass Strait at Marlo, on the coast of Victoria. Flow in the system is largely regulated by releases from Jindabyne Dam, a large upland storage. After leaving the dam, the river flows for 352 km and drains an area of 13,785 km<sup>2</sup> (Pigram, 2000). The Snowy River is one of the great Australian natural icons having featured in many books, movies and poetry. Unfortunately, much of the attention surrounding the Snowy River today is that of environmental degradation and the consequences arising from the completion of the \$800M Snowy Mountains Hydro-electric Scheme (SMHS) in 1974.

The SMHS was constructed to divert water to irrigation areas in the Murray and Murrumbidgee River and to provide hydro-electric power (Frost, 1983; Erskine *et al.*, 1999). The SMHS was a great engineering achievement consisting of 16 large storage dams and many smaller diversion structures, and hundreds of kilometres of tunnels, aqueducts and pipelines. The SMHS diverts water through a series of turbines and pools from the upper Snowy, Murrumbidgee and Tooma Rivers into either the Swampy Plain or Tumut River. Since the completion of the SMHS, average flow in the Snowy River at Jindabyne has been drastically reduced from 3,200 ML/day to 25 ML/day; this represents less than 1% of natural flow (Pigram, 2000).

The diversion of flows has led to dramatic changes to riverine habitats, the magnitude and frequency of floods and the complete loss of seasonal flow variability (Pendlebury *et al.*, 1996; Rose and Bevitt, 2003). These changes have substantially affected the distribution and abundance of native fish in the system (Gehrke, 2001). A framework has been established to restore up to 28% of natural flows to the Snowy River. In 2002, water flow was less than 1% and an increase to 4% was achieved by 2008. The overall objective is to reach a return of 21% (by 2012). The aim of the program is to improve the hydrology of the river to provide opportunities to rehabilitate the degraded aquatic community. Some species, however, have declined significantly and natural recruitment processes are insufficient to maintain their populations. Australian bass are included in this category.

#### **1.5. Fish communities in the Snowy River**

A total of at least 26 species of fish are recorded from the Snowy River catchment including two threatened species and a combination of native and introduced species (Table 1.1). One of these species, Australian bass (Figure 1.1), was once abundant in the Snowy River with reports that the species was historically caught by anglers as far upstream as Dalgety (altitude of 760 m and approximately 328 km from the river mouth). Reduced flows as a result of the SMHS are believed to have impacted largely on the capacity and frequency of natural spawning events.



**Table 1.1.** Fish species found in the Snowy River catchment. Sources: Raadik, 1992; 1995; Raadik and O’Conner, 1997; DLWC, 1998; Gehrke, 2000; 2001; Rose and Bevvitt, 2003.

Scientific Name	Common Name	Native/Alien
<i>Anguilla australis</i>	Short finned eel	Native
<i>Anguilla reinhardtii</i>	Long finned eel	Native
<i>Arenigobius bifrenatus</i>	Bridled goby	Native
<i>Atherinosoma microstoma</i>	Small-mouthed hardyhead	Native
<i>Carassius auratus</i>	Goldfish	Alien
<i>Gadopsis marmoratus</i>	River blackfish	Native
<i>Galaxius brevipinnis</i>	Climbing galaxias	Native
<i>Galaxius maculatus</i>	Common galaxias	Native
<i>Galaxius olidus</i>	Mountain galaxias	Native
<i>Galaxius truttaceus</i>	Spotted galaxias	Native
<i>Gambusia holbrooki</i>	Gambusia	Alien
<i>Geotria australis</i>	Pouched lamprey	Native
<i>Gobiomorphus australis</i>	Striped gudgeon	Native
<i>Gobiomorphus coxii</i>	Cox’s gudgeon	Native
<i>Herklotsichthys castelnaui</i>	Sprat	Native
<i>Percalates novemaculeata</i>	Australian bass	Native
<i>Mordacia mordax</i>	Short-headed lamprey	Native
<i>Nannoperca australis</i>	Southern pygmy perch	Native
<i>Oncorhynchus mykiss</i>	Rainbow trout	Alien
<i>Perca fluviatilis</i>	Rainbow trout	Alien
<i>Philypnodon grandiceps</i>	Flatheaded gudgeon	Native
<i>Philypnodon sp1</i>	Dwarf flathead gudgeon	Native
<i>Prototroctes maraena</i>	Australian grayling	Native
<i>Pseudaphritis urvilli</i>	Congolli	Native
<i>Retropinna semoni</i>	Australian smelt	Native
<i>Salmo trutta</i>	Brown trout	Alien

## 1.6. Snowy River Native Fish Recovery Plan

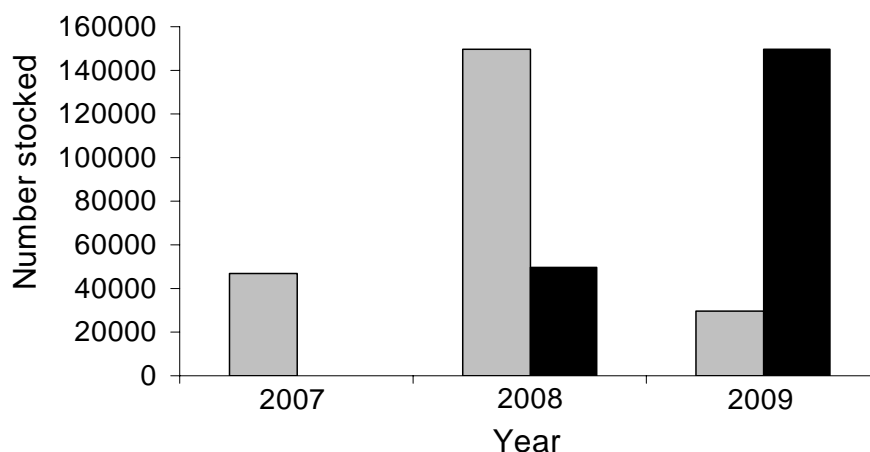
The Snowy River Native Fish Recovery Plan (Lugg *et al.*, 2006) was developed to assist in the recovery of the native fish population in the Snowy River from the estuary at Marlo upstream to Jindabyne dam. The Recovery Plan outlines the serious decline in fish populations particularly in the upper reaches of the Snowy River. Australian bass recruitment in the Snowy River is reported in the Recovery Plan as being ‘poor’ with the majority of the population being old fish (i.e. >20 years). Furthermore, the Plan reports Australian bass as a ‘rare’ species throughout the Snowy River and lists a number of factors that have most likely contributed to their decline including competition and predation from introduced species, de-snagging, siltation, sedimentation, loss of riparian vegetation, fish passage barriers, modification of flow regimes, reductions in flow and possible losses in water quality. The

Plan also lists two future threats including invasion by new alien fish species and diseases (Lugg *et al.*, 2006).

As a result of the decline in the Australian bass population in the Snowy River, attempts to re-introduce the species into the upland reaches have been made. A one-off stocking of 13,000 fingerlings was undertaken in the late 1990's by Native Fish Australia (East Gippsland) into the Victorian section of the Snowy River. The success of this stocking remains unknown. Following this, one of the major recommendations of the Snowy River Native Fish Recovery Plan was to enhance the population of Australian bass in the Snowy River through stocking. Since then, a large restocking effort has been undertaken in both the NSW section of the Snowy River (which extends 183 km below Jindabyne Dam) and in Victorian reaches by the Southern Rivers Catchment Authority (SRCMA) In total, 427,000 Australian bass fingerlings were stocked in the period 2007-2009 (Figure 1.2).



**Figure 1.1.** Australian bass (*Percales novemaculeata*).



**Figure 1.2.** Numbers of Australian bass fry stocked into the NSW (grey bars) and Victorian (black bars) sections of the Snowy River in the period 2007-2009 sponsored by Southern Rivers Catchment Management Authority.

### **1.7. Purpose of this study**

This current study was developed to determine the success of Australian bass restocking efforts in the Snowy River. Specifically, the project aimed to determine the survival, growth and potential sources of mortality of the stocked Australian bass in the upper Snowy River. We also sought to determine the habitat used by stocked Australian bass, assess how the stocked population interacts with wild fish communities, determine if stocked Australian bass disperse and migrate through the Snowy River away from the upper Snowy River and develop and trial a non-lethal marking technique which could be applied to Australian bass fry or fingerlings prior to future stocking efforts.

This study is one of the first of its kind and will provide a greater understanding of the factors influencing the success of Australian bass stockings specifically in the Snowy River. Clearly defining these factors and assessing measures to address them should lead to the development of more effective stocking plans in the future.

## **2. FISH SPECIES COMPOSITION AND THE GROWTH, SURVIVAL AND HABITAT USE OF AUSTRALIAN BASS IN THE SNOWY RIVER**

### **2.1. Introduction**

Stocking success can be influenced by many external factors. The size of fish at stocking can influence the ability to compete for resources or physiologically adapt to a new environment and can ultimately lead to varying levels of survival (McKeown *et al.*, 1999). Fish community structure in the receiving stream could render the stocked population susceptible to increased competition or predation (Wahl, 1995). The lack of suitable habitat or unsuitable water quality can also create substantial problems for fish in a new environment and may force population bottlenecks in fish (Elliott, 1990). Monitoring the survival, growth and integration of stocked fish into target habitats should therefore form an important component of any stocking program.

Unfortunately, limited resources usually restrict the amount of detailed stock assessments that can be done. This frequently creates situations where stocking success remains largely unknown. In many cases, no information exists to inform what stocking densities should be used, the objectives for the fishery (or population in the case of conservation stocking) or the overall impact of stocking activities on resident fish (Cowx, 1994). Stocking programs may continue for many years despite a lack of quantitative information on the status of the stocked population. Collection of baseline data, at set intervals following stocking events, could greatly assist fisheries management and help determine if the program was ultimately successful and the value in continuing stocking efforts.

The absence, or inadequacy, of monitoring programs following many stocking events does not allow for evaluating stockings and improvements in further stocking techniques (Agostinho *et al.*, 2010). Several aspects of monitoring can help determine whether a stocked population has established. Firstly, ongoing surveys using standardised methods are essential, if conducted at regular intervals to confirm the ongoing presence of stocked fish. Such monitoring studies may use methods such as electrofishing (Henry *et al.*, 2008), gillnetting (Hunt *et al.*, 2010) or even visual census (Santos *et al.*, 2006) to collect stocked fish. Once confirmed, the collection of length, weight and age information can be used to report relevant population statistics. A range of commonly used estimators are available and there are many software programs that can assist with the analysis of complex datasets. These data could then be augmented with prey availability and diet information to confirm that levels of nutrition in the waterbodies to be stocked are appropriate.

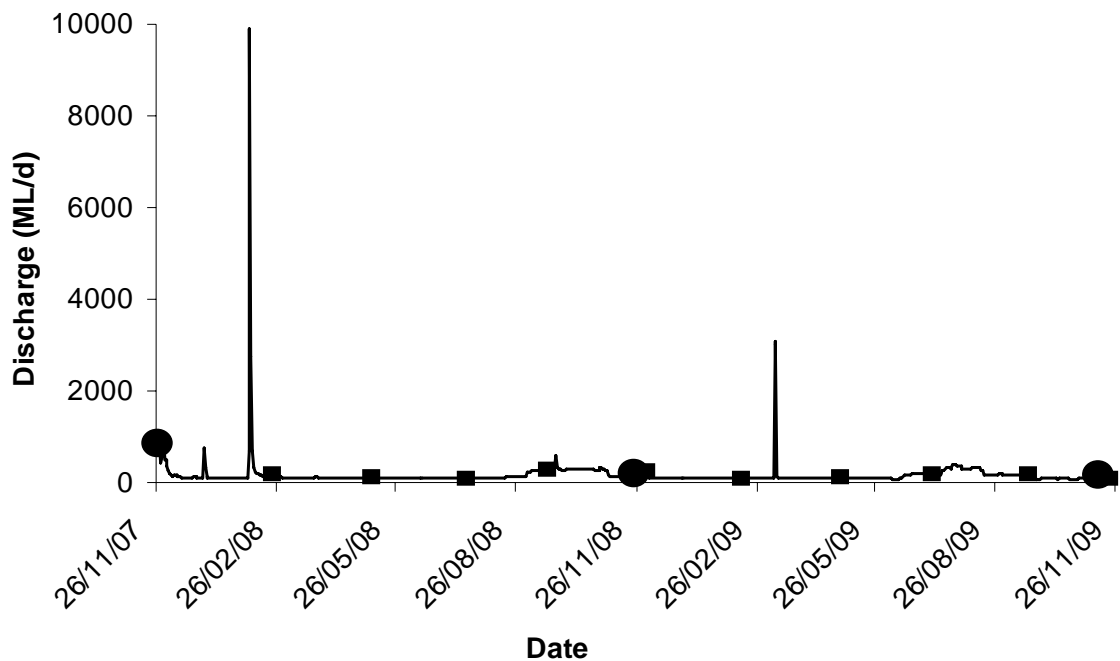
This study sought to perform a detailed, post-stocking assessment of an introduced Australian bass population in the Snowy River. A combination of electrofishing and netting was used to monitor fish community composition for two years following commencement of the stocking program. The study sought to determine the overall effect of stocking fish at two different densities (high and low) on growth rate and weight of individual fish. River reaches were sampled at regular intervals to investigate the effect of seasonality on the stocked population.

## 2.2. Methods

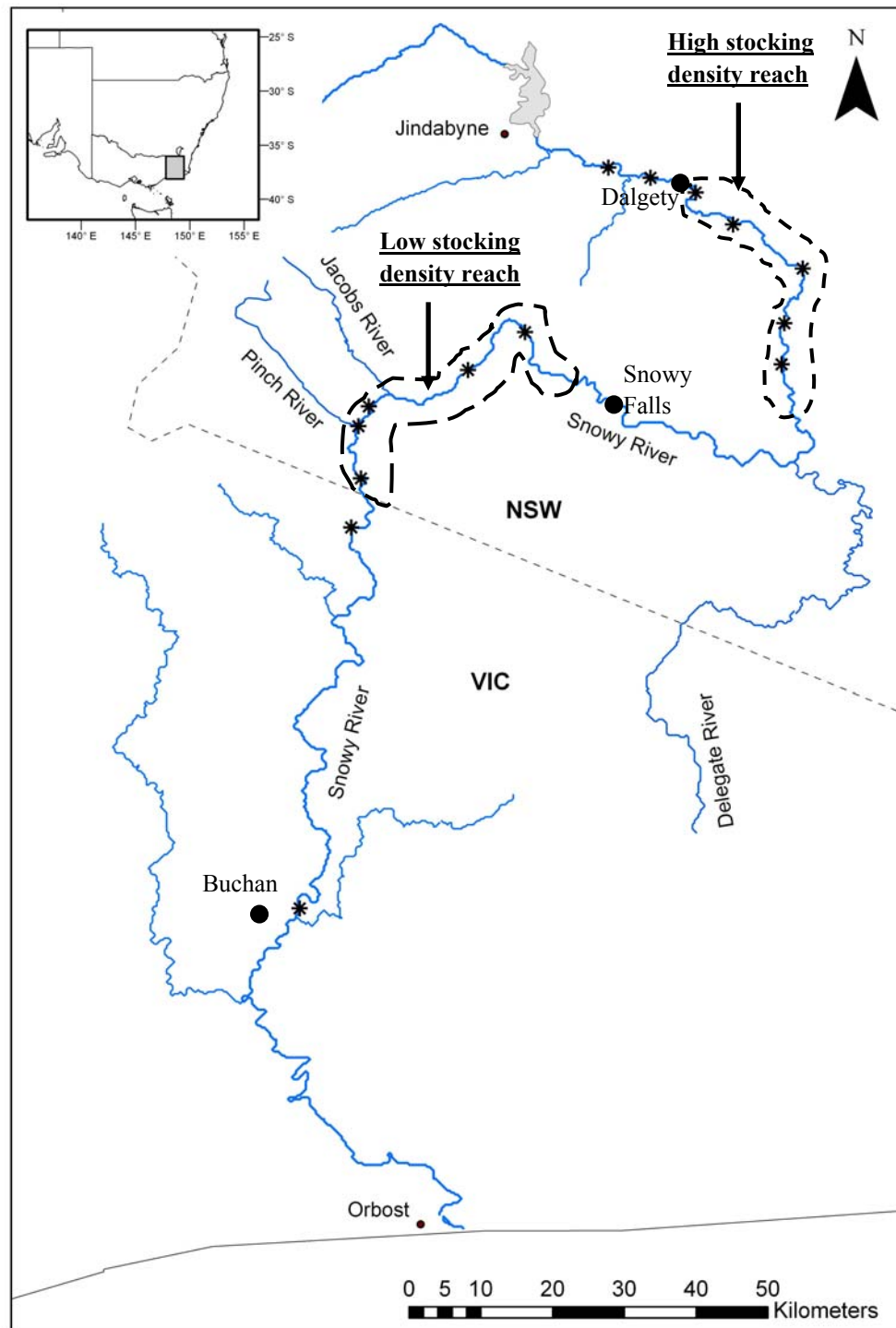
### 2.2.1. Study sites

Sampling was done between February 2008 and November 2009. Mean daily river flow during this period was 169 ML, and river flows generally remained at approximately 100-200 ML/day. However, there was one large increase in February 2008 when flows reached 9896 ML/day (shortly after the November 2007 stocking event) and another in March 2009 (Figure 2.1).

Fish were collected from 14 sites using standardised methods (Figure 2.2). Five sites were located within a high density stocking reach, where Australian bass were introduced at a rate of 1,000 fish/km. Five sites were located within a low density reach where stocking took place at 500 fish/km. Sampling was performed at an additional four sites outside the 2 main stocking zones. Two were upstream of the high density reach and two were downstream of the low density reach. The purpose of these additional sampling was to determine whether fish moved away from the stocking reaches. The sites also served as a non-stocking control for analyses seeking to determine density-dependent influences on growth.



**Figure 2.1.** Hydrograph of the Snowy River at Dalgety from the first stocking of Australian bass (November 2007) to the last date of sampling (November 2009). Black circles represent stocking dates and black squares represent sampling occasions.



**Figure 2.2.** A map of the Snowy River reach from Jindabyne dam to Orbost highlighting the location of the 14 sampling sites (\*), the major natural migration barrier (Snowy Falls) and the two reaches (high and low) where stocking was done.

### **2.2.2. Fish Collection**

Fish collections were based on the NSW Rivers Survey electrofishing method (Harris and Gehrke, 1997). At nine sites, fish were collected by using an electrofishing vessel (3.6 metre, flat-bottomed aluminium vessel) equipped with a 2.5 kW Smith-Root Model GPP 2.5 H/L electrofishing unit (Figure 2.3). The boat electrofisher was generally operated at between 200 and 1000 V DC, 2 - 5 amps pulsed at 120 Hz and 20 - 50% duty cycle, depending on water conductivity. One senior operator controlled the boat from the back and a second operator controlled fishing operations and dip-netted fish from the front of the boat. Back-pack electrofishing was performed at five sites where boat access was not possible (Figure 2.4). Pool and riffle habitats were sampled using a 400 W Smith-Root Model 12 backpack electrofisher (Figure 2.4). The pulsed DC output used was commonly between 300 and 500 V, 0.5 - 1.0 amps at 60 Hz. The sampling methods used comply with the Australian Code of Electrofishing Practice.

All electrofishing operations consisted of 15 x 2 minute operations per site, undertaken during daylight hours. At the end of each operation, all fish were identified, counted, measured and inspected for any external parasite or disease. Large bodied fish were stomach pumped (Chapter 3) and then released. All Australian bass collected were retained for ageing purposes. Length measurements to the nearest millimetre (mm) were taken as fork length for species with forked tails, and total length for other species. In addition to electrofishing, ten commercially available bait-traps were set in water depths of less than 1 m to sample small or benthic species typically under-represented by electrofishing. Bait-traps were set before electrofishing commenced and were removed at the completion of electrofishing. The minimum set time for bait-traps was 2 hours. The catches from all gear types were pooled for subsequent analysis.

### **2.2.3. Seasonality**

Fish collection events were stratified into Austral seasons. Fish were collected in each year during spring (September - November), summer (December - February), autumn (March - May) and winter (June - August). Fish collections were stratified so that at least one sample was collected from each season over the two year study.

### **2.2.4. Calculating fish growth**

Transverse sagittal otolith sections were examined using a compound microscope at 100× magnification, and counts of the opaque bands were used to estimate age. Thin sections of otoliths revealed a clear pattern of translucent and opaque bands when viewed under both transmitted and reflected light. Fish were aged by counting growth rings on the transverse section of one otolith from each Australian bass, based on the procedure used by Harris (1987). One ring was assumed to be laid down each year and each fish was assigned the nominal birthday of 1 September which is when fish were produced in the hatchery (Bruce Lawson, Pers Comm).



**Figure 2.3.** Fisheries technicians Leo Cameron and Tim McGarry operating a boat-mounted electrofisher on the Snowy River (Photo: Mick Bettanin, NSW DPI).



**Figure 2.4.** Fisheries technicians Jarrod McPherson and Mick Bettanin operating a backpack electrofisher on the Snowy River. (Photo: Leo Cameron, NSW DPI).



### 2.2.5. *Fish Condition*

Two indicators of fish health were used to determine the overall condition of Australian bass that were collected. The estimators were calculated on fish collected from both reaches (high and low stocking density zones) and among all seasons sampled (autumn, winter, spring, summer).

The two estimators were:

- 1) Logarithmic length-weight relationship; defined as  $W = aL^b$

which can be alternatively expressed as  $\log_{10} W = b(\log_{10} L_f) + \log_{10} a$

where  $W$  is the weight of the fish,  $L_f$  is the fork length of the fish,  $b$  is the slope (exponent), and  $a$  is the y-intercept determined from empirical data. Growth is considered isometric (i.e. body form and specific gravity do not change) when  $b$  (regression slope) = 3.

- 2) Condition factor; defined for Australian bass (Harris, 1987) as a modified version of Fulton's  $K$ , is expressed as:

$$C = \frac{0.1W}{(0.01L_f)^3}$$

where  $W$  is the weight of the fish,  $L_f$  is the fork length and  $C$  is the condition of the fish. These measures of length-weight and condition have been previously used for Australian bass so could be compared to other studies (Harris, 1987).

### 2.2.6. *Data Analysis*

Data were analysed using S-PLUS and PRIMER. General linear regression techniques were used to explore the relationship between log transformed length and weight relationships for Australian bass. Data were log (x+1) transformed data and Cochran's tests determined non-homogeneity of variances.

Two-Way Analysis of Variance was used to determine any differences in individual species abundance and condition factor between reaches (high and low density) and among seasons (autumn, winter, spring, summer). A variance stabilising log (x+1) transformation was applied to abundance data prior to analyses. No transformation was required for condition factor.

The number of individual fish species caught from each site was standardised for time (number.hour<sup>-1</sup>) and fish communities were compared by calculating Bray-Curtis similarity values (Clarke and Warwick, 1994). A two-way, nested Analysis of Similarities (ANOSIM) was used to test for fish community differences between reaches (high and low density) and season (autumn, winter, spring and summer), with sample differences plotted using non-metric multi-dimensional scaling ordinations. Twenty thousand Monte-Carlo randomisations were used to calculate approximate probabilities for the ANOSIM test. All statistical tests were considered significant at  $P < 0.05$ .

### 2.3. Results

A total of 2,850 fish from 10 species was collected during routine sampling between 2008 and 2009. Six species were considered native to the system and four were introduced (Table 2.1). The most commonly collected species were Australian smelt and gambusia. There was substantial seasonal variation in fish abundance. Gambusia and goldfish were collected in highest abundance during autumn. Australian smelt and Longfinned eel were more prevalent during winter and spring. No Australian bass, rainbow trout or gambusia were collected during spring.

More fish were collected from the low density stocking reach (n = 2,056) than the high stocked density reach (n = 794). No Australian smelt, Congolli or rainbow trout were collected from the high density reach. Furthermore, no more than four species were collected from this reach during spring and no Australian smelt, Longfinned eel, Congolli, gambusia or rainbow trout were sampled from spring or summer from this reach. All other species were collected from the low density reach, but the seasonal absences were less pronounced. Only Australian bass and rainbow trout were absent from spring and summer sampling. Species richness was highest in winter, but lowest in summer and greater total numbers of fish were collected during autumn and winter sampling. Shortfinned eel and goldfish were the only two species to be captured from both river reaches in all seasons.

**Table 2.1.** Total catches of individual species in each reach of a given stocking density (high or low) and season. All collected species are listed and data is not standardised to effort.

Species	High Density Reach				Low Density Reach			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
<i>Native Fish</i>								
Australian bass								
<i>Percalates novemaculeata</i>	2	40	0	24	4	2	0	0
Australian smelt								
<i>Retropinna semoni</i>	0	0	0	0	43	745	267	11
Longfinned eel								
<i>Anguilla reinhardtii</i>	0	6	0	0	71	108	68	83
Congolli								
<i>Pseudaphrities urvilli</i>	0	0	0	0	7	26	14	3
Mountain galaxias								
<i>Galaxias olidus</i>	1	5	1	1	0	0	2	0
Shortfinned eel								
<i>Anguilla australis</i>	52	57	42	62	1	9	4	1
<i>Alien Fish</i>								
Brown trout								
<i>Salmo trutta</i>	0	9	5	1	1	6	3	0
Gambusia								
<i>Gambusia holbrooki</i>	226	55	0	0	124	30	0	27
Goldfish								
<i>Carassius auratus</i>	136	52	4	13	287	81	6	24
Rainbow trout								
<i>Onchorhynchus mykiss</i>	0	0	0	0	0	1	0	0
Grand Total	417	224	52	101	538	1008	364	149

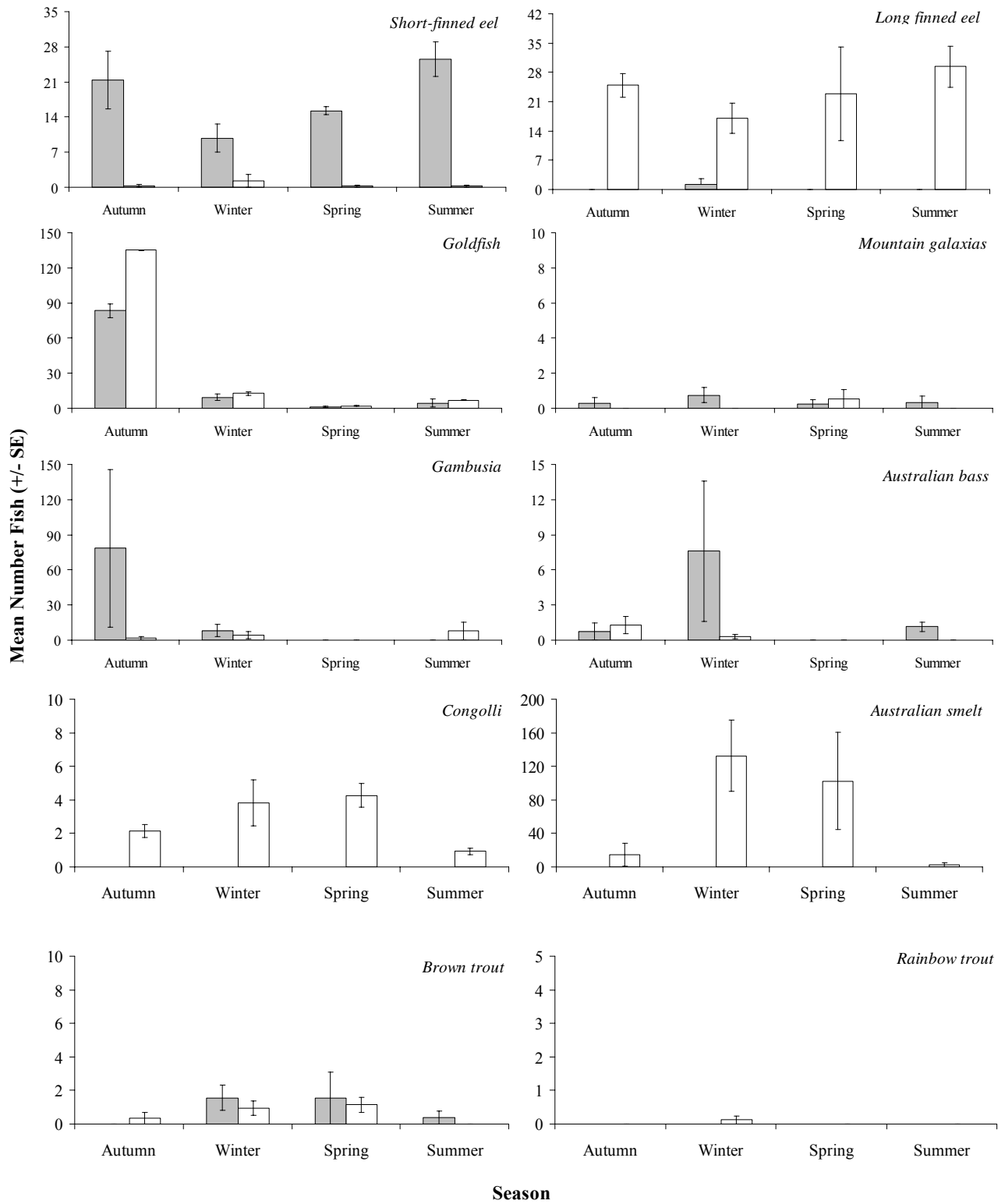
### 2.3.1. Seasons vs sites

Seasonal changes in fish abundance varied among species. Neither goldfish nor brown trout exhibited any significant spatial (among reaches) or seasonal (among austral seasons) changes in abundance (Two-Way ANOVA; Figure 2.5, Table 2.2). There were no spatial differences in the abundance of gambusia or Australian bass among the two different stocking reaches. However, there was substantial seasonal variation in abundance. Gambusia were more abundant in autumn (Table 2.2, Figure 2.5), and Australian bass abundances in spring combined with an elevated abundance in winter contributed to differences. Parametric analyses were only possible for four species due to non-homogeneous variances. Multivariate statistics were subsequently applied to investigate any multi-species distribution patterns. Multidimensional scaling revealed clear separation of fish communities between stocking reaches (Two-Way ANOSIM: Global R = 0.975;  $P < 0.001$ ; Figure 2.6). The relatively high R value indicates high degrees of multi-species similarity within the two stocking reaches. Variations in the abundance of six species (Australian smelt, Longfinned eel, Shortfinned eel, Congolli, gambusia and Australian bass) contributed to observed differences (SIMPER: 81.87% of cumulative contribution; Table 3.3). Australian smelt, Longfinned eel, gambusia and Australian bass were collected in greater abundances from high density reaches. Congolli and shortfinned eel were collected in higher abundance from low density reaches. Both Australian smelt and Congolli were totally absent from high density sites (Table 3.3).

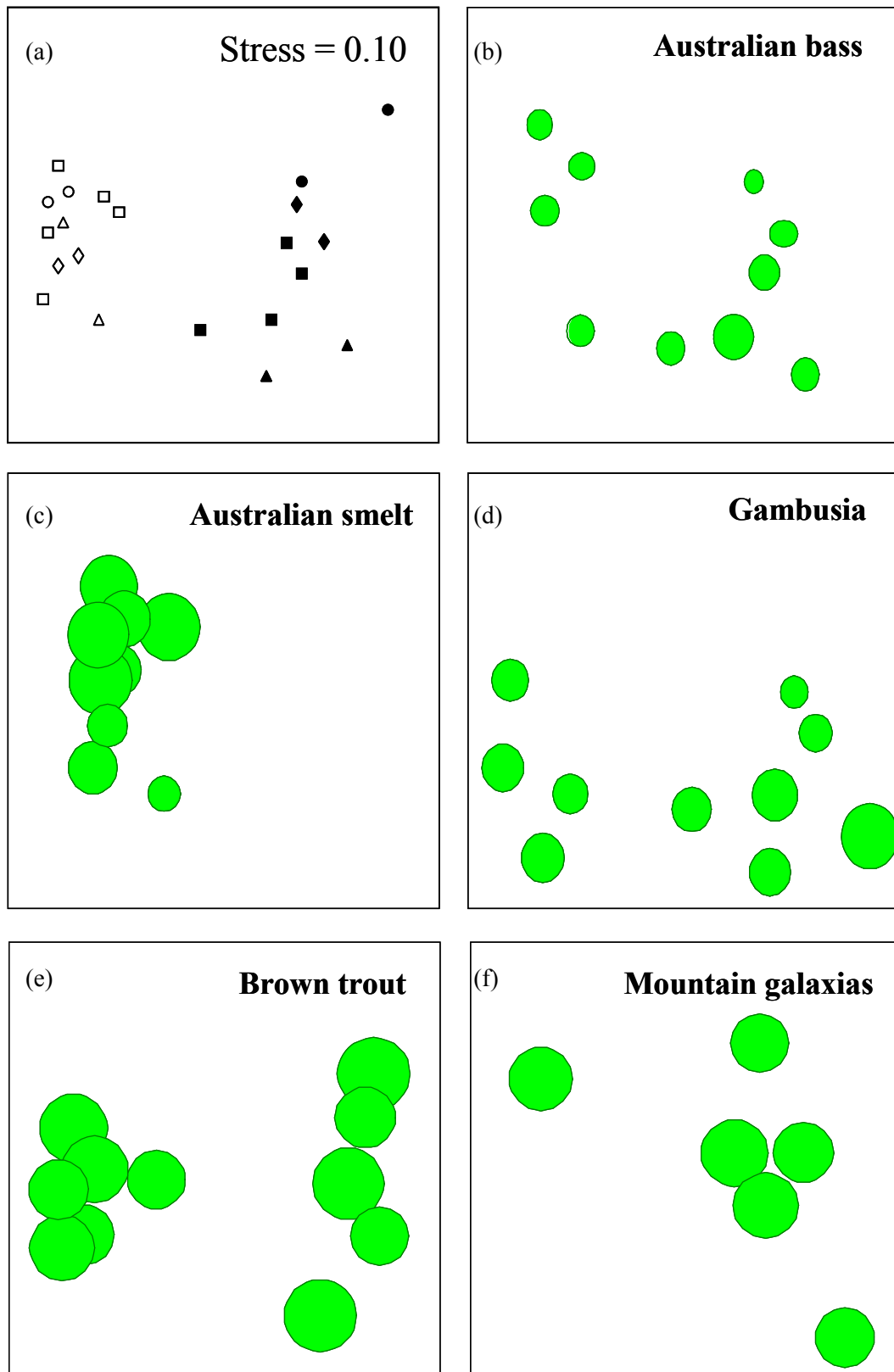
Significant differences among seasons were also detected (Two-Way ANOSIM: Global R = 0.293,  $P < 0.001$ ). Seasonal groups were tightly grouped in ordinal space, particularly from the high density stocking reach, indicating a substantial degree of similarity among species abundance (Figure 2.5). More variation was apparent from the low density stocking reach. There was substantial dissimilarity in species assemblages in autumn and winter, due largely to changes in the abundance of gambusia in low density reaches. This separation, however, was not statistically significant due to the much tighter grouping of these seasons in the analysis for the high density reach. This is suggestive of an interactive effect among these groups, which cannot be tested using ANOSIM. The lower value of R is suggestive of increasing similarity within and between groups which arises from low catches and absences of some species in certain seasons. Abundance (bubble) plots further identify a lack of any strong seasonal structure (Figure 2.6). Fish communities among winter and summer were the only significant pairwise comparison (ANOSIM: R = 0.339,  $P < 0.05$ ). The differences were largely driven by high winter abundances of gambusia, Australian smelt, brown trout and Australian bass (SIMPER: 64.25% cumulative contribution; Table 2.4). Shortfinned eel were the only species with higher summer abundance (Table 2.4).

**Table 2.2.** ANOVA results of the effect of stocking density (high or low) and season (autumn, winter, spring, summer) on the abundance of fish collected from the Snowy River. Tests were only conducted on abundant fish with homogenous variances. F-ratios are given and tests were considered significant at  $P < 0.05^*$ ,  $< 0.01^{**}$  or  $< 0.001^{***}$ .

Factor	df	Goldfish	Gambusia	Australian bass	Brown trout
Density	1	0.27	1.53	0.32	0.22
Season	3	1.78	22.63***	23.02***	1.33
Density:Season	3	0.15	1.69	0.68	0.14



**Figure 2.5.** Interaction plots showing differences in mean fish abundance between high (grey) and low (white) stocking density zones among seasons. All means are shown with one  $\pm$  S.E.



**Figure 2.6.** (a) Multidimensional scaling ordinations of Bray-Curtis similarity measures highlighting differences between low (black) and high (white) density stocking zones along with changes in season (autumn - triangle, spring - circle, summer - diamond, winter - square). Bubble plots (b - f) represent the contribution of individual species to the groupings.

**Table 2.3.** Similarity percentages (SIMPER) table showing differences in mean abundance of species collected from high and low density stocking zones. Only higher contributing species are shown. Diss/SD is the ratio of dissimilarity contribution to the standard deviation and is higher for species contributing more to observed differences. Cum % is the percentage contribution of each species to observed differences. Only comparisons deemed significant by ANOSIM are shown.

High density vs low density; Average Dissimilarity = 65.37%				
Species	High Density Abundance	Low Density Abundance	Diss/SD	Cum.%
Australian smelt	0.00	2.40	2.27	23.29
Longfinned eel	0.15	2.14	2.39	40.96
Shortfinned eel	1.96	0.45	2.11	54.66
Congolli	0.00	1.27	4.46	66.42
Gambusia	1.11	0.67	1.03	75.07
Australian bass	0.79	0.38	1.25	81.87
Goldfish	1.43	1.83	0.94	88.11
Brown trout	0.57	0.61	1.09	93.19

**Table 2.4.** Similarity percentages (SIMPER) table showing differences in mean abundance of species collected among different sampling seasons. Only higher contributing species are shown. Diss/SD is the ratio of dissimilarity contribution to the standard deviation and is higher for species contributing more to observed differences. Cum % is the percentage contribution of each species to observed differences. Only comparisons deemed significant by ANOSIM are shown.

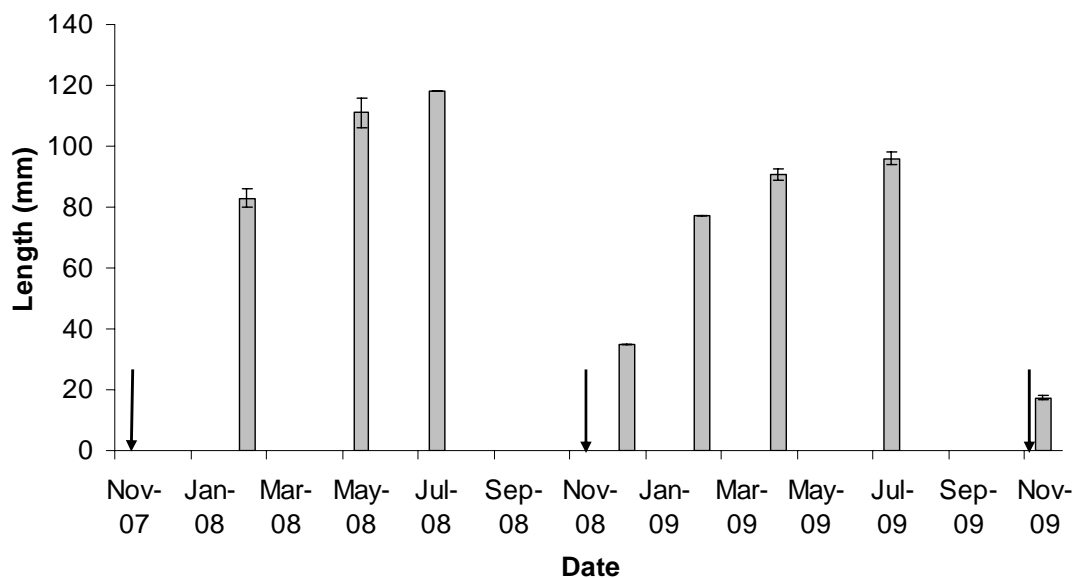
Winter vs Summer; Average dissimilarity = 35.89%				
	Winter Abundanc e	Summer Abundanc e	Diss/SD	Cum. %
Gambusia	1.15	0.5	1.66	22.47
Australian smelt	1.62	0.38	0.84	41.47
Brown trout	0.82	0.23	1.41	54.07
Australian bass	0.81	0.51	1.12	64.25
Shortfinned eel	1.05	1.33	1.44	74.26
Goldfish	1.64	1.53	1.39	81.38

### 2.3.2. Growth and condition

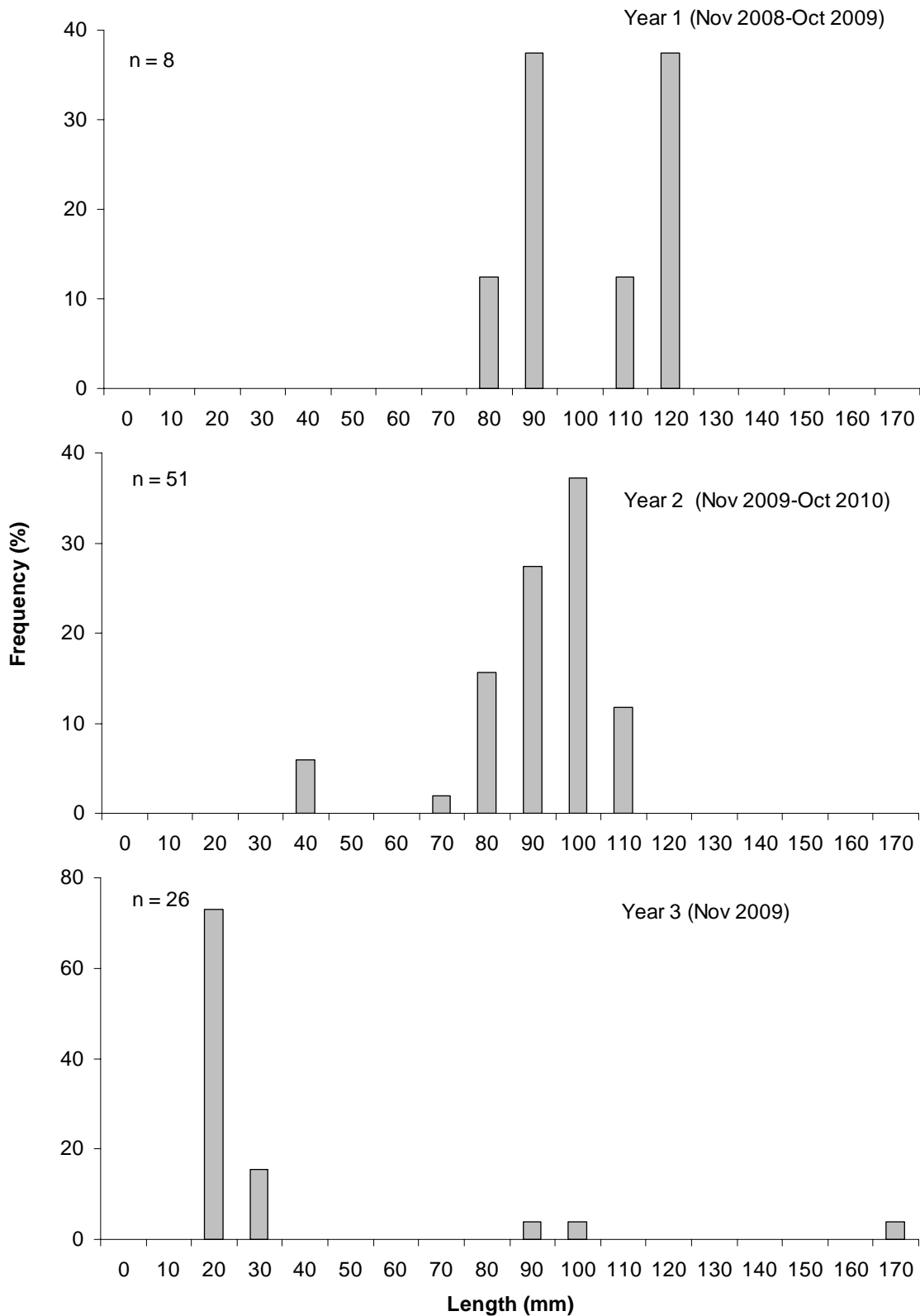
Growth and condition parameters were calculated to determine Australian bass population dynamics. Fish grew relatively quickly post-stocking, progressing quickly from the initial stocking size of approximately 15 mm to four times this length after 3 months at liberty. Growth rate then slowed substantially and it took fish a further 12 months to double in length again (Figure 2.7). Australian bass were collected in all seasons of the first year and displayed an expected increase in average length, but were subsequently absent from any sampling conducted after spring at which point in time they were approximately 10 months at liberty. Following spring each year, the average size of Australian bass dropped substantially as the existing population disappears from the catch and the newly stocked fish are collected.

Few stocked Australian bass were collected 12 months after the inaugural stocking (2007; Figure 2.8) which were likely to survivors of this initial stocking. Australian bass were collected approximately 3 months post stocking during summer sampling, and again approximately six months post-stocking during winter sampling. Substantially more fish were collected 12 months following the next stocking event (2008) but there was a notable absence of any fish from the initial stocking. Fish from this second stocking had grown rapidly (Nov 2008-09; Figure 2.8), but again no fish were collected after winter. A small number of fish were collected following the third stocking event, although these were collected after only one week at liberty (Nov 2009; Figure 2.8).

Condition factors showed some degree of variation among river reaches with different stocking densities, although no significant differences were detected (ANOVA:  $F_{2,83} = 2.67$ ;  $P > 0.05$ ). The lack of Australian bass collected from spring prevented any seasonal analysis of changes in condition. However, a few fish collected from the non-stocked reach ( $n = 2$ ) and high density stocking reach ( $n = 4$ ) had very low conditions factors ( $< 1$ ) which are indicative of extremely poor health (Figure 2.9). Most other fish were in relatively good condition (Figure 2.9).

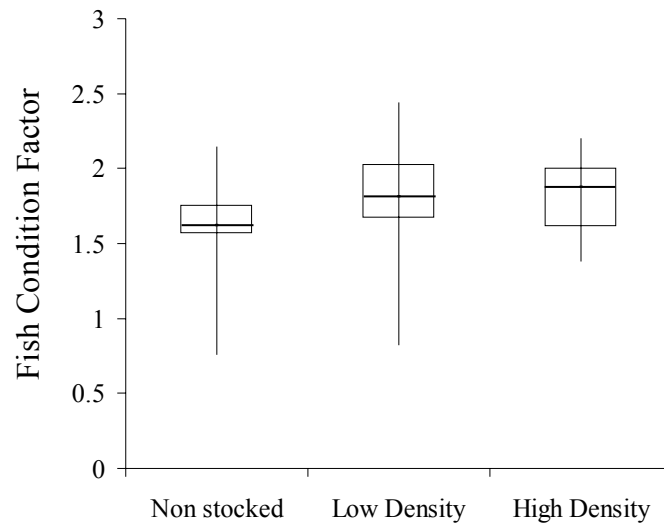


**Figure 2.7.** Mean length ( $\pm$  S.E.) of Australian bass collected each sampling event (February 2008 to November 2009) from the two stocked reaches combined. Arrows indicate stocking events. No data is presented for September sampling in either year as no Australian bass were collected.



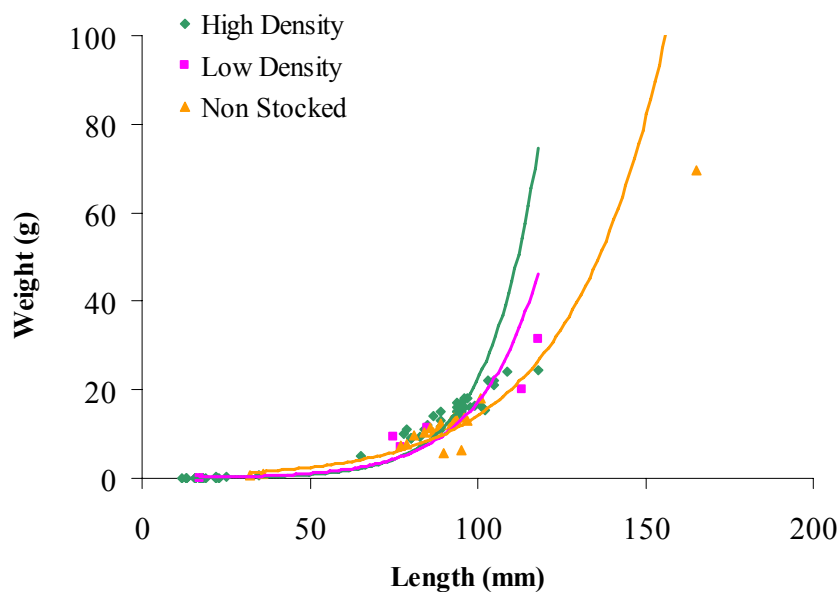
**Figure 2.8.** Length-frequency distributions of Australian bass caught in each year of the study. Each year reflects the 12 month period following the previous stocking event. The November 09 event occurred 5 days after the sampling event and no further data were collected from that year.



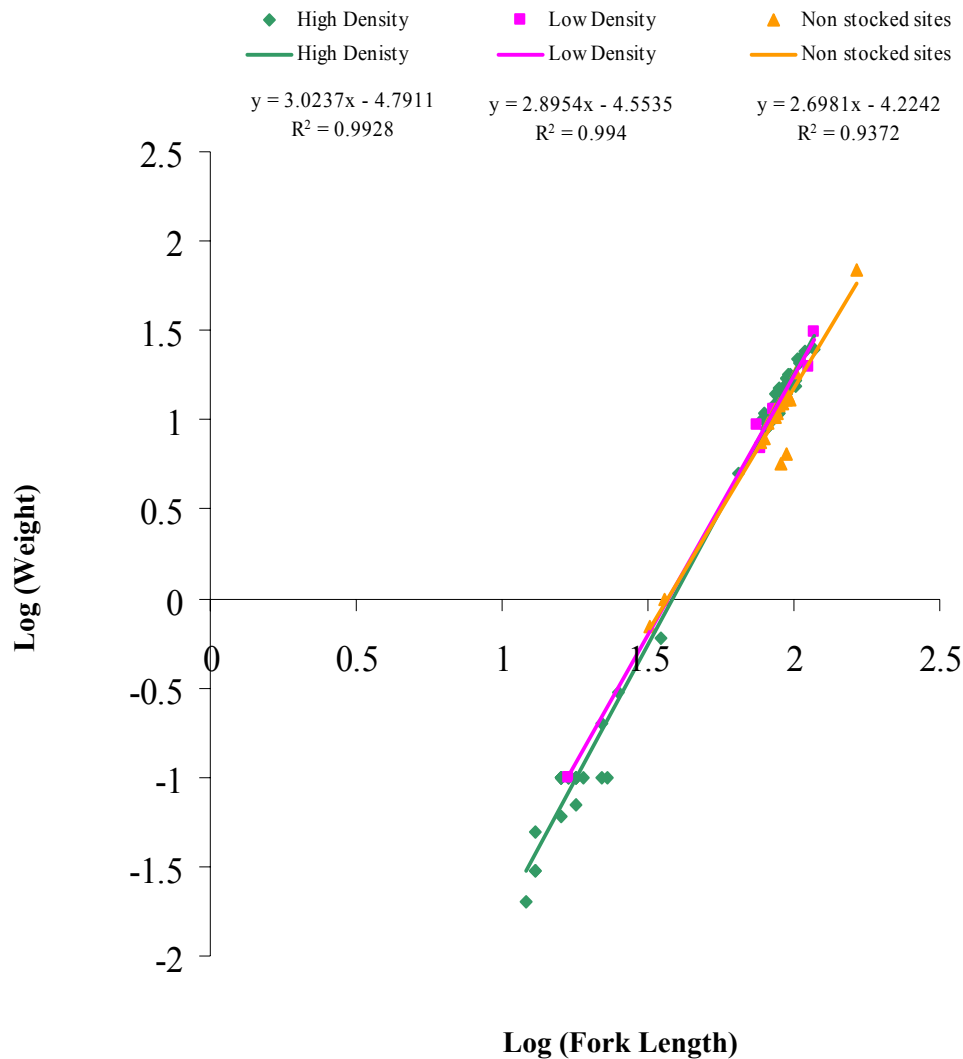


**Figure 2.9.** Box plot of median Australian bass condition factors from each of the three sampling locations in the Snowy River. Box plots are shown with upper and lower quartiles with maximum and minimum outliers.

Length-weight relationships were compared among Australian bass collected from the three river reaches. There was high agreement among growth rates for smaller sized fish (<100mm) but a lack of larger fish led to variation in estimates for larger size classes (Figure 2.10). Log-linear transformations of growth rates identified little difference in slope (exponent  $b$ ) among the three river reaches (Figure 2.11). In general, fish collected from the high stocking zone ( $b = 3.02$ ) were closest to isometric growth (estimated at  $b = 3$  for most fish species) suggesting that length and weight relationships were within expected values. Fish collected from the non stocking ( $b = 2.69$ ) and low density reach ( $b = 2.89$ ) are considered negatively allometric; and were underweight for the length classes that were collected.



**Figure 2.10.** Scatter diagram of the relationship between length and weight for Australian bass captured from high density (green), low density (pink) and unstocked (orange) reaches. Fitted lines are based on the assumption of isometric growth (i.e.  $b = 3$ ).

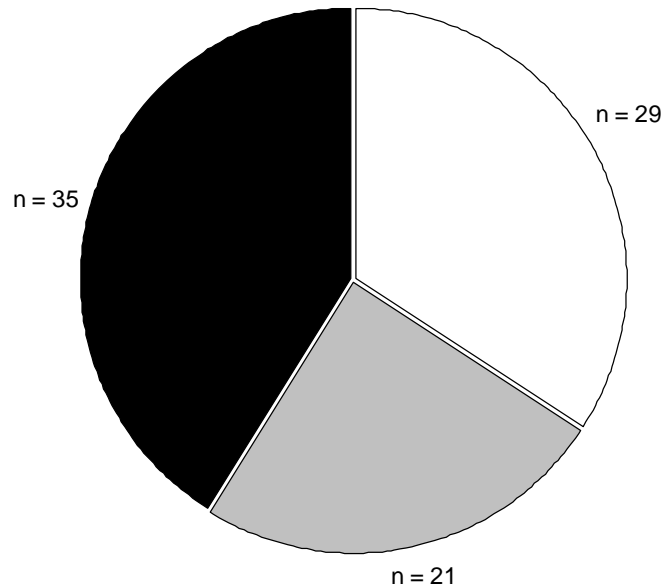


**Figure 2.11.** Rectilinear length-weight relationships for Australian bass using logarithmic transformation. Data are shown for the high density (green), low density (pink) and unstocked (orange) sites.

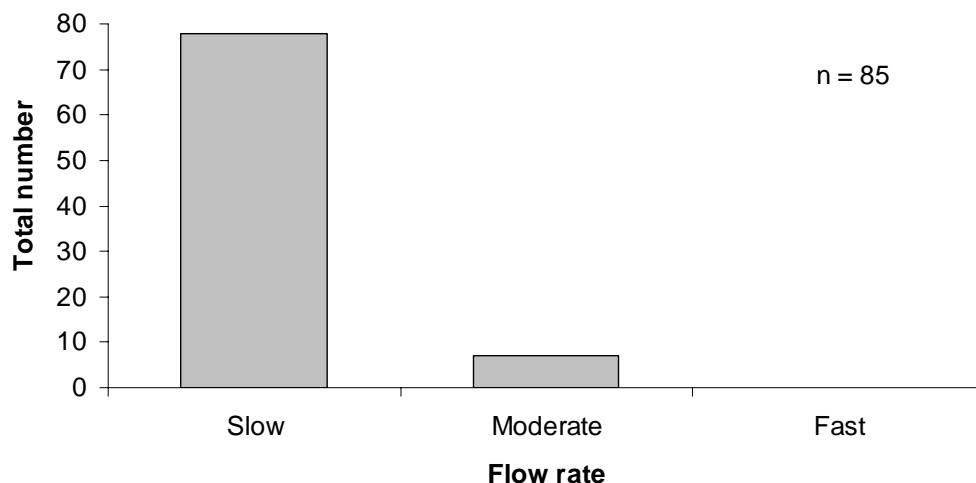
### 2.3.3. *Habitat use of Australian bass*

Detailed descriptions including water quality parameters, water depth, water velocity, cover and location of capture within the Snowy River were recorded each time Australian bass were collected. Individuals were collected in water depths of 0.3 to 4 m of water (Figure 2.12). Smaller Australian bass, such as those collected soon after stocking events (15 to 40 mm fish) were generally captured in shallow water (under 1 m) and larger fish which had been stocked for a longer period, were generally captured in deeper water up to several metres in depth. Whilst water depth among capture sites varied, there was a much more specific water velocity preference shown by Australian bass. Over 91% of the individuals captured were from sections with a slow water flow rate (<0.1 m/sec) (Figure 2.13). Australian bass had a preference for edge habitat (over 94%) compared to the middle sections of the river (6%). Edge habitats typically consisted of large bedrock, boulders (Figure 2.14), often with overhanging timber.

Irrespective of season sampled, Australian bass were collected from a wide range of water temperatures, pH levels, conductivities, turbidity and dissolved oxygen levels (Table 2.5). These observed water quality measurements are among some of the only available data on environmental tolerances for Australian bass. Of particular interest, these new data provide the lowest recorded water temperatures in which Australian bass are known to live in the wild; 5.6°C.



**Figure 2.12.** Percentage of Australian bass (n = 85) collected in 0-1 m water (white), 1-2 m water (grey) and 2-5 m water (black).



**Figure 2.13.** Flow rate at which Australian bass were collected during sampling. Slow (<0.1 m/sec), moderate (0.1 m/sec – 0.5 m/sec) or fast (>0.5 m/sec).



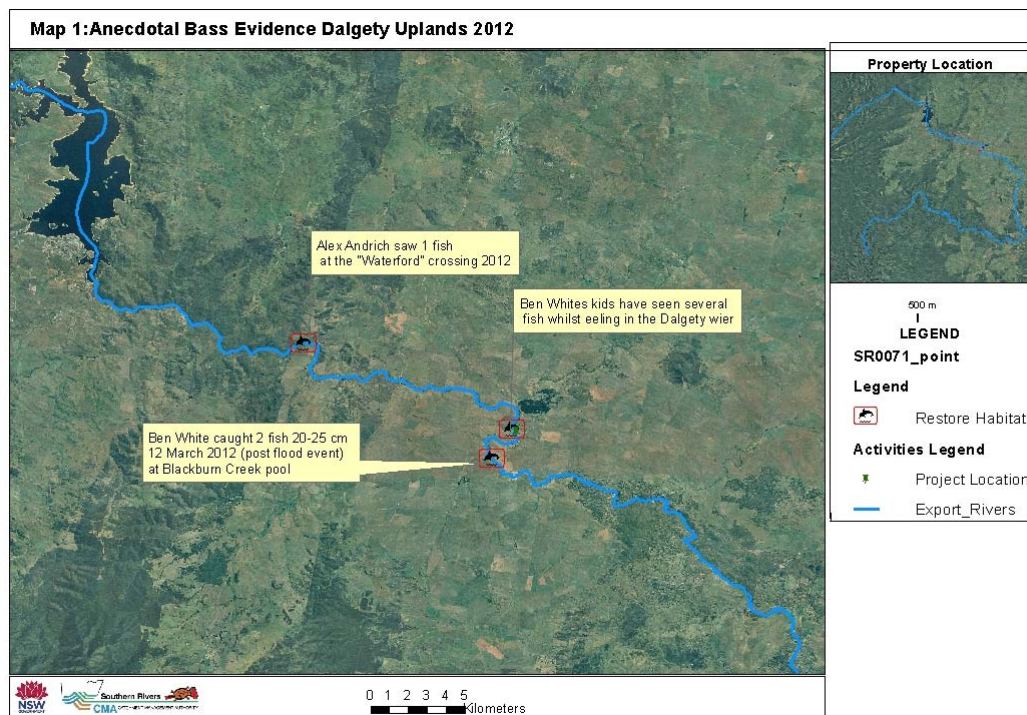
**Figure 2.14.** Habitat where juvenile Australian bass were collected on a number of sampling occasions in the upper Snowy River (large boulders on the edge of the river in low flow areas).

**Table 2.5.** Water quality parameters measured from sites where Australian bass were collected and not collected during 2007 and 2008.

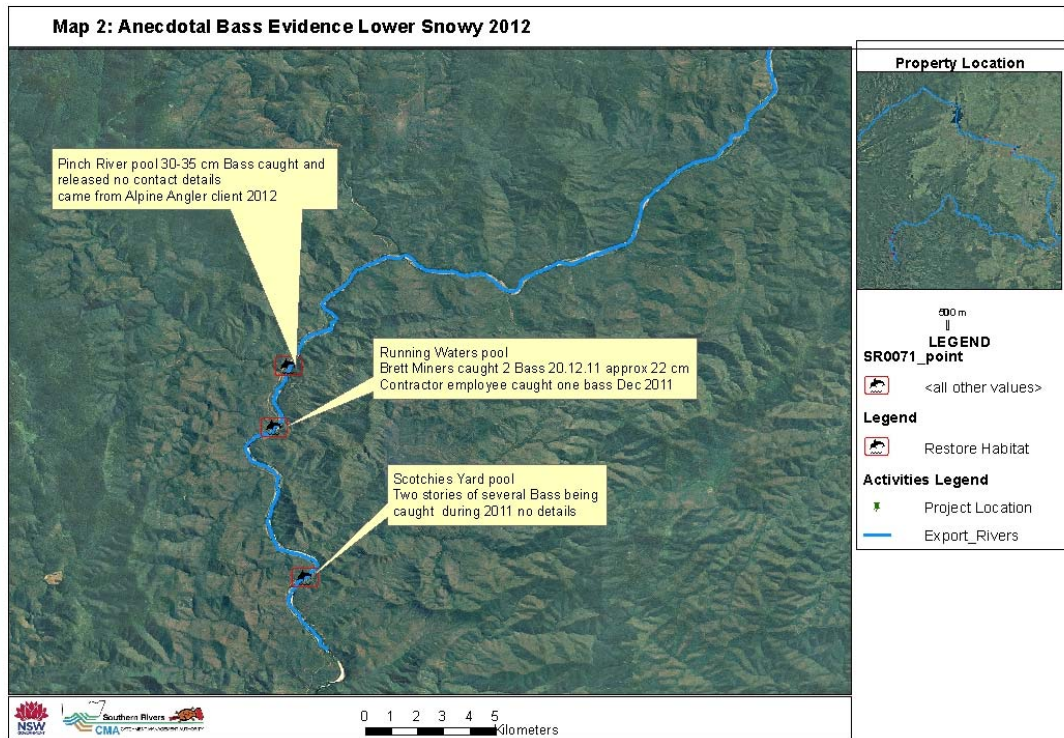
Parameter	Sites Australian bass were collected		Sites Australian bass were not collected	
	Minimum	Maximum	Minimum	Maximum
Water temperature (°C)	5.6	24.9	4.4	25.9
Dissolved oxygen (mg.L <sup>-1</sup> )	6.35	12.16	4.61	12.4
pH	5.79	8.07	6.2	12.6
Conductivity (µS.cm <sup>-1</sup> )	33	181	35	162
Turbidity (NTU)	3	243	1	310

### 2.3.4. *Anecdotal evidence of Australian bass in the Snowy River since monitoring program was completed*

A number of confirmed and unconfirmed reports of anglers catching Australian bass in the upper Snowy River have occurred since the monitoring program ended in November 2009. Confirmed catches of juvenile-sized Australian bass have occurred in the stretch of river above Dalgety, at the weir at Dalgety, in the stretch of river several km downstream of Dalgety (Figure 2.15), in the pool above the Pinch River - Snowy River junction, several km below this junction at Running Waters and at Scotchies Yard Pool approximately 10 km downstream of Running Waters (Figure 2.16). These captures and sightings indicate that survival in the upper Snowy River is occurring and this may provide a valuable recreational fishery in coming years. However, when these fish mature, downstream migration is likely for this catadromous species. Consequently, maintaining a fishery in the upper Snowy River will be reliant on continual stocking or upstream migration of recruits to the upper Snowy River.



**Figure 2.15.** Map showing three locations on the Snowy River in the Dalgety region where recent Australian bass sightings and captures have occurred since completion of the two-year monitoring program.



**Figure 2.16.** Map showing three locations in the lower end of the NSW reach where recent Australian bass captures have occurred since completion of the two-year monitoring program.

#### 2.4. Discussion

The stockings of Australian bass into the Snowy River appeared successful with individuals recaptured in each of the three years following stocking. Community analysis demonstrated that Australian bass had established at most sampling sites. In most instances, Australian bass co-existed with other species of similar sizes. Fish were collected from high density, low density and non-stocked reaches and there was anecdotal evidence to suggest anglers had started to collect small bass and a number of further sightings were reported. These reports of anglers catching Australian bass continued after this monitoring program into 2011 and 2012 which suggests a proportion of the stocked fish had survived a number of years post-stocking to a catchable size using angling methods.

Initial growth rates of Australian bass were high, and most individuals doubled in length every three months following stocking. Calculated condition factors were excellent and most fish were in good to excellent condition. These results contrast with earlier observations that Australian bass can experience slow growth rates in Gorge habitats (Harris, 1987). Length-weight relationships suggested fish within the high density reach were in good condition, but fish collected from other reaches were somewhat underweight. Presumably fish stocked into enclosed waters have reduced ability to disperse and self regulate densities among habitats, especially if stocking density is high for a given amount of habitat. This situation is less pronounced in river environments where a diversity of habitats exist and fish can redistribute longitudinally if resources become limiting. This is commonly observed in stocked salmonid populations (Serrano *et al.*, 2009) and may account for the relatively high observed condition in Australian bass. Whilst a large number of fish were stocked into the Snowy River, they

were manually introduced via helicopter over a large spatial range, which would reduce any density-dependent impacts on the acclimatising population.

A potentially confounding component of the experimental design was that the high density reach was upstream of the low density reach. The reaches were separated by a major natural migration barrier (Snowy Falls) which would have prevented upstream migration. However, it may have been possible throughout the study period for fish from the high density reach to have moved downstream into the low density reach, potentially accounting for decreases in condition throughout this reach. Downstream movement, if occurring, should have resulted in a concurrent increase in fish density from the low density reach but none was observed. In fact, catches from the low density stocking reach were always lower than from the high density stocking reach.

Australian bass abundance did not differ substantially between the two stocking reaches suggesting that the effect of stocking density may not be responsible for observed fish community differences. Snowy Falls is a relatively large natural barrier (> 3m) which is only open to fish passage during relatively high flow events. This barrier has previously been shown to have an affect on fish communities below and above (Rose and Bevitt, 2003). Several species collected upstream of the barrier are those with either a known ability to climb (e.g. eels; Linton *et al.*, 2007) or that possess substantial jumping ability (e.g. salmonids; Kondratieff and Myrick, 2006). The absence of Australian smelt and Congolli are the likely result of an inability to surmount the falls. Natural barriers are known to cause fragmentation in other river systems (Northcote, 2010) and the absence of data before Australian bass were stocked makes it difficult to determine if resultant differences arose from the stocking or from other natural factors.

Shortly after commencement of the experiment, a high flow event occurred throughout the entire system. The event was substantial and was driven by substantially high inflows from tributary streams. Flows passed through both the high and low density stocking sites but were relatively short-lived, only lasting a few days. It is possible that the event could have displaced 0+ Australian bass from upland to lowland reaches and could account for low catches of Australian bass during the first year of sampling. The displacement of highly susceptible 0+ fish following flood or high flow events (Peirson *et al.*, 2008) has previously been reported. However, it could be expected that other species were equally susceptible to displacement, but these were collected from both reaches following the event. Further, it could be expected that any Australian bass that were displaced would eventually migrate back upstream and recolonise when they had attained a sufficient size. Such fish were not detected during any sampling event, and there was a distinct lack of older Australian bass collected overall. Further fish collections from estuarine reaches would be required to confirm the presence of Australian bass further downstream. Obtaining age data from several fish could then confirm whether these fish were displaced by the flooding. Whilst this could provide additional information on the survival of stocked fish, that work was beyond the scope of the present study.

The study also sought to investigate patterns of movement of Australian bass away from the stocking site, by collecting fish from upstream and downstream of stocking zones. Additional sites were established outside the stocking area and monitored twice annually. This component of the experiment was confounded during the second stocking event, when fish were deliberately stocked into the upper zone at the high density rate. This created a situation where it was impossible to determine whether fish collected from that zone were upstream migrants or stocked individuals. The upper sites were therefore removed from any subsequent comparisons of fish condition with the unstocked reference sites. Even when the confounding effects of downstream movement and additional stocking are considered, the seasonal trends

in Australian bass abundance are difficult to interpret. Very few individuals were actually collected during routine sampling and the total absence of Australian bass from spring samples was of concern. Furthermore, the substantial drop in average size in summer, when considered along with age data that demonstrated no fish greater than 2 years of age, suggests that survival of stocked individuals may be low. When considering factors which influence survival, it is worth considering ecological aspects of life history to assist with possible interpretations.

Australian bass is a catadromous species and adults migrate seaward to spawn and then return to freshwater. Return migrations of adult Australian bass have been confirmed via otolith microchemistry, and it is now known that adults perform numerous return movements over many years (Miles, 2009). Following spawning, larvae and juveniles spend several months in estuarine reaches before colonising freshwater reaches (Harris, 1986). So at very early life history stages, the natural location for Australian bass juveniles is within estuarine reaches of rivers (Harris, 1986). Fish with marine life histories often benefit from access to productive nursery areas (Martinho *et al.*, 2007) with a more stable thermal regime. The time and size that Australian bass were introduced into freshwater reaches of the Snowy River coincides with a marine life phase and any one of these parameters could account for low recapture rates. Low re-captures following two subsequent stockings of over 200,000 fry indicate conditions within the stocked reaches were not suitable for long-term residency beyond several months for Australian bass at this age and size. It is difficult, however, to determine exactly which factors contributed.

Fish are especially susceptible to changes in thermal regime during early life and there is a vast amount of literature discussing the importance of temperature (Beitinger *et al.*, 2000). Most species have a good capacity to tolerate large changes over broad temporal scales (i.e. months) and are able to adapt physiologically. Rapid rates of change can occur under natural conditions over relatively small temporal scales (i.e. hours), and these are difficult to tolerate physiologically. Under such conditions, rapid increases or decreases in temperature are often lethal (Donaldson *et al.*, 2008). The Snowy River is typical of a temperate Australian stream. It progresses from a relatively cool, montane climate in the headwater zones, and meanders downstream to a climatic zone where summer and winter temperatures are more stable. Sudden short-term temperature changes can occur in montane reaches, especially during winter. It is common for ambient temperatures to fall below 0°C and for water temperatures in the upper Snowy River to range between 3 °C and 10 °C for a period of up to 3 months and remain between 3 °C and 6 °C for several weeks. These ranges could potentially impact lethally on juvenile Australian bass (see Chapter 4). Temperature variations in estuarine reaches would be less extreme as sea temperatures vary much less than fresh water temperatures in areas such as those chosen for these stockings. As water temperatures will drop with increased altitude (Johnson, 1971; Hari *et al.*, 2006) the likelihood of survival for the species may be directly related (See chapter 4)

Survival of early life history stages could also be influenced by predatory interactions with resident fauna. Routine sampling revealed a number of predatory fish within the system including brown trout, rainbow trout, Longfinned eel and Shortfinned eel. Salmonids are known to eat Australian native fish (Crowl *et al.*, 1992). Eel species are also known as fish predators (Sloane, 1984). It is also known that hatchery-reared fish often have reduced escape capabilities and are sometimes more susceptible to predation than wild fish (Weber and Fausch, 2003). Whilst predation could potentially account for some losses of Australian bass, the overall catch rates of predatory fish within the Snowy River were relatively low. It is considered unlikely, therefore, that predation rates were sufficiently high to substantially impact upon the long term survival of stocked fish (See Chapter 3).



Australian bass generally preferred slow flowing areas with bedrock as substrate or cover. It is likely that the preference for low flow areas is due to the age of the Australian bass population within the Snowy River with the greater proportion of individuals collected under 100 mm. Larger individuals may take up refuge in faster flowing habitats but an absence of fish collected during sampling did not allow this comparison. Much of the upper Snowy River experiences stable flow regimes throughout the year. This potentially results in stable and generally low flows which may suit stocked juvenile bass as they find suitable cover away from predators in the susceptible period immediately following stocking.

## **2.5. Conclusion**

This study demonstrated that Australian bass were successfully recorded from the Snowy River beyond the initial stocking event in 2007. Although Australian bass were present, they were not recaptured in high abundances relative to the number of fish stocked. There were no overall differences in the numbers of Australian bass observed among high and low stocking reaches but there was substantial evidence to suggest that Snowy Falls was acting as a natural barrier to resident fish growth rates and condition of Australian bass were consistent with previous studies but no fish older than 18 months were collected throughout the entire study. Further, no Australian bass were collected in any spring sampling event suggesting that residency beyond winter in the upper Snowy River may be impacted. Although several factors influencing residency and survival were considered, no single factor could be isolated. Further experiments on the environmental tolerances of Australian bass are investigated in subsequent chapters.

### **3. AN ASSESSMENT OF THE DIETARY OVERLAP BETWEEN AUSTRALIAN BASS AND OTHER FISH SPECIES IN THE UPPER SNOWY RIVER**

#### **3.1. Introduction**

Studies which examine the diet of fishes and quantify food habits are common in fish ecology (Hyslop, 1980), are a fundamental part of fisheries science and provide information about foraging behaviour and ecology of species (Clarke and MacLeod, 1976). An understanding of these ecological concepts is essential for informed management and conservation. Food habits and diets are frequently assessed to describe competitive relationships among fish species which co-exist. This information can then be used for a range of purposes such as quantifying predator-prey relationships (Fraser and Cerri, 1982), assessing fish predation (Buckmeier *et al.*, 2005) or determining trophic niche overlap between native and non-native fish species (Pilger *et al.*, 2010).

Studies of the dietary overlap among species are used to gauge the intensity of competition by estimating some measure or overlap in resource use (Pusey and Bradshaw, 1996). Dietary overlap and competition are frequently recognised as mechanisms that influence fish populations and communities (Matthews, 1998; Raborn *et al.*, 2004). If species are to compete, they must overlap in exploitation of a limiting resource (Gotelli and Graves, 1996). Partitioning of food resources among fish species is regarded as being more important as a mechanism for aiding the co-existence of different species than either space or time (Ross, 1986).

Competition and dietary overlap between stocked and wild fish have frequently been cited as important ecological interactions. However, this has rarely been tested rigorously (Weber and Fausch, 2003). Previously, a number of studies have examined the competition and resource use between co-existing wild and domesticated fish, but usually of the same species (e.g., Weiss and Schmutz, 1999; Kahilainen and Lehtonen, 2001) with few studies investigating dietary overlap between stocked species and other fish species found within the stocked area.

The aim of this study was to examine the stomach contents of Australian bass and other fish species of the upper Snowy River (stocked and wild) to assess the degree of dietary overlap. In addition to documenting diet overlap among fish species, prey abundance and availability were also measured to determine if seasonal shifts in diet could be attributed to seasonal changes in the availability of food resources.

## 3.2. Methods

### 3.2.1. Collection of fish

Fish were collected from a number of pre-selected sites on the Snowy River (Chapter 2: Figure 2.2). Collection of large-bodied fish (>200 mm) for dietary analysis was completed during regular electrofishing surveys (Chapter 2) on ten occasions from February 2008 to November 2009. Fish large enough to be stomach pumped were placed into a 60 L plastic bin filled with 50 mg l<sup>-1</sup> ethyl-p-aminobenzoate solution to induce anaesthesia prior to stomach content evacuation. Smaller individuals (<200 mm) were preserved as whole specimens and stored in 100% ethanol for later analysis under laboratory facilities. Stomach contents were extracted using a modified Seaberg pump (Seaberg, 1957; Hyslop, 1980; Strange and Kennedy, 1981) as detailed in Baumgartner (2005). All displaced prey items were collected on a 500 µm sieve and stored in 100% ethanol and later examined under a dissecting microscope and identified to at least family level and even to species level for some of the larger, more common items.

### 3.2.2. Stomach flushing

The stomach flushing pump consisted of rubber tubing connected to a rigid oesophageal tube that penetrated the stomach. A one-way pressure bulb controlled water flow into the stomach to displace items. Repeated depressions using the one-way pressure bulb force water from a bucket through the tubing into the stomach and out through the mouth of the fish (Figure 3.1). All prey items were collected on a 500 µm sieve and stored in 100% ethanol. Following extraction of stomach contents, fish were placed into an aerated live well to recover and then released into the river.

### 3.2.3. Prey item identification

Stomach samples were spread out onto a petri dish and contents were then examined under a dissecting microscope. Each item was identified to the lowest possible taxonomic level (usually family) with the aid of several identification guides (Allen *et al.*, 2002; Gooderham and Tsyrlin, 2003). Due to high levels of digestion of some items, identification was not possible and these were recorded as unidentifiable. All individual items were counted. A wet weight to the nearest 0.01 g was measured and recorded for each different family of dietary items recovered.

In order to quantify the dietary habits of all species and provide an overall description of their diets, two diet indices were used - frequency of occurrence (*O<sub>i</sub>*) and proportion by weight (*W<sub>i</sub>*) (Chipps and Garvey, 2007). Due to the relatively small numbers in several taxa, items were combined into seven broader groups for statistical analysis; aquatic insects, crustaceans, fish, arachnids, molluscs, aquatic vegetation and other (including sub categories; worms and amphibians).

### 3.2.4. Collection and count of prey availability

An estimate of prey availability was obtained following the protocols for the Australian River Assessment System (AUSRIVAS) in New South Wales (NSW) (Turak and Waddell, 2002). Samples were collected by the kick-and-sweep method which effectively collects more

species than techniques such as coring and plankton tows. Secondly, this method samples both the water column and the sediment bed of streams (Cheal *et al.*, 1993).

Samples consisted of a 10 m sweep using a d-framed 250 µm mesh net with a length of 38cm, width of 30cm (0.114m<sup>2</sup>) held downstream whilst disturbing the bed-sediments (Figure 3.2). One single ‘sweep’ was completed at a randomly selected ‘riffle’ type habitat and one was collected from the ‘edge’ of the river at each sampling site during all sampling events. Due to fluctuating river levels during this study, samples could not be taken in riffle habitats at several sites on a number of occasions. A second sweep from the edge was taken in this case. Samples were immediately preserved in 70% ethanol and later identified under a dissecting microscope, counted and weighed to the nearest 0.01 g in a laboratory. Organisms were generally identified to family level with the aid of the identification guides mentioned above.

### 3.2.5. Data analysis

To assess dietary overlap among species, Schoener’s similarity index (Schoener, 1970) was calculated:

$$D = 1 - 0.5 \left( \sum |P_{xi} - P_{yi}| \right)$$

where:  $P_{xi}$  and  $P_{yi}$  are the proportions (percent weight or proportion of occurrence) of food item ( $i$ ) in the diet of species  $x$  and  $y$ . Schoener’s index was used as it requires relatively few assumptions and is frequently applied (Bowen, 1996). Furthermore, Schoener’s index varies from 0 to 1, where 0 indicates no overlap and 1 indicates identical diets. Diet similarity is biologically similar when  $D$  is greater than 0.6 (Wallace, 1981).



**Figure 3.1.** Fisheries Technicians Jarrod McPherson and Mick Bettanin extract stomach contents from a Longfinned eel (*Anguilla reinhardtii*) on the Snowy River (Photo: Leo Cameron, NSW DPI).



**Figure 3.2.** Collecting available prey using the kick sampling method (Photo: Prue McGuffie, NSW DPI).

### 3.3. Results

#### 3.3.1. *Dietary analysis*

The stomach contents of 398 fish from six species were collected and analysed. In total, 98 of these individuals examined (24.62%) were found to have empty stomachs (Table 3.1). A total of 7,029 dietary items (combined weight of 209.82 g) were removed, identified and subsequently used in the analysis. Only a small proportion of the dietary items found were not positively identified to family level including aquatic vegetation, segmented worms, unidentifiable fish, copepods and seedshrimp.

##### *Australian bass*

Australian bass consumed aquatic insects and crustaceans (Figure 3.3). This generally included small prey items including non-biting midge larvae (*chironomidae*), freshwater shrimp (*atyidae*), copepods (*copepoda*) and unidentified aquatic insects. Freshwater shrimp were found in over half (53.33%) of the Australian bass stomachs examined, which comprised 65.64% of the diet by weight. A small number of individuals consumed fish (1.66%) which could not be identified. However, fish accounted for only a small percentage by weight of the overall diet (0.33%). Australian bass was the only species for which no aquatic vegetation were present in the diet, and no arachnids or molluscs were found either (Figure 3.3). The overall diet consisted of 15 different families of prey groups which was one of the least varied among species

### *Longfinned eel*

Longfinned eels were found to have the most varied diet by number of different prey items. Dietary items were found belonging to all seven major food groups (Figure 3.3). Aquatic insects were present in almost half of the stomach examined (47.07%) but these items only represented 23.73% of the diet by weight. Among the most common aquatic insects were non-biting midge larvae, freshwater shrimp, diving beetles (*dytiscidae*) and other unidentified aquatic insects, all of which occurred in over 10% of stomachs. Arachnids (spiders) were found in 1.71 % of individuals and represented 2.11% of the diet by weight. Fish including Australian smelt (*Retropinnidae*), brown trout (*Salmonidae*), and unidentified species were found in 4.12% of stomachs but accounted for only 3.59% of the diet by weight. Aquatic vegetation was common in the diet, being recorded in 33.33% of individuals, but it only accounted for 8.55% of the overall diet by weight. The most commonly found prey item were Freshwater snails (*Physidae*) which were found in 65.78% of all individuals.

### *Shortfinned eel*

Overall, the diet of Shortfinned eels was most similar to that of Long-finned eels, overlapping on 24 families of prey with a similar proportion in each of the 7 major prey groups (Figure 3.3). Shortfinned eels consumed 28 different families of prey/prey groups with high frequencies of aquatic insects (including non-biting midge larvae, freshwater shrimp, diving beetles, free-living caddis (*Hyrobiosidae/Ecnomidae*), freshwater snails (*Physidae*) and aquatic vegetation. The most frequently recorded item were freshwater snails, found in 55.67% of fish (20.48% by weight) and aquatic vegetation at 51.554% of stomachs (32.35% by weight). Shortfinned and Longfinned eels were the only species found to consume tree frog (*hylidae*), pea shells (*sphaeriidae*), wolf spiders (*lycosidae*), caddis larvae (*limnephilidae*), stick caddis (*leptoceridae*) and crane fly larvae (*tipulidae*). Shortfinned eel consumed fewer large prey including fish compared to Longfinned eel.

### *Congolli*

The diet of congolli was made up of small dietary items including high frequencies of non-biting midge larvae, freshwater shrimp, stonefly nymph (*Notonemouridae*), mayfly nymph (*Baetidae*), unidentified aquatic insects, seedshrimp (*ostracoda*) and freshwater snails. Freshwater shrimp alone made up almost half of the diet by weight (45.64%) which occurred in 16.66% of stomachs examined. Similar to Australian bass, Congolli consumed a low number (11) of different families of prey. Absent in the diet were fish and arachnids (Figure 3.3). Some physidae were recorded in the diet but in lower occurrence than the other three species (Longfinned eel, Shortfinned eel and brown trout) which consumed this dietary item. Congolli consumed aquatic vegetation less frequently at 8.33% of individuals compared to other species which consumed this dietary item, namely Longfinned eel (33.33%), Shortfinned eel (51.54%), goldfish (15.38%) and brown trout (40%).

### *Goldfish*

Goldfish was only found to contain mayfly nymph (*Leptophlebiidae*), unidentified aquatic insects and aquatic vegetation representing 88.88% of the diet by weight. This suggests an almost completely herbivorous diet and is quite different from all other species examined. A detailed description of the diet of goldfish was not possible because of the large number of empty stomachs encountered for this species (81.25%) (Table 3.1).

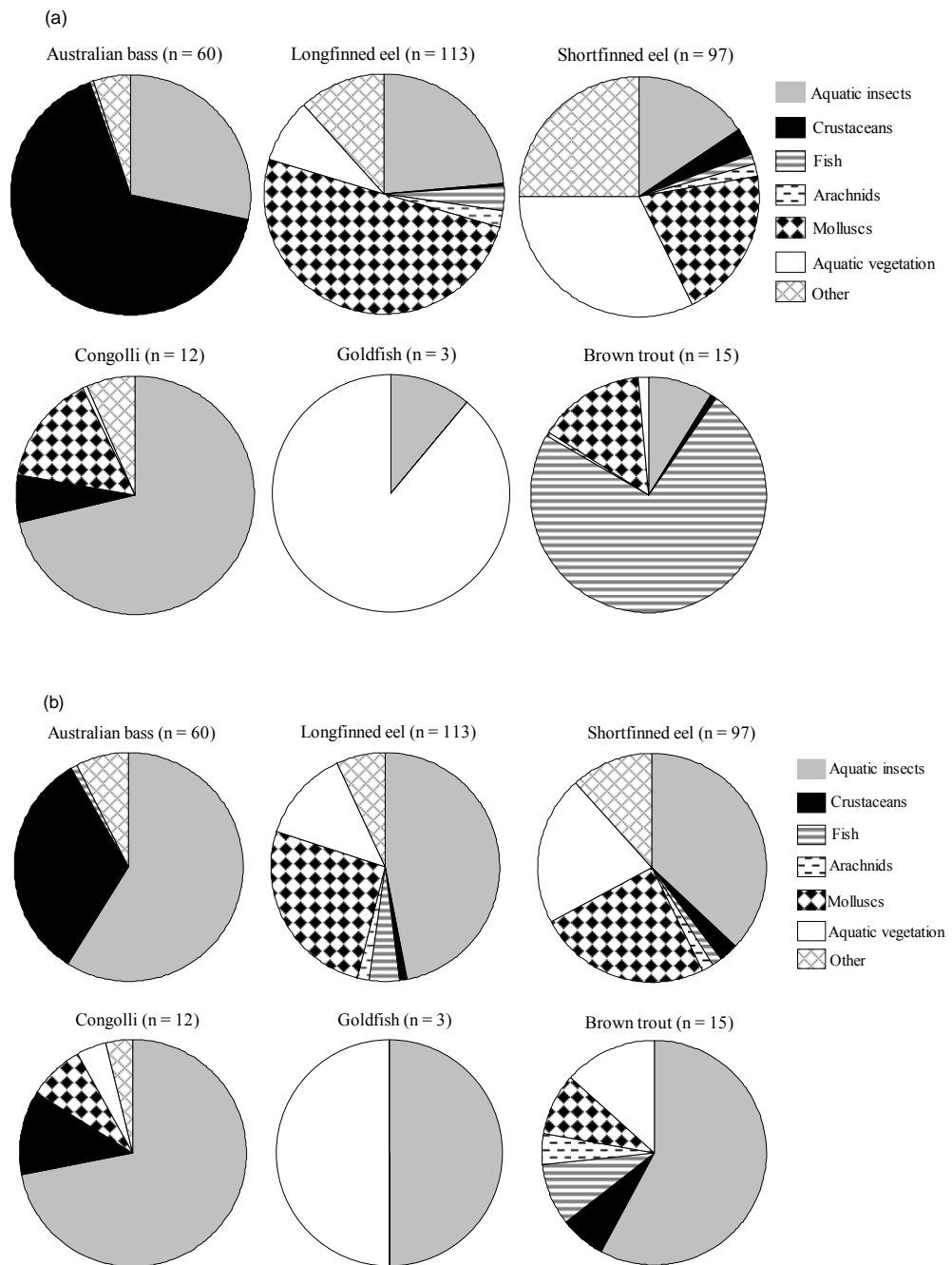
### *Brown trout*

Brown trout was the only species found to consume Australian bass (*Percichthyidae*) fingerlings. Three juvenile Australian bass were recovered from two stomachs of the 18 brown trout examined (11.11%). The largest brown trout which consumed Australian bass were 478 mm (consumed one Australian bass). Another smaller brown trout caught at the

Dalgety site in July 2008 had consumed two Australian bass. Although accurate measurements of these Australian bass were difficult due to digestion, the length of all three individuals was estimated at 70-100 mm. The piscivorous brown trout also consumed other fish including Australian smelt, with fish making up a large proportion of the diet by weight (73.77%). This is a considerably higher percent by weight for consumption of fish in the diet than any other fish species sampled - Australian bass (0.09%), Longfinned eel (3.59%), Shortfinned eel (1.41%), Congolli (0%) and goldfish (0%).

**Table 3.1.** Length range of fish, number of each species examined and the number of empty stomachs.

<b>Species</b>	<b>Length range (mm)</b>	<b>Number of stomachs collected</b>	<b>Empty stomachs (%)</b>
<u>Native</u>			
<i>Australian bass</i>	32-165	62	3.22
<i>Longfinned eel</i>	245-1100	148	23.64
<i>Shortfinned eel</i>	300-745	141	31.20
<i>Congolli</i>	122-250	13	7.69
<u>Exotic</u>			
<i>Goldfish</i>	57-263	16	81.25
<i>Brown trout</i>	86-517	18	16.67



**Figure 3.3.** Percentage of occurrence (a) and percent by weight (b) of dietary items determined for fish species sampled from the Snowy River.



### 3.3.2. *Dietary overlap*

#### Dietary overlap

Generally, overlap values were higher among all species by occurrence of prey in the diet compared to weight of dietary items in the diet. The only exception was that of the overlap between Congolli and Longfinned eel which was 0.43 by occurrence and 0.44 by weight.

There was little dietary overlap between Australian bass and any of the other fish species examined by either occurrence of dietary items (Table 3.2) or weight of items found in the diet (Table 3.3). Schoener's index suggests that the diet of Australian bass is most similar to Congolli by both occurrence (0.44) and weight (0.23) of items found in the diet. However, biologically, these levels of overlap are considered insignificant. In general, the overlap indices were higher between Australian bass and other native fish species (Longfinned eel, Shortfinned eel and Congolli) and most dissimilar to exotic fish species (brown trout and goldfish).

Significant dietary overlap existed between Longfinned eel and Shortfinned eel by occurrence (0.75) but not by weight (0.51). A major factor separating the overlap in terms of weight is the difference in proportion by weight of freshwater snails in the diet; Longfinned eel (50.36%) had much more than Shortfinned eel (20.48%). This dietary overlap by occurrence between these two eel species was the only significant overlap among all 15 combinations of fish species examined. This significant dietary overlap exists as a result of the high frequencies of non-biting midge larvae, diving beetles, free-living caddis, unidentified aquatic insects, freshwater snails and aquatic vegetation in their diets. Overlap values between all other combinations of fish species examined were low to moderate (<0.6) which suggests little dietary overlap and a tendency for specialized dietary habits among these fish species in the Snowy River.

**Table 3.2.** Schoener's similarity index of dietary overlap (by occurrence) in fishes from the Snowy River. Values approaching 0.00 indicate dissimilar diets, values approaching 1.00 indicate similar diets (Wallace 1981).

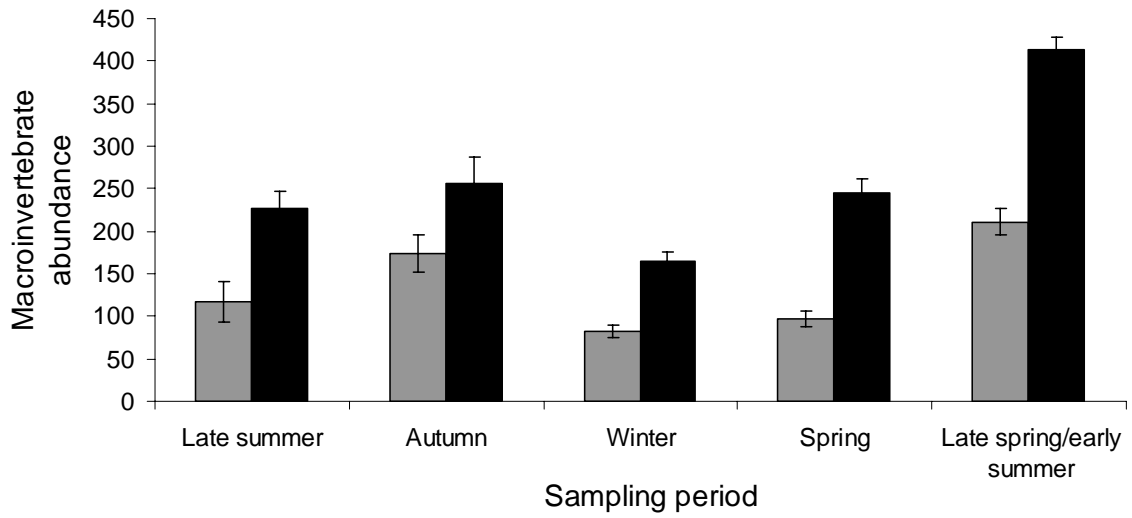
Species	<i>Longfinned eel</i>	<i>Shortfinned eel</i>	<i>Congolli</i>	<i>Brown trout</i>	<i>Goldfish</i>
<i>Australian bass</i>	0.35	0.32	0.44	0.26	0.22
<i>Longfinned eel</i>		0.75	0.43	0.48	0.22
<i>Shortfinned eel</i>			0.35	0.47	0.28
<i>Congolli</i>				0.32	0.20
<i>Brown trout</i>					0.18

**Table 3.3.** Schoener's similarity index of dietary overlap (by weight) in fishes from the Snowy River. Values approaching 0.00 indicate dissimilar diets, values approaching 1.00 indicate similar diets (Wallace 1981).

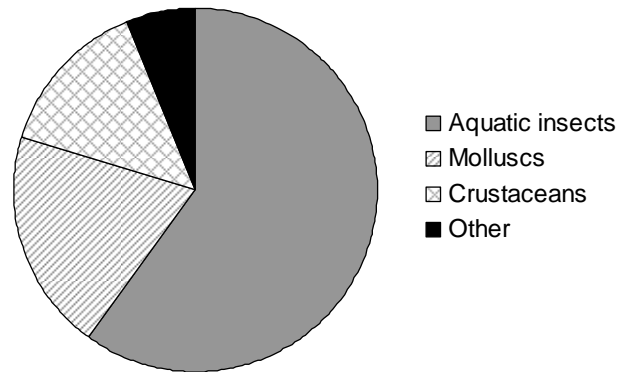
Species	<i>Longfinned eel</i>	<i>Shortfinned eel</i>	<i>Congolli</i>	<i>Brown trout</i>	<i>Goldfish</i>
<i>Australian bass</i>	0.12	0.17	0.23	0.03	0.06
<i>Longfinned eel</i>		0.51	0.44	0.22	0.12
<i>Shortfinned eel</i>			0.33	0.22	0.36
<i>Congolli</i>				0.17	0.04
<i>Brown trout</i>					0.02

### 3.3.3. Prey availability (all sites combined)

Thirty-three identified families of macroinvertebrates were collected. In total, an estimated 40,275 invertebrates weighing 268.91g were collected during the sampling with every sample containing some level of macroinvertebrates. Significant differences were found between mean numbers of macroinvertebrates sampled between years (ANOVA:  $F_{1,8} = 6.90$ ;  $P < 0.05$ ) but not between seasons (ANOVA:  $F_{4,5} = 1.13$ ;  $P > 0.05$ ) (Figure 3.4). The most collected family was the *Chironomidae* (non-biting midge larvae) with 7,332 sampled across all sites. Other highly abundant macroinvertebrates were seedshrimp (family unknown), *Sphaeriidae* (pea shells) and *Caedidae* (Mayfly nymph). Less abundant families were *Tipulidae* (Cranefly larvae), *Coenagrionidae* (Damselfly larvae) and *Gripopterygidae* (Stonefly nymph). Overall, aquatic insects were the most abundantly collected group of macroinvertebrates (Figure 3.5). Mean numbers of macroinvertebrates collected in each sample varied among seasons but were greatest in the late-spring/early summer and lowest in winter during both years. The abundance of macroinvertebrates per sample was higher in all seasons during 2009 compared to 2008.



**Figure 3.4.** Mean ( $\pm$ S.E.) available prey abundance per single sample across all sites (grey bars = 2008 and black bars = 2009).



**Figure 3.5.** Percentage of each available prey group collected during all sampling events combined. ‘Other’ includes unidentified fish eggs and fish. Fish collected were gambusia (*Gambusia holbrooki*) and Australian smelt (*Retropinna semoni*).

### 3.4. Discussion

The diets of the six co-occurring fish species examined were generally dissimilar. Analysis of dietary items indicated three feeding strategies; one herbivorous species (goldfish), two opportunistic general feeders which could be considered omnivores (Longfinned eel and Shortfinned eel) and three species whose diets indicated carnivorous feeding habits (Australian bass, brown trout and Congolli).

The herbivorous goldfish mostly consumed aquatic vegetation which consisted of over 90% of the diet by weight. A small number of aquatic insects were found in the stomachs of goldfish but it is possible that these were not intentionally consumed but were incidentally eaten whilst grazing on aquatic vegetation. Dietary items for goldfish conform with previously reported feeding habits for this species elsewhere (Jenkins and Burkhead, 1993) with the only major difference being an absence of fish in the diet of goldfish from the Snowy River. Interestingly, goldfish were the only species found to not eat the most abundant taxon available, Chironomidae.

Omnivorous fish generally consume a wide variety of dietary items consisting of an array of plants and animals. The diets of the two eel species (Longfinned eel and Shortfinned eel) could be described as omnivorous as they consumed a diverse range of prey including aquatic insects, crustaceans, fish, arachnids, molluscs and aquatic vegetation. Large frequencies of aquatic insects were previously reported in the diet of both these eel species in the Douglas River, Tasmania (Sloane, 1984) along with the presence of fish in the diet of Longfinned eels in a study which focussed largely on the diet of eels less than 400 mm in length. Interestingly, Sloane, (1984) reported no aquatic vegetation or molluscs in the diet of Longfinned eels or Shortfinned eels in the Douglas River. This current study showed that aquatic vegetation and molluscs constituted a large percentage of the diet of Longfinned eels from a much wider range of sizes (245-1100 mm). These results possibly reflect the opportunistic feeding regime of these eel species which allows them to adapt their diet to the available prey types in different locations as previously suggested (Sloane, 1984; Jellyman, 1989). Alternatively, aquatic vegetation are consumed whilst feeding on molluscs.

Australian bass, brown trout and Congolli were found to have more specialised diets which focussed on great proportions of aquatic insects. Although these species could be described as being carnivorous in the Snowy River, the prey types consumed by each of these species differed, suggesting that each of these species had a somewhat specialised diet. Australian bass were found to have the least varied diet by number of different dietary groups, with aquatic insects and crustaceans combined accounting for over 90% of the diet by weight. Brown trout differed from all other species with the presence of fish in the diet. Interestingly, over 10% of brown trout stomachs that were assessed contained Australian bass. The diet of brown trout has frequently been reported as being highly piscivorous (e.g., Garman and Nielson, 1982; Jensen *et al.*, 2004). Piscivory has been observed more frequently in larger individuals of this species compared to smaller individuals (Garman and Neilsen, 1982). As the majority of individuals of this species collected for stomach content examination were large fish (i.e. >300 mm) it could have been expected that if this species was highly piscivorous in the Snowy River, higher frequencies of fish would have been present in the diet of brown trout. However the diet of this species in the Snowy River was evidently more dominated by aquatic insects than fish by frequency of occurrence in the diet. Despite the widespread distribution of Congolli, limited published data are available describing the diet for this species. One comprehensive study by Hortle and White (1980) from several streams in south-eastern Tasmania, found Congolli to be mainly carnivorous, consuming a wide

variety of aquatic insects which conforms to this current study and suggests a somewhat similar feeding strategy between different areas.

Schoener's similarity index, (Schoener, 1970) indicated dietary overlap among these co-occurring fish species was low. The only significant overlap detected was between the two species of eel in terms of occurrence of dietary items (0.75). In contrast, dietary overlap between these two species was not significant by weight (0.51). The insignificant value by weight was most likely a result of the differences in consumption rates of two dietary items. The first was Physid snails, which accounted for 50.36% of the diet by weight for Longfinned eels but only 20.48% by weight for Shortfinned eels. The second was aquatic vegetation, which Shortfinned eels consumed in greater proportions by weight (32.35%) than Longfinned eels (8.55%). Sloane, (1984) previously reported dietary overlap between these two species and found similarities between diets with an overall dietary overlap value of 0.57.

Overall, dietary overlap between the stocked Australian bass and the five other species examined was low to moderate by occurrence and low by weight. One possible explanation for the diet of Australian bass overlapping most with Congolli is the similar size range of the individuals collected for these two species (Table 3.1). These two species and goldfish shared the most similar length range of the individuals collected for the six species compared to individuals of both eel species and brown trout which were generally much larger in size. Interestingly, the percentage of empty stomachs of Australian bass and Congolli were the lowest among all six species at 3.52% and 7.69% respectively (Table 3.1).

Low to moderate dietary overlap was found between the stocked brown trout and the other species examined with the greatest dietary overlap found between brown trout and Longfinned eel and lowest overlap between the two stocked species, brown trout and Australian bass. A number of dietary items separated the diet of these stocked fish species. Brown trout frequently consumed Dytiscidae, Baetidae, Leptophlebiidae, Gripopterygidae, Percichthyidae, Physidae and vegetation which were all rare or absent from the diet of Australian bass. There appears to be little scope for competition in the Snowy River between these stocked species as their diets suggest completely different feeding patterns, with brown trout consuming larger prey items such as fish and Australian bass consuming the smaller items such as aquatic insects and crustaceans.

There was a high correlation between the available prey types collected in the kick samples and the diets of several species. The most abundant prey type, aquatic insects, was also the most consumed by Australian bass, Congolli and brown trout. The reason for the significant difference in mean collections of available prey between years may be as a result of the more stable flows in the Snowy River during 2009 compared to 2008. During early 2008 there was one major flood event in the upper Snowy River (Figure 2.1) but no similar flows were recorded in 2009. Macroinvertebrate richness is known to decrease after flooding under regulated conditions (Rader *et al.*, 2008) and the recovery of many species following these events occurs over large temporal scales (Robinson *et al.*, 2003).

### **3.5. Conclusion**

This study confirmed that each of the six fish species examined possesses an individual feeding strategy and this is a likely reason for their co-existence. Four different feeding strategies were evident which categorise these species as one of the following; carnivorous, omnivores, piscivorous or herbivorous. Diet was most similar between the two species of eel and this relationship by occurrence of items in the diet was biologically significant. Australian bass diet was most similar to Congolli, one of the smallest species in body size of the species studied, but of a similar size range to the Australian bass examined. Whilst the diet of Australian bass was closest to Congolli, it was dissimilar to all other species. This suggests

that dietary overlap between stocked Australian bass and resident fish species in the Snowy River is unlikely to limit survival of stocked fish or impact negatively on the existing fish population. Furthermore, kick sampling to estimate macroinvertebrate numbers and prey availability during this study showed a considerable abundance of available prey during all sampling events. This suggests that survival of stocked Australian bass is unlikely to be limited by a lack of available prey or from predation by larger fish species.

## 4. POTENTIAL EFFECTS OF WINTER WATER TEMPERATURES ON JUVENILE AUSTRALIAN BASS STOCKED POPULATIONS INTO THE UPPER SNOWY RIVER

These data have been published in Fisheries Management and Ecology:

Cameron, L., Baumgartner, L.J., Bucher, D.B. and Robinson, W. (2012). Critical Thermal Minima of age-0 Australian bass, *Macquaria novemaculeata*, fingerlings: implications for stocking programs. *Fisheries Management and Ecology* **19**, 344-351.

### 4.1. Introduction

Temperature can be a limiting factor for survival in fish (Brett, 1956) as it influences virtually all biochemical, physiological and life history activities (Beitinger *et al.*, 2000). The impacts of temperature on fish have been extensively studied, with many of the very earliest accounts examining temperature tolerance (e.g., Heath, 1884). Determining thermal tolerance of a species is critical to understanding distribution and ecology (Fernandes and Rantin, 1986). Many previous studies have examined temperature tolerance and effects on fishes at high temperatures (Beitinger *et al.*, 2000). However, most fish kills in nature are the result of exposure to low temperatures as fish develop a tolerance for heat stress more rapidly than cold tolerance (Doudoroff, 1942).

A major source of mortality in age-0 fish is winter mortality, which has received much attention with countless studies have assessed the impacts of low temperatures on age-0 individuals (e.g., Hoang *et al.*, 2002; Kooka *et al.*, 2007; Michaletz, 2010). Over-winter mortality can vary substantially among years in age-0 fish and can be influenced by many factors including starvation, predation, disease, parasites and thermal stress (Hurst, 2007). Subsequently, the first winter is regarded as a critical period which determines year class strength of fishes (May, 1974; Garvey *et al.*, 1998). Few studies have specifically determined if critical temperature values of stocked or translocated fish influence subsequent survival.

Little or no information exists regarding critical thermal limits for most Australian freshwater fish including Australian bass (*Percales novemaculeata*). Stocking programs have been implemented over the past few decades to re-habilitate this popular recreational fish species in many river systems and freshwater impoundments in south-eastern Australia. Impoundments mostly exist in upland reaches of these streams but under natural conditions Australian bass would spawn and, juveniles develop, in estuarine reaches. Some stockings into upland reaches as fry and fingerlings (e.g., 10-80 mm) are therefore occurring outside the natural range of these juveniles which are usually only several weeks of age at time of stocking. It is unknown whether differences in thermal regime may be limit survival for individuals at this size and age. Understanding the thermal tolerance of Australian bass could firstly determine the suitability of current stocking locations. Secondly, this information could aid future stocking events to ensure stocking occurs in suitable areas.

The aim of this current study was to determine Critical Thermal Minima (CTMin) for age-0 Australian bass using controlled laboratory experiments. The impact of acclimation temperature and rates of temperature decline were assessed to determine subsequent survival of the fish. This information was compared with historical and recent water temperature data

from the Snowy River to assess if survival of stocked Australian bass may be influenced by changes in thermal regime.

## 4.2. Methods

### 4.2.1. Source and care of Australian bass prior to experiments

Age-0, hatchery-reared Australian bass used in these experiments were obtained from Aquablue seafoods, Pindimar, New South Wales (NSW) (S 32.630: E 152.083). Experiments were completed at Grafton Fisheries Centre, NSW (S 29.612: E 152.956). Fingerlings were housed in two 1000 L tanks between August and November 2009 and were fed a combination of frozen bloodworms (Kong's Pty Ltd, Ingelburn, NSW, Australia) and 3 mm commercial fish pellet (Ridley Aqua-feed, Narangba, QLD, Australia) provided twice daily. Water supply was from the Clarence River.

### 4.2.2. Acclimation of Australian bass

For acclimation, fish were randomly selected from the 1000 L tanks and stocked at a density of 15 individuals into each of 12, 56 L glass aquaria. The fish were not fed during the seven day acclimation period. A single micro compact chiller unit CL650, 1/4 hp, 650 W, (RESUN, Shenzhen, China) each delivered water to three separate aquaria. An overflow hose attached to the top of each set of three aquaria directed water back into a sump when aquaria were full (Figure 4.1). A submersible pump (Aqua One, maxi 104, Ingleburn, NSW, Australia) was fitted to a sump tank which delivered water to the chiller unit for cooling. This provided a fully closed, temperature-controlled system for each set of three aquaria (treatment). Chillers were set at 8°C or 15°C respectively. All aquaria were aerated during acclimation, and fish were observed daily at 07:00, 12:00 and 17:00 h for visual signs of abnormal behaviour. Photoperiod was 14 h light: 10 h dark. Water quality variables were measured daily including pH and dissolved oxygen. All aquaria were aerated to provide sufficient oxygen and water quality parameters were monitored daily.

### 4.2.3. Critical Thermal Minima trials

The experiment involved all 12 aquaria, three for each of the four treatments. Following acclimation, water temperature was decreased at a rate of 1°C.h<sup>-1</sup> or 1°C.d<sup>-1</sup> at the predetermined temperatures (8°C or 15°C) in which water temperatures were controlled electronically by adjusting the chiller output. Temperatures were decreased at predetermined rates until a non-lethal point, loss of equilibrium (LOE), was reached for all fish within each treatment (Figure 4.2). LOE was used as the critical thermal endpoint and defined as the point where an individual lost the ability to control position and failed to maintain dorsal-ventral orientation for a period of one minute (Currie *et al.*, 1998). At LOE, time and temperature were recorded. The affected individual was removed from the aquaria, weighed ( $\pm 0.01$  g), measured (fork length  $\pm 0.1$  cm) and placed into a separate aquaria at a stocking density of 15 per aquaria and monitored for any delayed mortality. No heating or cooling was applied to these aquaria, which were maintained at ambient temperature ranging from 15.0-16.4°C for a period of 14 days following LOE, then all surviving fish were weighed and measured (Table 4.1).



#### 4.2.4. Winter water temperatures in the upper Snowy River

Monthly minimum water temperature data were sourced from the Dalgety weir logging station (No: 222026) to compare with CTMin values of juvenile Australian bass and to gauge the impacts of known CTMin with temperatures throughout the stocked reaches. Nine years of data commencing the year 2000 were sourced in order to determine if low temperatures in 2008 and 2009 were representative of long term temperatures for that region.

#### 4.2.5. Statistical Analysis

All statistical analyses were completed using SAS® version 9.2 (SAS Inc. 2009) with the level of significance for all tests at 0.05. Unless otherwise indicated, data are presented as means  $\pm$  S.E. A mixed linear model was calculated to test for differences in temperature at LOE between the two acclimation temperatures (8°C and 15°C) and two rates of temperature decline (1°C.h<sup>-1</sup> and 1°C.d<sup>-1</sup>). Acclimation temperature and rate of temperature decline were treated as fixed factors and aquaria considered as a random factor nested within treatments. Residual plots confirmed the assumptions of homogeneity of variances and normality. Where significant effects were found between levels of a treatment, multiple comparisons between the least squares means were conducted whilst controlling the type 1 error rate using Scheffe's adjustment. The survival distribution function of the 45 Australian bass used in each treatment was modelled using the lifereg procedure (SAS® Institute Inc. 2009. This modelled the life expectancy for Australian bass fingerlings using data that include right-censored values (as LOE was the endpoint used, not death) and then compared survivor functions between acclimation temperature and rate of temperature decline among treatments using the Wilcoxon non-parametric test and Scheffe's adjustment for multiple comparisons (Westfall *et al.*, 1999).



**Figure 4.1.** Three replicate aquaria (top) for one of the four treatments used during experiments, the chiller (bottom left) used to control water temperatures and the sump tank (bottom right) used to deliver water to the chiller for cooling.



**Figure 4.2.** Australian bass fingerlings commencing LOE at 5°C.

### 4.3. Results

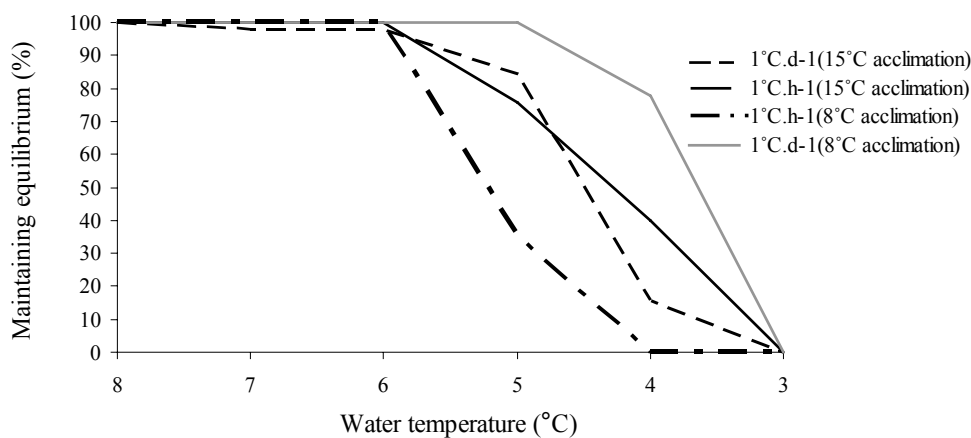
#### 4.3.1. Critical thermal minimum

Dissolved oxygen ranged between 7.4-9.3 mg L<sup>-1</sup>, pH 7.4-7.9 and water temperature 14.0-19.6°C in the two 1000 L tanks prior to the experiments commencing. Dissolved oxygen and pH were 9.2-10.1 mg L<sup>-1</sup> and 7.0-7.4, respectively, during acclimation. Highest recorded temperature of any individual at LOE was 7°C. There were substantial lethal impacts of water temperature below 6°C on age-0 Australian bass (mean 64.4 ± 0.4 mm FL and 3.8 ± 0.8 g). A variation in water temperature from 7°C to 6°C had no impact on age-0 Australian bass as none of the 180 fish used reached LOE at 6°C, but LOE for 179 of the 180 (99.44%) fish occurred between 3-5°C (Figure 4.3). Final CTMin values were 3.22 to 4.64°C and these were influenced by acclimation temperature and rate of temperature decline. Overall mean LOE values for fish subjected to the two rates of decline (1°C.h<sup>-1</sup> and 1°C.d<sup>-1</sup>) were 3.53°C and 4.35°C respectively. However, the overall mean LOE between the acclimation temperatures (8°C and 15°C) were more similar at 3.93°C and 3.95°C respectively.

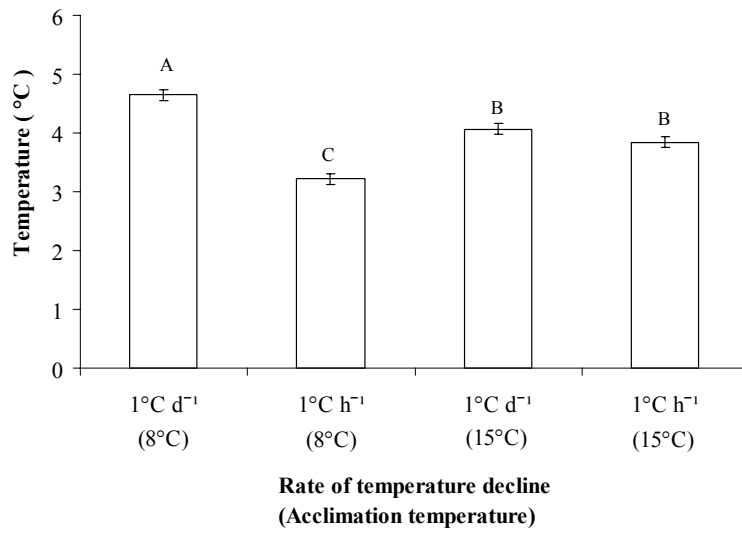
There was a significant difference in the temperature at LOE for Australian bass between the 1°C.h<sup>-1</sup> and 1°C.d<sup>-1</sup> rates of decline; however, acclimation temperature did not have a significant effect (Table 4.2). A significant interaction between acclimation temperature and rate of temperature decline was also found (Table 4.2). The mean temperature at LOE for Australian bass acclimated at 8°C with the 1°C.d<sup>-1</sup> rate of decline was 4.64°C which was significantly higher than all other treatments (Figure 4.4). In total, 29 of the 45 (64.44%) Australian bass reached LOE at 5°C. A total of 16 out of 45 (35.56%) reached LOE at 4°C following the 8°C acclimation and 1°C.d<sup>-1</sup> rate of temperature decline treatment. However, Australian bass acclimated at 8°C with a rate of decline of 1°C.h<sup>-1</sup> had a significantly lower LOE than all other treatments (Figure 4.4) with 35 of the 45 (77.78%) individuals reaching LOE at 3°C and 10 of the 45 (22.22%) reached LOE at 4°C.

### Survival

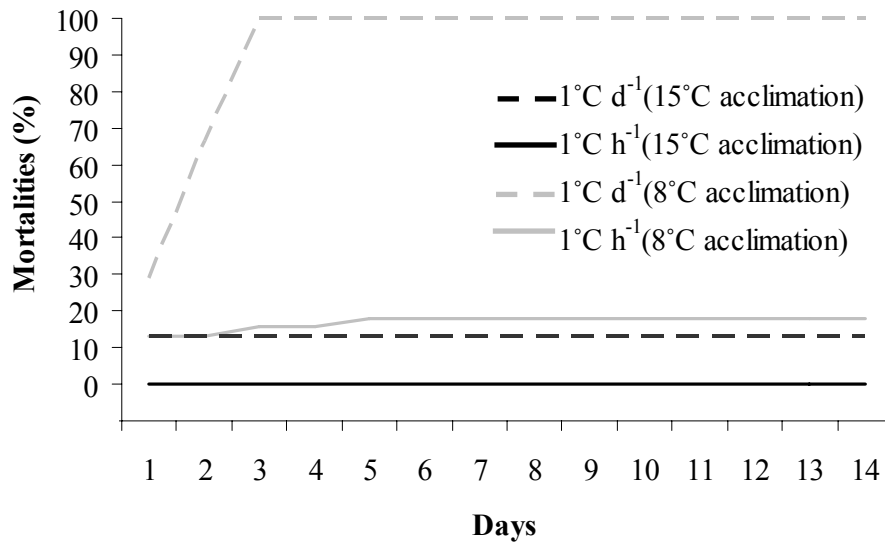
A greater proportion of Australian bass survived when tested under the faster rate of temperature decline ( $1^{\circ}\text{C}\cdot\text{h}^{-1}$  compared to  $1^{\circ}\text{C}\cdot\text{d}^{-1}$ ) in both  $8^{\circ}\text{C}$  and  $15^{\circ}\text{C}$  acclimation treatments. No fish survived  $8^{\circ}\text{C}$  acclimation when the  $1^{\circ}\text{C}\cdot\text{d}^{-1}$  temperature decline, whereas most (82%) survived the faster rate in decline ( $1^{\circ}\text{C}\cdot\text{h}^{-1}$ ) at the  $8^{\circ}\text{C}$  acclimation. No Australian bass died in the  $15^{\circ}\text{C}$  acclimation;  $1^{\circ}\text{C}\cdot\text{h}^{-1}$  treatment (Figure 4.5) but only 86% of individuals survived when the rate was decreased at  $1^{\circ}\text{C}\cdot\text{d}^{-1}$ . All mortalities among treatments occurred within 5 days following LOE, with no mortalities between days 6 and 14. Mean survival time for treatments in which Australian bass died following LOE ranged from  $1.41 \pm 0.08$  to  $38.38 \pm 13.15$  hours (Figure 4.6). There was a significant difference in the survival distribution functions (Wilcoxon's  $\chi^2 = 126.3$ ,  $\text{df} = 3$ ,  $p < 0.0001$ ). Follow up analysis found that the Australian bass acclimated at  $8^{\circ}\text{C}$  with a  $1^{\circ}\text{C}\cdot\text{d}^{-1}$  rate of decline had significantly lower survival rates than all other treatments (Figure 4.7).



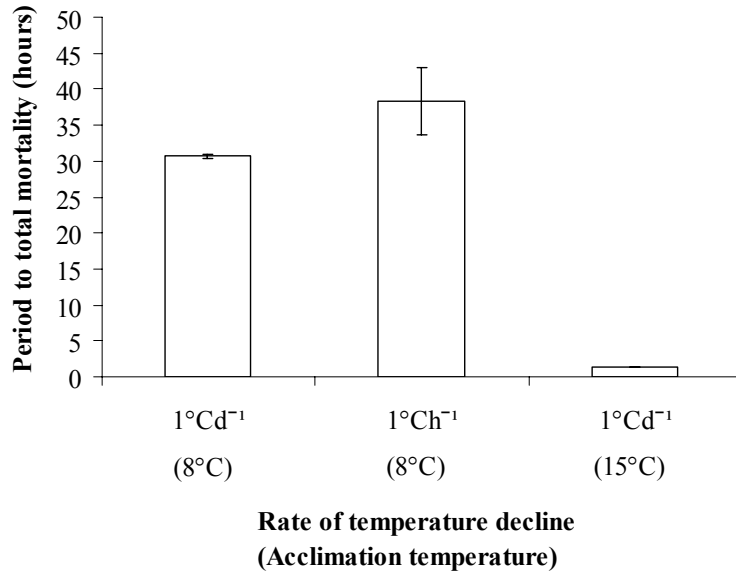
**Figure 4.3.** Equilibrium of Australian bass fingerlings acclimated to 8 or  $15^{\circ}\text{C}$  and then subjected to a decline of  $1^{\circ}\text{C}\cdot\text{h}^{-1}$  or  $1^{\circ}\text{C}\cdot\text{d}^{-1}$ .



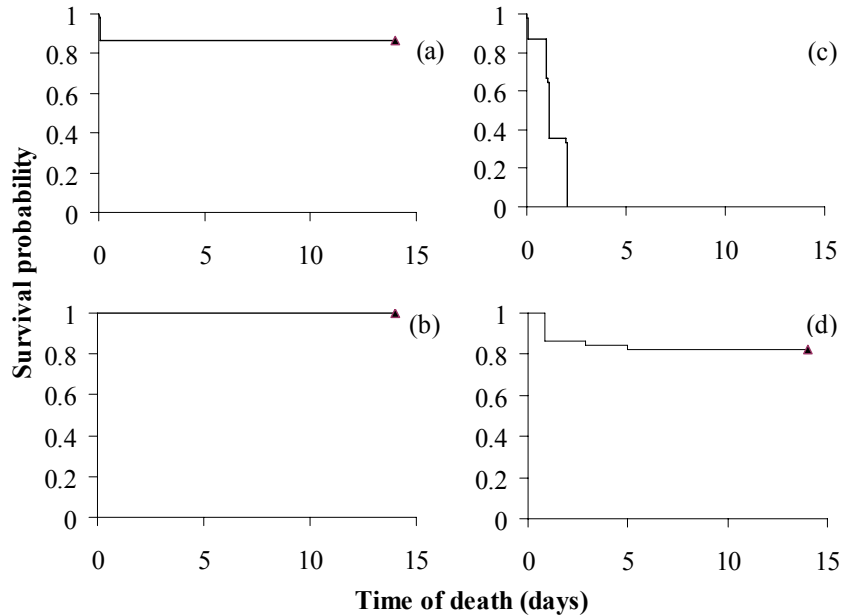
**Figure 4.4.** Critical Thermal Minima (CTMin) of Australian bass fingerlings subjected to different temperature regimes. Data are mean ± S.E. Treatments with the same letters represent no significant difference at the 0.05 level after Scheffe’s correction for multiple comparisons.



**Figure 4.5.** Cumulative mortality of Australian bass fingerlings after loss of equilibrium at different temperature regimes.



**Figure 4.6.** Periods to total mortality after loss of equilibrium of Australian bass fingerlings. Treatment: 15°C acclimation 1°C.h<sup>-1</sup> decline is not included as all Australian bass survived.



**Figure 4.7.** Product survival curves for Australian bass fingerlings. Triangles (▲) indicate censored data, that is, survival time beyond the 14 days is unknown as it was not tested in this study. Treatments: 15°C acclimation 1°C.d<sup>-1</sup> temperature decline (a), 15°C acclimation 1°C.h<sup>-1</sup> temperature decline (b) 8°C acclimation 1°C.d<sup>-1</sup> temperature decline (c) and 8°C acclimation 1°C.h<sup>-1</sup> temperature decline (d).

**Table 4.1.** Lengths and weights of Australian bass fingerlings used in this study. Data are means  $\pm$ S.E.

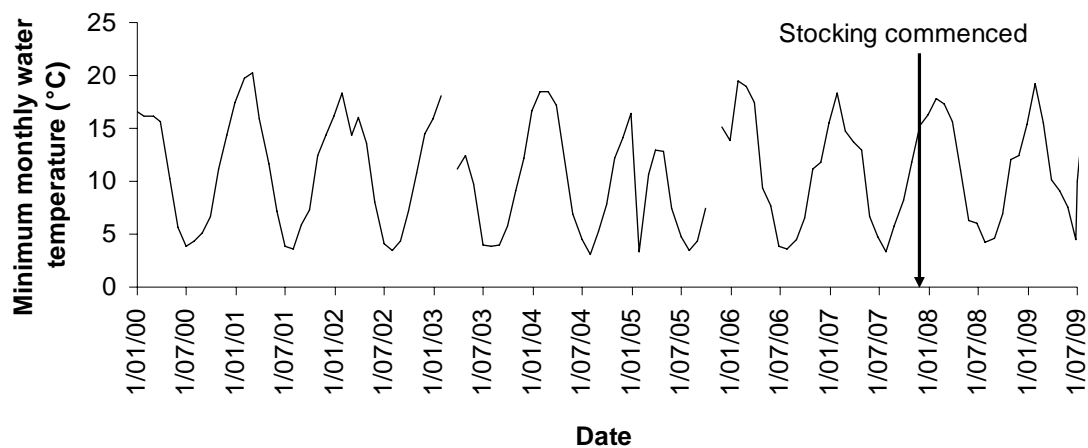
Parameter	Overall size of fingerlings
Length (mm)	64.41 $\pm$ 0.40
Length range (mm)	56-86
Weight (g)	3.77 $\pm$ 0.80
Weight range (g)	2.26-9.25

**Table 4.2.** Results of mixed linear model to calculate for differences in temperature at LOE between acclimation temperature (8°C and 15°C) and rate of temperature decline (1°C.h<sup>-1</sup> and 1°C.d<sup>-1</sup>).

Source	df	F-value	p
Acclimation temperature	1,8	0.06	0.8149
Rate of temperature decline	1,8	80.15	< 0.0001
Acclimation temperature X Rate of temperature decline	1,8	42.68	0.0002

#### 4.3.2. Winter water temperatures in the upper Snowy River

Minimum monthly water temperatures for the winters of 2008 and 2009 (post-stocking winters) were marginally higher than previous years (Figure 4.8). The coldest minimum monthly water temperature recorded during this period was the winter of 2007 (several months prior to stocking). In July of 2007, water temperature dropped to 3.1°C. During the post-stocking period assessed, the coldest minimum monthly water temperature recorded was considerably higher at 4.21°C in July 2008. The winter of 2009 was not as cold as previous years with the coldest water temperature recorded as 4.77°C in June. In general, there appears to be a trend annually in minimum monthly water temperatures dropping to below 5°C during winter.



**Figure 4.8.** Monthly minimum water temperatures for the Snowy River at Dalgety (Source: Waterinfo NSW 2010). Breaks in the data indicate where data was lost due to logger and sensors failing.

#### 4.4. Discussion

Australian bass fingerlings (0+) were found to have a precise critical thermal range. Irrespective of acclimation conditions, temperatures below 6°C resulted in loss of equilibrium, but impacts were not always lethal. Rate of temperature decline was significant, particularly when fish were acclimated at 8°C and the rate of temperature decline was slow (1°C.d<sup>-1</sup>). The effects of acclimation temperature has previously been reported to have a significant influence on CTMin values for fingerlings of other fish species including Texas pinfish (*Lagodon rhomboids*) (Bennett and Judd, 1992) and Silver catfish (*Rhamdia quelen*) (Chippari-Gomes *et al.*, 1999). The rate of change however, could be greatly influenced under natural conditions by spatial location within a river system and this may have a major affect on survival rates. Acclimation at lower temperatures had a more substantial effect on LOE and subsequently mortality. These treatments sought to replicate the effect of stocking fingerlings into an upstream reach such as that of the Dalgety region in the upper Snowy River prior to attaining an equivalent size in nature. The increased mortality rate suggested that stocking hatchery-reared age-0 Australian bass in upland reaches in areas where water temperatures drop below 6°C would adversely influence survival. Sudden overnight drops in temperature at water can be extreme and may vary markedly in upland high-altitude areas (Johnson, 1971; Pennak, 1971). Consequently, this may lead to winterkill and significantly reduce the success and value of the stocking efforts. Cooler temperatures reduce antibody production (Burreson and Frizzell, 1986; Klesius, 1990; Hrubec *et al.*, 1996) and hinder non-specific immune functions (Scott *et al.*, 1985; O'Neill, 1986; Hrubec *et al.*, 1996) and this could render individuals susceptible to infection such as winter saprolegniosis (“winter kill”) a fungal condition known to affect silver perch (*Bidyanus bidyanus*) (Rowland *et al.*, 2007). Additionally, low temperatures could substantially influence the ratio of energy stores to metabolic rates which ultimately leads to winter starvation (Byström *et al.*, 2006), another potential source of mortality for stocked Australian bass under critical conditions.

Delayed mortality among the 4 treatments varied substantially. Greatest delayed mortality occurred in the slowest rate of temperature decline following a coldest acclimation. A possible explanation for high mortality in this treatment is that due to the slow decrease in temperature decline (1°C.d<sup>-1</sup> compared to 1°C.h<sup>-1</sup>) following a cold acclimation (8°C compared to 15°C) the period of time in which an individual is exposed to cold conditions

before LOE is prolonged compared to other treatments. The long-term effects of the 8°C acclimation temperature combined with the 1°C.d<sup>-1</sup> reduction in temperature appeared to result in detrimental effects which limited the ability of the fish to recover post LOE. In the faster rate of temperature decline (1°C.h<sup>-1</sup>) fish reach LOE more rapidly and are removed from these critical conditions (i.e. within several hours). A second possible explanation for the 100% mortality observed in this treatment is cold thermal shock. This is defined as an acute decrease in temperature leading to a rapid reduction in body temperature, eventually resulting in a cascade of behavioural and physiological failures (Donaldson *et al.*, 2008). However, this seems unlikely as mortality was far lower at only 13.33% in the 15°C acclimation at the same rate of temperature decline. If cold thermal shock was the cause of the significantly higher mortality in one of the treatments used, greater levels of mortality would have been expected in the remaining three treatments as the range of LOE temperatures observed were not substantial.

Sexual maturity of Australian bass occurs at 3+ years for males and 5-6+ years for females (Harris, 1986). Australian bass have a catadromous lifecycle and as a result, mature fish migrate in schools out of upstream reaches, downstream to brackish estuarine waters during the winter months and immature fish remain in the headwaters and freshwater reaches of rivers. Spawning of sexually-mature fish then occurs in the estuaries when water temperature is 11-18°C (Harris, 1986). Young-of-the-year individuals remain in estuaries at the spawning site, before migrating upstream during early spring as juveniles aged 0+ (Jerry, 1997). Naturally-recruited Australian bass are therefore well-developed before occurring in upstream freshwater environments. This study demonstrated that fish acclimated to higher ambient conditions were resilient to sudden decreases in temperature. It would be a rare event for coastal temperatures to fall below the critical values identified by this study. This may explain a lack of winter mortality observed for fish located within estuarine environments, as seawater temperatures would largely buffer any sudden changes to ambient conditions.

Hatchery programs seeking to successfully establish Australian bass populations should consider several factors to increase the likelihood of success. Firstly, the temperature regime of the receiving stream and, in particular intended stocking sites, should be known prior to any stocking activities. This should be considered as a fundamental step in assessing the suitability of the proposed waterway. Secondly, if stocking into upland reaches is deemed necessary, then grow out of individuals, to 1+ age prior to stocking would be more closely aligned with natural migratory behaviour of this species. Thirdly, Australian bass are generally confined to lowland and slopes regions of south-eastern Australian rivers. Large scale fish surveys have failed to detect the species above approximately 700 m elevation (Harris and Gehrke, 1997). The thermal tolerances identified within this study are consistent with this distribution for Australian bass as temperatures would be expected to drop with increases in altitude (Johnson, 1971; Hari *et al.*, 2006). Agencies seeking to stock this species should consider adopting strategies that mimic these natural distributions at various life history stages.

Further research is essential to determine if CTMin changes for Australian bass during different life stages and sizes. This study was limited to investigating the thermal tolerances of 0+ individuals which was designed to replicate the age and size stocked fish approach their first winter after being stocked into upstream environments such as that of the Snowy River around the Dalgety region. However, in the event of on-going stocking, it is inevitable that a number of age classes may occur at any one time throughout stocked areas (e.g. 0+, 1+, 2+, 3+ fish). Currently, no studies have determined CTMin for Australian bass for 1+ or older fish. Previous studies involving other fish species have found differences in CTMin across size ranges and ages (e.g. Barrionuevo and Fernandes, 1995) and others have not (e.g. Young and Cech, 1996; Ospina and Mora, 2004). If this was determined for Australian bass, it would



then be possible to more confidently assess the suitability of this species across a wider size range and age for stocking in the upper Snowy River.

Findings from this current study will have implications for future stocking programs for age-0 hatchery-reared Australian bass for the Snowy River and beyond. Furthermore, this study showed that mortality of age-0 Australian bass fingerlings can occur once exposed to water temperatures of 7°C but should be expected at 5°C and below. Minimum monthly water temperature from the Snowy River at Dalgety indicate that survival of stocked Australian bass was most likely impacted over the winters of 2008 and 2009 and this finding is supported by the results of Chapter 2. Historical data indicated that the winters of 2008 and 2009 could not be considered exclusive. Available data suggests that minimum monthly water temperatures were actually slightly higher than previous years. This information should be used to guide future stocking efforts for Australian bass in the Snowy River. Future stockings of age-0 Australian bass should be concentrated in areas where this species has the ability to withstand minimum water temperatures.

#### **4.5. Conclusion**

Findings from this study present the only available information on the cold thermal tolerance of juvenile Australian bass. This study showed that high mortality rates for Australian bass are most likely to occur in the wild if water temperatures remain low and stable for long periods, or continue to decrease below a threshold level of 6°C. Unless fish can move to warmer areas, LOE is inevitable and mortality is likely. As seen in these experiments once a fish has reached LOE, it essentially loses any capacity to escape conditions that can rapidly result in death. Stocking of hatchery-reared 0+ Australian bass into upland reaches of the Snowy River, particularly where low temperatures will persist below critical levels may result in high mortality rates during the coldest months which are June, July and August for that region.

## 5. AN ASSESSMENT OF CALCEIN TO CHEMICALLY-MARK AUSTRALIAN BASS TO DIFFERENTIATE STOCKED FROM WILD FISH

These data have been published in Australian Journal of Zoology:

Cameron, L., Baumgartner, L.J., Bucher, D.B. and Robinson, W. (2012). Optimising chemical marking techniques for Australian bass (*Macquaria novemaculeata*) fry and fingerlings prior to restocking. *Australian Journal of Zoology*. **59**, 242-248.

### 5.1. Introduction

In many stocking programs it can be difficult to determine the survival of stocked individuals due to difficulties in distinguishing stocked from wild individuals. It is therefore essential when monitoring programs are established, that a suitable fish tagging or marking method is used on fish to allow later separation between the two groups. This problem accentuates when fish are stocked into areas within their natural range, as stocked (often hatchery-reared) fish need to be determined from wild individuals (Russell, 2008) or overestimating natural recruitment and underestimating stocking success may occur.

Recently, a stocking program was established in order to boost numbers of Australian bass in the Snowy River. As with many stocking programs, no tagging or marking was applied to fish prior to stocking to allow for identifying these stocked individuals. One reason for this is the lack of information regarding the suitability of potential marking techniques for use on Australian bass. This raises difficulties in determining the overall proportion of stocked fish in a river population. Due to the size of stocked Australian bass (~ 10 - 80 mm) some marking methods are simply not possible. Additionally, the large numbers of individuals which are frequently stocked in programs, often 10,000's or 100,000's, poses major limitations for several marking techniques which do not allow for mass-marking such as many tagging methods.

There are many types of methods used to mark or tag fish such as finclipping, external tagging broodstock injection, isotope immersion and osmotic induction. Recently, there has been increased attention and application towards chemical marking which rely on fluorescing compounds that label calcified or bony tissues to fish. Chemical markers have considerable application because they are suitable for marking large numbers of hatchery-reared fish at minimal labour costs. Also, they do not require the handling of individual fish (Nielsen, 1992). Calcein is a commonly-used fluorescent compound (Mohler, 2003) which binds with alkaline earth metals and causes calcified parts of organisms (e.g., otoliths, fin rays and scales) to fluoresce when examined under a suitable light source (Wilson *et al.*, 1987). Calcein has been shown to be a reliable, non-lethal detectable marking technique for salmonids (see Mohler, 2003; Negus and Tureson, 2004) and more-recently in Australia, calcein was assessed as a possible marking technique for golden perch (*Macquaria ambigua*) (see Crook *et al.*, 2009).

Future restocking programs for Australian bass in the Snowy River would benefit from the development of a reliable batch-marking technique which allows for differentiating between stocked and wild fish. Any such method would provide the ability to determine particular

'batches' of stocked fish as part of any future monitoring programs and ultimately the degree of stocked fish survival. The aim of this study was to assess and optimise a method for batch-marking Australian bass and to assess the detection effectiveness of a calcein-mark by non-lethal methods up to 115 days post-marking. Osmotic induction delivery of a calcein-mark was applied to juvenile Australian bass from two size classes, fry and fingerlings. The interactive effects of different calcein immersion times, calcein concentrations and salt immersion times were compared to determine the best combination for producing a detectable calcein-mark on Australian bass. A non-lethal method was assessed for discriminating between calcein-marked and unmarked fish. Impacts of calcein marking on growth rates and post-marking survival were also investigated.

## **5.2. Methods**

Two separate experiments were completed in attempt to cover the broad size range of Australian bass stockings in south-east Australia. Australian bass were sourced from two separate fish farms and two experiments were run independently to achieve the objectives (Table 5.1). Experiment 1 was completed at Narrandera Fisheries Centre, Narrandera (S 34.777; E 146.568) with fingerlings and experiment 2 was completed at Grafton Aquaculture Centre, Grafton (S 29.612; E 152.956) with fry (Table 5.1). Methods described below were followed during both experiments. Any differences such as feeding between the two batches of fish are described in Table 5.1. A total of 27 glass aquaria (56 L) were used for each experiment, fingerlings were stocked at a rate of 15 per aquaria.

### **5.2.1. Stock solutions**

A salt solution consisting of 5% (50 g of commercially available natural salt/L water) was prepared. A 0.5% solution of calcein (5 g of calcein powder in 1 L of water) and an additional 1% calcein solution were prepared (10 g of calcein powder in 1 L water). Both calcein and the salt solution were aerated to maintain sufficient oxygen levels during marking activities. A separate salt immersion solution (also 5%) was prepared and used for each calcein solution (0.5 or 1%).

### **5.2.2. Marking procedures**

Fish from one aquaria per time (selected randomly) were dip netted and placed into a 1 L plastic container with a fine mesh bottom and immersed into the salt solution (2.5 or 5 min), rinsed in aquaria water for 5 s, and then immersed in the selected calcein solution for either 5 or 10 min at either 0.5 or 1%. Following the marking procedure, fish were stocked into one of the 27 glass aquaria (Figure 5.1). Unmarked control fish were left in the aquaria in which they were stocked during the marking procedures. All fish were held for a period of 115 days and all aquaria were cleaned weekly and approximately 25% of the water was replaced in each aquaria.

### 5.2.3. *Survival and growth*

Fish were viewed daily and any mortalities were removed, weighed, measured and date of death recorded. After 115 days, all surviving fish were removed from the aquaria and euthanased. Each fish was weighed (to the nearest 0.1 g) and measured (to the nearest mm). Mean growth over the 115 day period was estimated between all nine treatments by calculating the specific growth rate (Busacker *et al.*, 1990), as:

$$\text{specific growth rate} = 100 \cdot \left( \frac{\log_e Y_2 - \log_e Y_1}{t_2 - t_1} \right),$$

where  $Y_1$  is the initial weight of the fish,  $Y_2$  is the final weight of the fish, and  $t_2 - t_1$  is the number of days over which growth occurred.

### 5.2.4. *Detection of calcein marks*

A battery-operated portable self-contained general purpose modulated probe fluorometer (GFP meter; Opti-Sciences Inc., Hudson, New Hampshire) (Figure 5.2) was used to obtain values given in relative units of fluorescence known as ‘tics’ on all fish. The GFP meter was designed for a wide array of applications including measuring green fluorescent protein. In this study, a GFP meter was used to assess both the ability of the unit to correctly distinguish between calcein-marked and unmarked ‘control’ fish and to assess the variation in fluorescence among calcein-marked treatments. Nine readings (three readings from each of the operculum area, lateral line and the caudal peduncle region) were taken from each fish. Readings were obtained by lightly placing the fiberoptic probe on the individual at the selected location and a measurement was taken. Mean number of tics of fluorescence was calculated for each fish. Readings that were above the output of the GFP meter, classified as signal overload, were assigned the maximum signal limit of the unit (1,800 tics).

### 5.2.5. *Data analysis*

The level of significance for all tests was 0.05. One-way analyses of variance (ANOVA) were performed to test for differences in survival among all nine treatments (calcein-marked and unmarked fish). ANOVA were also performed to test for differences in survival between calcein-marked and unmarked fish and to test for differences in mean specific growth rates among all nine treatments.

The upper level of detection of the tics of fluorescence was 1800 (maximum value) and subsequently the data did not follow a distribution suitable for either a traditional ANOVA or a generalised linear model. No transformations could rectify this condition so a factorial ANOVA was performed on the actual data compared to 10,000 randomised tests. Observations were randomised across all treatment combinations and the f-value of the tests were used for comparisons, a method shown to be suitable for testing main effects and their interactions in factorial ANOVA’s on non-normal data (Gonzalez and Manly 1998). ANOVA’s included three fixed factors; salt immersion time (2.5 or 5 min), calcein immersion time (5 or 10 min) and calcein concentration (0.5 or 1%) and only the mean tics of the fish in each glass aquaria (replicate) was used in the analysis which was performed using SAS version 9.2 (SAS 2004).

**Table 5.1.** Details of Australian bass fry and fingerlings used in experiments. Lengths and weights are means  $\pm$ SE., ranges are shown in parentheses. Water quality data are ranges.

Parameter	Fingerling Experiment	Fry Experiment
Source of Australian bass	Narooma Aquaculture	Searle Aquaculture
Age (months)	9	2
Length (mm)	64.49 $\pm$ 0.46 (38-80)	21.36 $\pm$ 0.21 (16-32)
Weight (g)	4.18 $\pm$ 0.08 (1-7.2)	0.21 $\pm$ 0.01 (0.18-0.28)
Food supply	Bloodworms	Live food (day 1-34) Bloodworms (day 35-115)
Feeding rate (per aquaria)	~6 g (6 times per week)	~3 g (12 times per week)
DO (mg L <sup>-1</sup> )	6.98-7.75	6.40-7.66
pH	7.40-7.85	7.01-7.98
Water temperature (°C)	18.30-23.00°C	18.50-25.10°C

### 5.3. Results

#### 5.3.1. Fingerling experiment

No fingerlings died or displayed signs of distress during calcein-marking. The first mortality of calcein-marked fish occurred 35 days post-marking (treatment: 2.5 min salt immersion, 10 min calcein immersion and 0.5% calcein concentration). The first unmarked mortality (control fish) occurred after 55 days. Survival was not significantly different among treatments (ANOVA:  $df = 1,8$   $F = 0.51$ ,  $p = 0.83$ ). Of the 360 calcein-marked fish, 343 survived and there were 17 mortalities (95.3% survival). Percentage of fish surviving the 115 days was similar for the 45 unmarked fish with 43 surviving and 2 mortalities (95.6% survival). There was no significant difference in survival between calcein-marked and unmarked fish (ANOVA:  $df = 1$ ,  $F = 0.06$ ,  $p = 0.93$ ).

Mean specific growth rates ranged from 0.06 to 0.11% but were not significantly different among treatments (ANOVA:  $df = 1,8$   $F = 0.45$ ,  $p = 0.87$ ) (Figure 5.3a). There were no significant differences in mean specific growth rates between marked and unmarked fish (ANOVA:  $df = 1$ ,  $F = 0.78$ ,  $p = 0.38$ ). Mean final weights of the calcein-marked and unmarked fish were  $4.62 \pm 0.08$ g and  $4.62 \pm 0.26$  g respectively. Mean final lengths of calcein-marked and unmarked fish were  $66.54 \pm 0.47$ mm and  $67.72 \pm 1.28$  mm respectively.

Mean tics of fluorescence per individual ranged from 697 to 1800 and 0 to 74 for calcein-marked and unmarked fish respectively. Calcein-marked fish consistently retained much greater measurements of mean tics of fluorescence. Among the calcein-marked fish, 231 of the 343 (67.34%) surviving individuals had a final mean tics of fluorescence of the maximum value (1800 or signal overload). For calcein-marked fish, all three main effects; salt immersion time, calcein immersion time and calcein concentration had no significant effect on the mean tics of fluorescence (Table 5.2).

#### 5.3.2. Fry experiment

No individuals died during the calcein-marking or showed any signs of distress. The first mortality of calcein-marked fish (two individuals) occurred two days following the calcein-marking (treatment: 5 min salt immersion, 10 min calcein immersion and 0.5% calcein

concentration). Survival of fish ranged from 86.7 to 100% and was significantly different among treatments (ANOVA:  $df = 1,8$   $F = 5.35$   $p = <0.001$ ). Survival among the 360 calcein-marked fish was high with 345 surviving and 15 mortalities (95.8% survival). Survival of unmarked fish was 100%. However, no significant differences in survival between marked and unmarked fish (ANOVA:  $df = 1$ ,  $F = 0.63$   $p = 0.43$ ).

Mean specific growth rates varied among treatments (1.32 - 1.47%) but were not significantly different (ANOVA:  $df = 1,8$   $F = 0.77$   $p = 0.63$ ) (Figure 5.3b). Calcein-marked fish recorded higher mean specific growth rates compared to non-marked fish, however, this was not significantly different (ANOVA:  $df = 1$ ,  $F = 0.62$ ,  $p = 0.43$ ). Mean final weights of the calcein-marked and unmarked fish were  $1.11 \pm 0.02$  g and  $1.09 \pm 0.05$  g respectively. Mean final lengths of the calcein-marked and unmarked fish were  $41.52 \pm 0.21$  mm and  $41.68 \pm 0.80$  mm respectively.

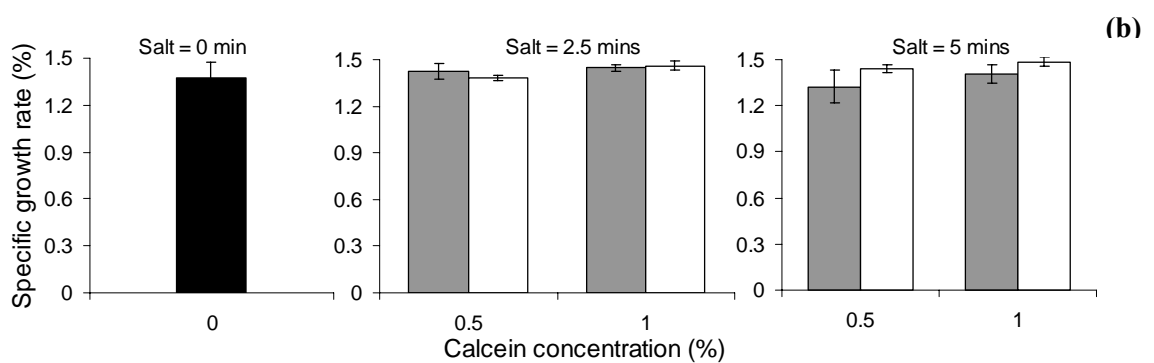
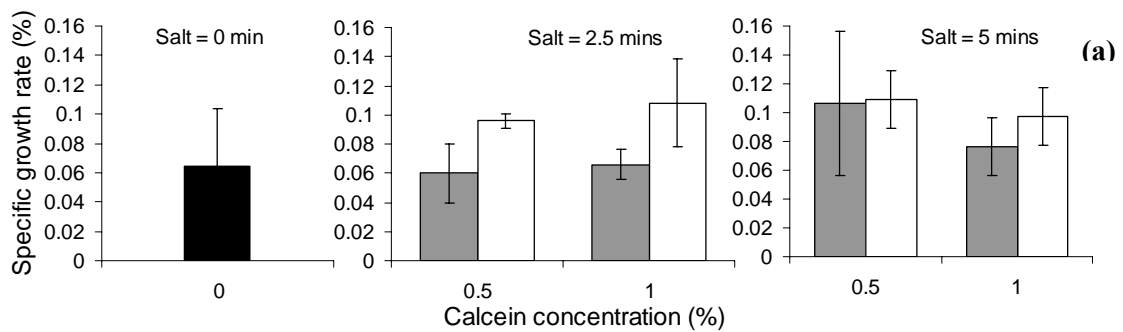
Mean tics of fluorescence ranged from 4 to 1800 and 0 to 3 for calcein-marked and unmarked fish respectively. Mean tic measurements were greater in calcein-marked fish compared to unmarked fish. For the calcein marked fish, 2 of the 345 (0.57%) had a final mean tics of fluorescence of the maximum value (1800 or signal overload). For calcein-marked fish, the main effects; salt time and calcein immersion time had no significant effect on mean tics of fluorescence. However, calcein concentration did have a significant effect ( $F = 5.05$ ,  $df = 1,16$   $p = 0.03$ ) (Table 5.3). Highest mean tics of fluorescence was measured in the treatment: 5 min salt immersion, 10 min calcein immersion and 1% calcein concentration (mean  $1020 \pm 41$ ) and lowest in treatment: 2.5 min salt immersion, 10 min calcein immersion and 0.5% calcein concentration (mean  $719 \pm 35$ ). Mean tics of fluorescence of fish marked with 1% calcein was  $941 \pm 22$ , whereas for fish marked with 0.5% calcein the mean was  $797 \pm 23$ .



**Figure 5.1.** Australian bass fingerlings stocked into aquaria following calcein-marking.



**Figure 5.2.** Fibre-optic (GFP) Meter used to determine differences in levels of fluorescence among fish during experiments.



**Figure 5.3.** Mean ( $\pm$ S.E.) specific growth rates for treatments examined in fingerling experiment (a) and fry experiment (b). S=salt immersion time, CT=calcein immersion time and CC=calcein concentration.

**Table 5.2.** Results of factorial ANOVA examining the effects of salt immersion time, calcein immersion time, calcein concentration and all interactions on means of fluorescence among calcein-marked fingerlings. \* = adjusted P-value calculated after 10,000 randomisations of the raw data.

Source	df	F ratio	<i>Prob&gt;F</i>	<i>Prob&gt;F*</i>
Salt time	1, 16	1.15	0.29	0.30
Calcein immersion time	1, 16	0.16	0.69	0.69
Salt time X Calcein immersion time	1, 16	0.61	0.44	0.45
Calcein concentration	1, 16	2.04	0.17	0.17
Salt time X Calcein concentration	1, 16	0.13	0.72	0.72
Calcein immersion time X Calcein concentration	1, 16	0.00	0.94	0.94
Salt time X Calcein immersion time X Calcein concentration	1, 16	0.01	0.91	0.91

**Table 5.3.** Results of factorial ANOVA examining the effects of salt immersion time, calcein immersion time, calcein concentration and all interactions on means of fluorescence among calcein-marked fry. \* = adjusted P-value calculated after 10,000 randomisations of the raw data.

Source	df	F ratio	<i>Prob&gt;F</i>	<i>Prob&gt;F*</i>
Salt time	1, 16	2.61	0.12	0.12
Calcein immersion time	1, 16	0.33	0.57	0.57
Salt time X Calcein immersion time	1, 16	0.26	0.62	0.62
Calcein concentration	1, 16	5.05	0.03	0.04
Salt time X Calcein concentration	1, 16	0.27	0.61	0.61
Calcein immersion time X Calcein concentration	1, 16	3.54	0.07	0.07
Salt time X Calcein immersion time X Calcein concentration	1, 16	2.28	0.15	0.15



#### 5.4. Discussion

This current study demonstrated that calcein-marking has no detectable effect on the survival of hatchery-reared Australian bass fry or fingerlings for up to 115 days. Although there were differences in survival rates between treatments in the fry experiment, overall survival of calcein-marked Australian bass was still high. Generally, results from these experiments showed that survival of calcein-marked fish were similar to or higher than that of unmarked 'control' fish. Mohler (2003) also reported no differences in mortality rates between calcein-marked and unmarked Atlantic salmon, (*Salmo salar*) up to 47 d post-marking and Crook *et al.*, (2009) reported low mortality rates of calcein-marked golden perch, up to 100 days post-marking.

No signs of distress or mortalities during calcein-marking in either of the fry or fingerling experiment suggests that the handling, exposure to salt, calcein or a combination of these factors did not have any short-term negative effects on the health of Australian bass. Similar observations were reported by Wilson and Beckman (1987) who found no mortality of red drum, (*Sciaenops ocellatus*), Atlantic croakers, (*Micropogonias undulates*) and spot croakers, (*Leiostomus xanthurus*) during calcein immersion. Crook *et al.*, (2009) also reported low mortality rates of calcein-marked golden perch up to 100 days post-marking which strengthens the basis that calcein has minimal physiological impact on fish.

The lack of differences in growth rates of calcein-marked and unmarked fish indicates that the calcein marking procedure does not inhibit post-marking growth of Australian bass at fry or fingerling stage. This finding is valuable for those involved in future calcein-marking and stocking of Australian bass as it suggests that growth will not be affected, at least over the short term. A number of studies have also reported similar trends in un-affected growth of calcein-marked fish (Frenkel *et al.*, 2002; Bashey, 2004; Crook *et al.*, 2009).

The GFP meter used in this study to differentiate between calcein-marked and unmarked fish showed that it can sufficiently and reliably distinguish between the two groups. Mean tics of fluorescence per fish was consistently higher for calcein-marked fish compared to unmarked fish in both the fry and fingerling experiments. Overall, higher mean readings were obtained from the calcein-marked fingerlings compared to the fry. As calcein binds to alkaline earth metals and calcified structures of fish such as vertebrae and otoliths (Wilson *et al.*, 1987; Leips *et al.*, 2001), it is possible that larger fish with a greater bone size have an increased ability to uptake greater amounts of the chemical during immersion. Consequently, this may result in higher tics of fluorescence in larger fish compared with smaller fish as seen in this study.

There appeared to be little or no benefit applying greater salt immersion times (5 min compared to 2.5 min), greater calcein immersion times (10 min compared to 5 min) or greater concentrations of calcein (1% compared to 0.5%) on the Australian bass fingerlings for detection purposes. Whilst slightly higher mean tics of fluorescence were measured in treatments which contained the maximum of each of these factors, the effects were not significant. Additionally, the levels of fluorescence detected were still significantly higher than unmarked fish, suggesting that discrimination between marked and unmarked fish would still be achievable. Therefore, the extra time and costs associated with marking fish under these higher treatments is most likely not justifiable.

Findings were similar in the fry experiment, except for the significant effect of calcein concentration. As calcein-marked Australian bass fry and fingerlings were all easily detectable after immersion in calcein at either 0.5 or 1% concentration, the decision of which

concentration to apply when calcein-marking Australian bass fry in the future may be influenced by the cost of calcein. However, more work is needed to determine the effect of calcein concentration beyond 115 days. Calcein concentration may impact on the ability to distinguish marked from unmarked fish over a longer time period such as several years. Due to the current cost of calcein (approximately AUS\$800 for 25 g), further work is also required to determine if calcein is detectable on fish using a GFP meter if fish are calcein-marked at lower concentrations (i.e. 0.1% or 0.25%). If detection is possible at lower concentrations, this would reduce the costs associated with these methods and possibly enhance the future use of calcein.

While the GFP meter showed valuable results in this current study, further research is required to determine the ability of the GFP meter under field conditions to ensure similar detection capabilities are achievable. Secondly, it would be useful to identify the period of time that the unit can positively detect calcein on fish. Previously, Crook *et al.*, (2009) reported calcein-marked golden perch reared in aquaria could be readily detected more than two years post-marking. We have since found that calcein-marked Australian bass fingerlings stocked into glass aquaria under laboratory conditions are detectable up to at least two and a half years post-marking with the GFP meter (L Cameron, unpublished data). However, as many stocked fish species, including Australian bass are long-lived (> 20 years) (Harris, 1987), it would be beneficial if longer term detection was possible.

One of the major advantages of using calcein to chemically-mark fish is the ability to discriminate calcein-marked fish via techniques other than the GFP meter, such as examining the otolith, anal fins or caudal fins. If further studies reveal that the GFP meter cannot detect calcein for the life of the marked fish, other methods of detection such as the examination of these structures may provide as a reliable alternative. These methods have been previously examined and have demonstrated that this technique for distinguishing calcein-marked fish is reliable using a GFP3 filter set with the appropriate excitation filter (Crook *et al.*, 2009). The ability to distinguish calcein-marked fish, for its entire life using a GFP3 filter set through examination of these body structures enhances the potential future uses of calcein to chemically-mark Australian bass as detection is relatively easy and possible under field conditions with on-the-spot recognition. This long-term capacity to detect marked fish may improve the ability to assess the effectiveness of stocking programs even if the GFP meter cannot reliably detect calcein-marked fish over a longer period of time such as several years.

In order to calcein-mark Australian bass and stock them into the wild, a legislative process is required. A major concern is the potential breach of environmental and or food/safety laws depending on the jurisdiction (Crook *et al.*, 2009). Calcein is presently being assessed for use under an Investigative New Animal Drug exemption file issued by the U.S. Food and Drug Administration for the chemical marking of fish (USFWS, 2004; Honeyfield *et al.*, 2008). Recent examinations of the legislative requirements for the use of calcein in Australia have found potential requirements for registration by Food Standards Australia New Zealand and the Australian Pesticides and Veterinary Medicines (Sanger and Crook, 2007; Crook *et al.*, 2009). Chemically-marking Australian bass has proved to be an uncomplicated method and the process does not reduce survival or growth and can be correctly detected. This could be applied to greatly enhance our knowledge and quantify the success of Australian bass stocking programs.

## **5.5. Conclusion**

This study identified that calcein-marking hatchery-reared Australian bass fry and fingerlings via osmotic induction techniques had no negative impacts of the growth or survival during marking and for a period of up to 115 days post-marking. A hand-held GFP meter used to distinguish unmarked from calcein-marked fish correctly separated marked from unmarked

fish by detecting high levels of fluorescence in calcein-marked fish. These levels of fluorescence did not vary greatly among calcein-marked treatments suggesting that even the lowest concentrations and immersion times in calcein would result in a reliable detectable calcein-mark on fish. Further work is necessary under natural conditions to comprehensively assess the ability of the GFP meter particularly in regards to its long-term ability to detect calcein in fish.

## 6. SOURCE AND MIGRATION PATTERNS OF AUSTRALIAN BASS IN THE SNOWY RIVER

### 6.1. Introduction

Otolith microchemistry is useful for determining the natal origin of individual fish and discriminating between stocks (Zitek *et al.*, 2010). Several studies have used microchemical structure at the otolith nucleus (referred to hereafter as 'core') to determine the birth location generally by examining strontium:calcium (Sr:Ca) and barium:calcium (Ba:Ca) ratios (e.g., Veinott and Porter, 2005; Swan *et al.*, 2006). For example, large-scale collection of otoliths from Snapper (*Pagrus auratus*) that were subjected to microchemical analysis indicated that most recruitment on the east coast of New Zealand occurred within a single region (NIWA, 2009).

The examination of fish movements between freshwater and marine environments has been greatly enhanced by the use of otolith microchemistry. The basic assumption of this technique is that ambient levels of microchemical trace elements are encapsulated within the otolith as a fish grows (McCulloch *et al.*, 2005). Correlation of the otolith microchemistry with known concentrations of trace-elements in the environment can be used as a way of documenting fish movements (e.g., Zitek *et al.*, 2010). For instance, Sr is a trace element which is naturally in much higher concentrations in seawater, than freshwater. Therefore, whenever a fish enters a saltwater environment, the ratio of Sr to Ca trapped within an otolith will subsequently increase (Crook *et al.*, 2006). Conversely, the trace element Ba is present in much higher concentrations from freshwater environments. Plotting the chemical changes in the ratio of Sr:Ca and Ba:Ca therefore provides a powerful and accurate method of determining fish movement patterns.

One of the most commonly-used techniques for otolith microchemistry is laser-ablation inductively-coupled-mass-spectrometry (LA-ICPMS) which has the ability to detect numerous elements in otoliths simultaneously (Tresher, 1999; Zitek *et al.*, 2010). LA-ICPMS analysis involves setting an otolith in resin and then taking a thin section for analysis. The section is then 'ablated' by pulsing a high-powered laser onto the otolith. Aerosols generated by the ablation are transported into the core of the plasma which are mass analysed for trace elements. Studies using this technique have identified valuable information on the life histories of many catadromous (e.g., Shiao *et al.*, 2006) and marine species (e.g., Hamer *et al.*, 2006). The major assumption of this technology is that fish reside in saltwater long enough to enable detection. Spot size of beams can be as small as 30 $\mu$ m (e.g., Waite *et al.*, 2008) and fish deposit otolith rings at known rates of between 1-5 $\mu$ m per day (Miles, 2009). This suggests that fish must reside within seawater for at least 10-50 days for this technique to be useful.

Otolith chemistry can be applied to Australian bass from the Snowy River to assist in determining information about fish origins and movement patterns. It was assumed that all recruited fish have arisen from either the estuary or a hatchery environment. The major assumptions of this study is that Australian bass have not recruited in the Snowy River for approximately 20 years (Lugg *et al.*, 2006) and any individuals collected from the upper Snowy River (NSW waters) are likely to have originated from a hatchery (see Chapter 1). The microchemistry of any wild fish should be different as these fish would have resulted from

subsequent spawning that took place in estuarine regions. Subsequently, fish that were spawned and recruited in hatchery environments should also have a substantially different signature to wild fish.

Low captures of Australian bass were recorded from the Snowy River following major stocking efforts (Chapter 2). In this current study, the chemical compositions (Sr:Ca and Ba:Ca) of wild-caught Australian bass otoliths were analysed (i.e. the region that reflects the first period of life) to determine whether fish were likely to be of hatchery (stocked) or wild origin (natural recruits) in order to determine the contribution of stocking. Secondly, transects were analysed across the otoliths from core to edge (first to the last period of life) from known hatchery fish, fish of unknown source (but suspected of originating from a hatchery) and wild caught fish (considered to be natural recruits, too old to have originated from stocking programs) to assess for evidence of any movement between environments throughout the life of these fish.

## 6.2. Methods

### 6.2.1. Fish Collection

Australian bass collected during regular sampling (Chapter 2) were retained for otolith microchemistry analysis. These included fish collected from the high density reach (NSW waters), low density reach (NSW waters) and downstream of the low density reach (sites within VIC waters). Low recaptures of Australian bass, particularly large individuals during regular sampling (Chapter 2) necessitated the collection of additional fish from outside the stocking zone. A specific one-off collection was subsequently performed in January 2011 and fish were collected from two sites (Table 6.1) (Figure 6.1). A combination of gill netting and electrofishing was performed at these sites to collect Australian bass. Fish were also returned and used as part of this study from anglers who were actively fishing during this period.

**Table 6.1.** Additional sites sampled to collect Australian bass for otolith microchemistry analysis.

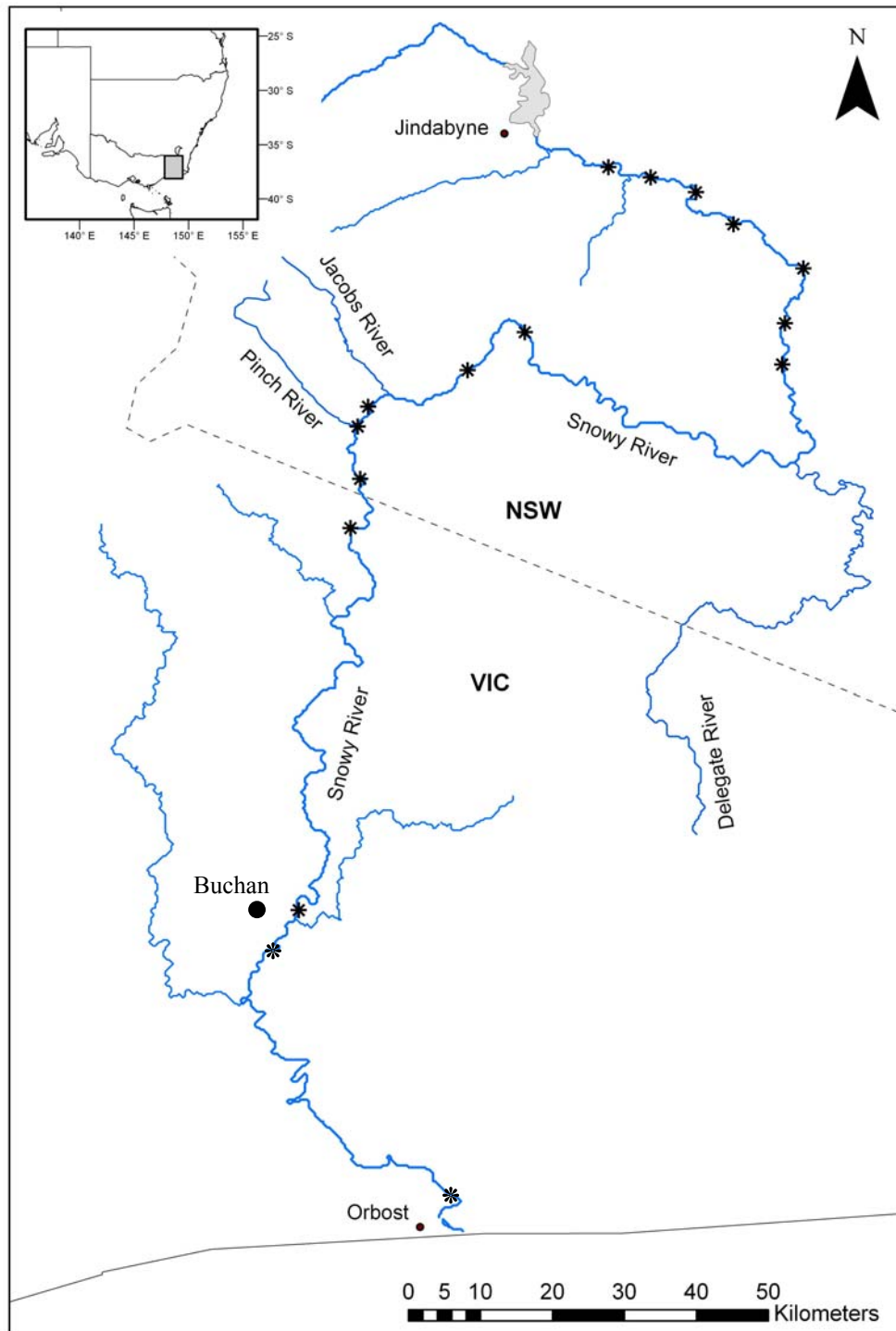
Site Name	Latitude	Longitude	Nearest town	Methods used
Bridge at Orbost	37.71694	148.45258	Orbost	Electrofishing
Harper's property	37.50759	148.27429	Buchan	Electrofishing/gillnetting

### 6.2.2. Rationale for microchemistry tests

Microchemical analyses were performed to answer two specific research questions:

- (1) Were individuals collected throughout this study likely to be of hatchery or wild origin?
- (2) Was there any evidence of movements between freshwater and saltwater regions?

For question 1, all fish collected as part of routine sampling (2008-2009), angling events (2008-2011) and the additional fish collections (2011) were used (n = 133) (Table 6.2). Otoliths from these fish were subjected to an individual comparison of the nucleus (core). It is assumed that microchemical composition of the nucleus would represent ambient conditions at the time of hatching.



**Figure 6.1.** A map of the Snowy River highlighting the location of the 14 sampling sites used to collect fish for this study (\*).

For question 2, a random sample of fish collected from both upper and lower reaches of the Snowy River were compared. LA-ICPMS were used performing a transect technique across a sectioned otolith. A transect scan ablation was carried out from the core to the edge of the otoliths ( $n = 40$ ) (Table 6.3). Any subsequent change in ratios would determine the frequency, if any, of movements between freshwater and seawater.

**Table 6.2.** Details of Australian bass used for otolith core examination (question 1).

Source	Collection location	Date of collection	Size range of fish (mm)	Number used
Narooma Aquaculture	Narooma Aquaculture	2009	12-26	19
Assumed as wild fish-Snowy River	Lower Snowy River	2011	~400-485	10
Unknown	Snowy River (NSW waters)	2008-2010	32-169	62
Unknown	Snowy River (VIC waters)	2008-2011	77-303	42

**Table 6.3.** Details of Australian bass used for transitional analysis (question 2).

Source	Collection location	Date of collection	Size range of fish (mm)	Number used
Assumed as wild fish-Snowy River	Lower Snowy River	2011	~400-500	10
Unknown	Snowy River (NSW waters)	2008-2010	101-170	16
Unknown	Snowy River (VIC waters)	2008-2011	101-303	14

### 6.2.3. Otolith Preparation

Sagittal otoliths were initially removed from all fish, washed in distilled water, and all tissue removed with fine forceps. Otoliths were then placed into 100% high grade ethanol for 30 seconds then air-dried. The dry clear otoliths were individually embedded in a silicon mould with polyester resin to produce a solid block. Otoliths in resin blocks were sectioned transversely through the core by a Buehler ISOMET low speed saw to produce a 450  $\mu\text{m}$  thickness of otolith section. The sections were then ground on 600 and 1200-grade carborundum paper, and polished with 0.3  $\mu\text{m}$  alpha alumina powder before being mounted on glass slides with polyester resin for microchemical analysis.

### 6.2.4. Laser ablation ICPMS

All LA-ICPMS work used a Coherent GeolasPro 193 nm ARF Excimer laser coupled to a Varian-Bruker 820-MS Inductively Coupled Plasma Mass Spectrometer. Laser energy was tuned to 6 J/cm<sup>2</sup>, the system was optimized to the maximum sensitivity while keeping the elemental fractionation to the minimum and ThO<sup>+</sup>/Th<sup>+</sup> to ~ 0.6%, ultrahigh purity. Ca, Sr and

Ba were measured and Ca was used as the internal standard for quantification purpose. For the otolith core analysis, the laser beam was focused on the core of the fish otolith sample, single point ablation was used and Ca, Ba and Sr were measured. The laser was operated at 10 Hz, beam diameter used was 32  $\mu\text{m}$ . For the otolith transect analysis, a transect scan ablation was carried out from the core to the edge of each otolith. A pre-scan cleaning step was applied before the analysis scan in order to remove any possible surface contamination. Step and repeat scanning mode was used, laser beam diameter of 60  $\mu\text{m}$  and 2 Hz were used for the cleaning step, beam diameter of 32  $\mu\text{m}$  and 10 Hz was used for the analysis scan. All measurements were recorded in ppm and later converted to  $\text{mmol mol}^{-1}$  (Sr:Ca) or  $\mu\text{mol mol}^{-1}$  (Ba:Ca).

### 6.2.5. Data Analysis

Non-parametric permutational multivariate analysis of variances (PERMANOVA) was used to identify differences in chemical ratios measured from the core of otoliths among collection locations (Australian bass juveniles collected from the Snowy River in NSW, Australian bass juveniles collected from the Snowy River in VIC, mature sized Australian bass collected from lower Snowy River in VIC and Australian bass fry from a known source - Narooma Aquaculture in 2009). Where significant differences were revealed by PERMANOVA *post-hoc* pair-wise comparisons were used to determine which collection sites differed from each other. Prior to analysis chemical ratios (Sr:Ca or Ba:Ca) were fourth root transformed and a resemblance matrix was constructed using the Bray-Curtis index. All tests were considered significant at  $p < 0.05$ . All multivariate analysis was performed using PRIMER-E statistical software (Clarke and Warwick, 2001).

The otolith core was defined as the region within 100 $\mu\text{m}$  of the focus of concentric growth. The otolith edge was defined as the area within 100 $\mu\text{m}$  of the outer edge. Otolith core and edge Sr:Ca and Ba:Ca were compared using one-way ANOVA.

## 6.3. Results

### 6.3.1. Otolith core analysis

Mean Sr:Ca ratios were greatest in samples from Narooma hatchery in 2009 and lowest in mature-sized fish from collected from the lower Snowy River which were assumed as 'wild' fish (Figure 6.2). Conversely, Ba:Ca ratios were greatest in mature-sized fish from the lower Snowy River and lowest in samples from the Narooma hatchery (Figure 6.3).

Significant differences occurred in Sr:Ca core ratios among fish from the four collection locations (PERMANOVA:  $F = 9.13$ ,  $P = < 0.01$ ). Post-hoc comparisons revealed that there were significant differences in Sr:Ca ratios between juveniles Snowy NSW and juveniles Snowy VIC (PAIR-WISE TEST:  $t = 2.45$ ,  $P = 0.01$ ), juveniles Snowy NSW and Narooma hatchery 2009 (PAIR-WISE TEST:  $t = 4.45$ ,  $P = < 0.01$ ), juveniles Snowy VIC and Narooma hatchery 2009 (PAIR-WISE TEST:  $t = 4.31$ ,  $P = < 0.01$ ) and mature lower Snowy and Narooma hatchery 2009 (PAIR-WISE TEST:  $t = 5.97$ ,  $P = < 0.01$ ). There was no significant interaction between juveniles Snowy NSW and mature lower Snowy (PAIR-WISE TEST:  $t = 0.35$ ,  $P = 0.81$ ) or juveniles Snowy VIC and mature lower Snowy (PAIR-WISE TEST:  $t = 1.60$ ,  $P = 0.11$ ).

Significant differences were found in Ba:Ca core ratios among collection sites (PERMANOVA:  $F = 14.81$ ,  $P = > 0.01$ ). Post-hoc comparisons revealed that there were significant differences in Ba:Ca ratios between all collection locations; juveniles Snowy NSW and juveniles Snowy VIC (PAIR-WISE TEST:  $t = 2.34$ ,  $P = 0.02$ ), juveniles Snowy NSW



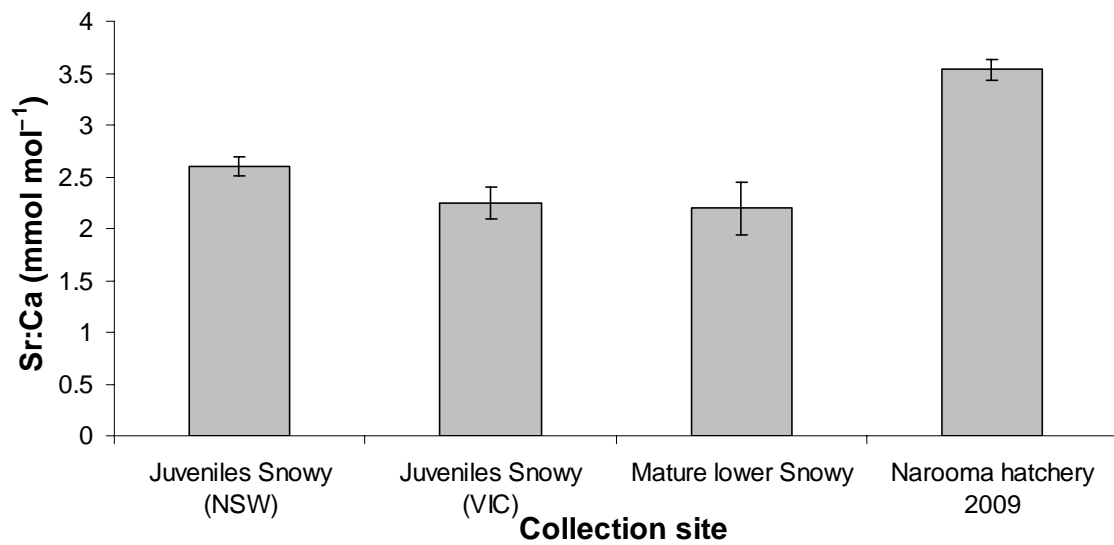
and mature lower Snowy (PAIR-WISE TEST:  $t = 4.31$ ,  $P = <0.01$ ), juveniles Snowy NSW and Narooma hatchery (PAIR-WISE TEST:  $t = 4.28$ ,  $P = <0.01$ ), juveniles Snowy VIC and mature lower Snowy (PAIR-WISE TEST:  $t = 2.15$ ,  $P = <0.03$ ), juveniles Snowy VIC and Narooma hatchery 2009 (PAIR-WISE TEST:  $t = 4.98$ ,  $P = <0.01$ ) and mature lower Snowy and Narooma hatchery 2009 (PAIR-WISE TEST:  $t = 8.79$ ,  $P = <0.01$ ).

### 6.3.2. *Otolith transitional analysis*

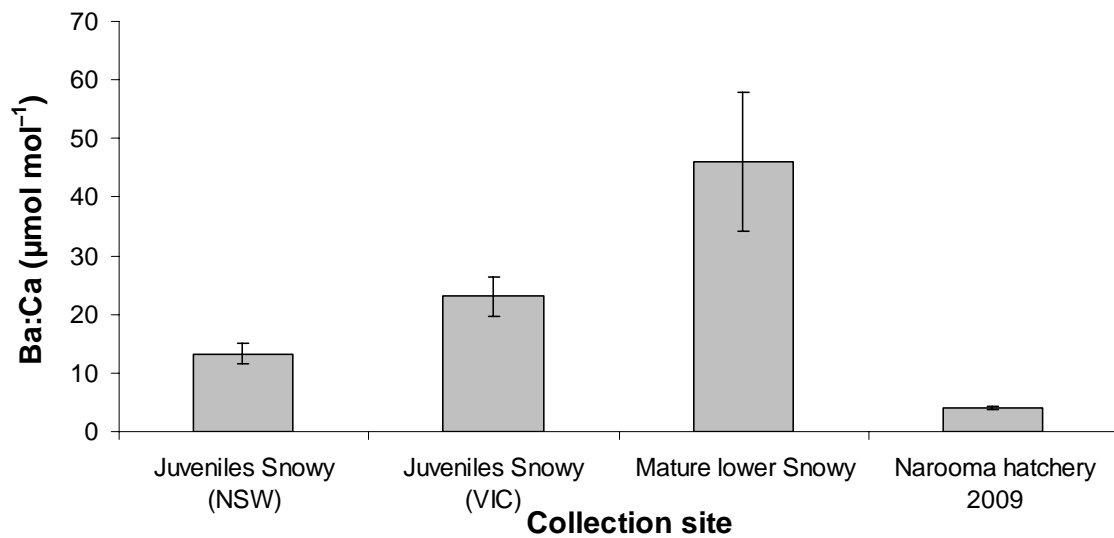
Otoliths examined from individuals collected throughout the upper Snowy River indicate a change of environmental conditions during the early stage of life, based on the distance from the core that these changes occurred (Figure 6.4). Across the otolith transect, there is a reduction in Sr:Ca ratios and an increase in Ba:Ca ratios simultaneously. Mean Sr:Ca ratios were  $2.74 \pm 0.07$  at the core and  $1.12 \pm 0.03$  at the otolith edge. Mean Ba:Ca ratios were  $13.34 \pm 0.62$  at the core and  $32.07 \pm 1.31$  at the otolith edge. These results indicate that the first period of life is spent in water with elevated salinities (i.e. held in hatchery conditions, in saline water), but later life was spent in waters consisting of lower salinities, typical of the water in the upper Snowy River where these fish were collected from. These findings support the presumption that Australian bass collected from NSW waters of the Snowy River would have spent the first part of life under hatchery conditions followed by later life spent in freshwater environments of the upper Snowy River.

Examination across the otolith of individuals collected from the lower Snowy River (>400 mm) indicate fluctuating Sr:Ca and Ba:Ca ratios through the otolith. A number of peaks in Sr:Ca ratios indicate movement to moderately saline environments as expected in mature-sized Australian bass (Figure 6.5). Mean Sr:Ca ratios at the core were  $2.21 \pm 0.07$  and  $1.83 \pm 0.04$  at the otolith edge. The peaks in Sr:Ca ratios, and the lower Sr:Ca ratios at the edge compared to the core, indicate that early life was spent in higher salinity waters, possibly in the estuary in the lower Snowy River followed by more freshwater environments later in life. Mean Ba:Ca ratios were  $43.68 \pm 3.37$  at the core and  $22.32 \pm 1.39$  at the otolith edge. The highly variable Sr:Ca ratios across the otolith transect (i.e. throughout the life of these individuals) suggest frequent movements between freshwater and marine environments.

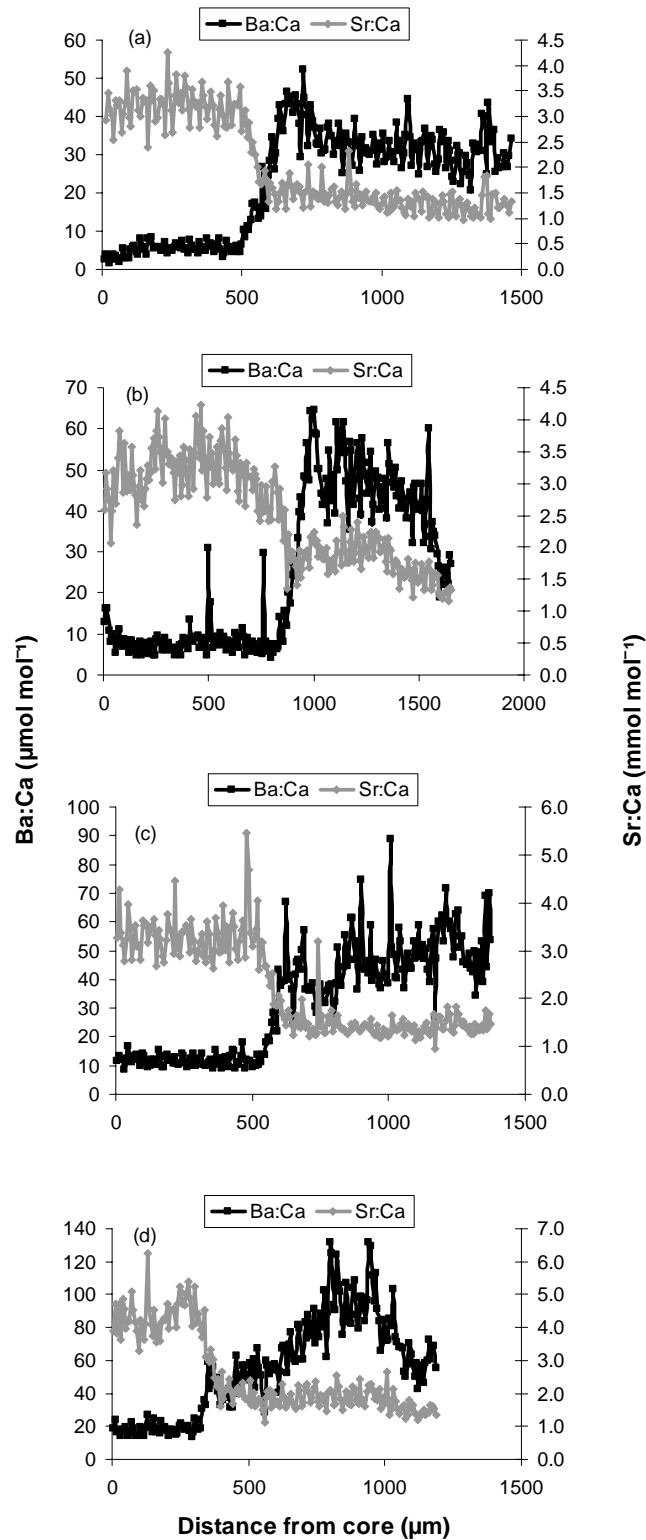
Mean otolith Sr:Ca ratios from Australian bass (<300 mm) collected from VIC waters of the Snowy River were  $2.18 \pm 0.07$  at the core and  $0.94 \pm 0.04$  at the otolith edge indicating early life was spent in water of high salinities (Figure 6.6). The otolith core Sr:Ca ratio is very similar to that of the mean for the >400 mm (assumed mature wild samples) from the Snowy River (Figure 6.2). Ba:Ca ratios were  $19.81 \pm 1.28$  at the core and  $13.96 \pm 1.58$  at the otolith edge. Sr:Ca ratios fluctuate throughout the otolith in these samples (Figure 6.6), similar to those movement patterns observed in the mature samples (Figure 6.5). These results most likely indicate movement throughout the lower Snowy River, in and out of freshwater and saltwater regions as expected by this catadromous fish species.



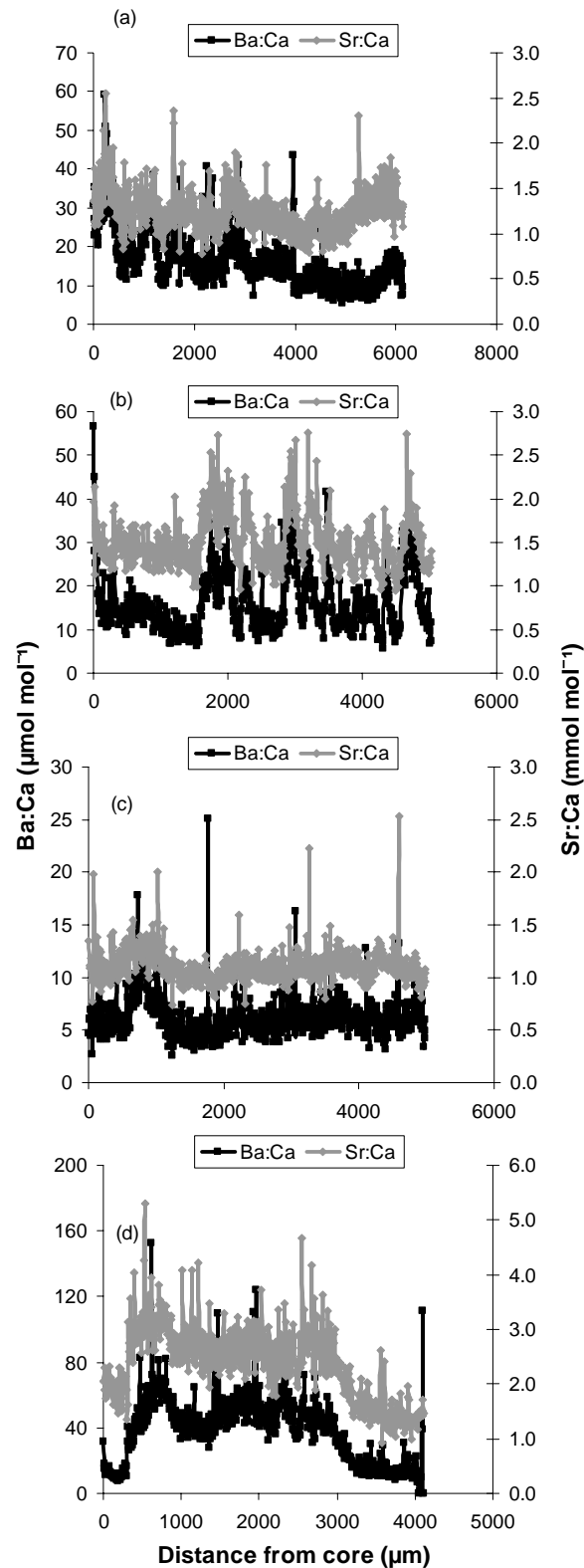
**Figure 6.2.** Mean Sr:Ca ratios  $\pm$  S.E. at the core of all samples examined. Juveniles were  $<300$  mm and mature samples were  $>400$  mm.



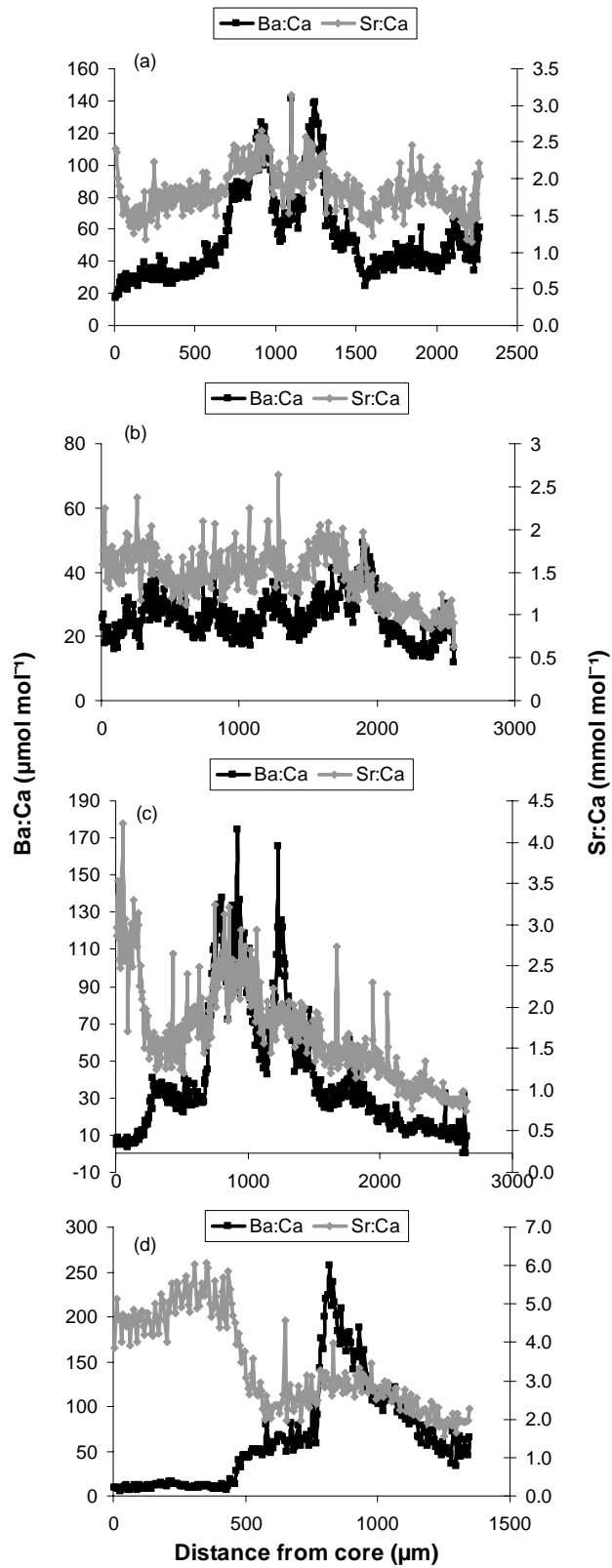
**Figure 6.3.** Mean Ba:Ca ratios  $\pm$  S.E. at the core of all samples examined. Juveniles were  $<300$  mm and mature samples were  $>400$  mm.



**Figure 6.4.** Otolith Sr:Ca and Ba:Ca ratios of transects across otoliths from core to edge of a random sub-set of Australian bass collected from NSW waters of the Snowy River. (a) 102 mm, (b) 102 mm, (c) 109 mm and (d) 103 mm.



**Figure 6.5.** Otolith Sr:Ca and Ba:Ca ratios of transects across otoliths from core to edge of a random sub-set of Australian bass collected from VIC waters of the Snowy River. (a) 482 mm, (b) 485 mm, (c) 444 mm and (d) 470 mm.



**Figure 6.6.** Otolith Sr:Ca and Ba:Ca ratios of transects across otoliths from core to edge of a random sub-set of Australian bass collected from VIC waters of the Snowy River. (a) ~250 mm, (b) ~250 mm, (c) 290 mm and (d) 101 mm.

#### 6.4. Discussion

Otolith microchemistry was examined in this current study to make inferences about the origin of Australian bass in the Snowy River. Sr:Ca ratios at otolith core suggest that juvenile Australian bass throughout the lower Snowy River have a similar early life history to mature lower Snowy individuals. As Sr is generally more available for uptake in otoliths in saltwater (Gillanders, 2005), it is likely that these two groups both originate from a marine environment. The non-significant interaction in mean Sr:Ca ratios between juveniles Snowy NSW and mature lower Snowy is difficult to explain, however one possible explanation is that both 'groups' were likely to be in environments consisting of high salinities during early life (i.e. originating from a hatchery or recruiting in an estuary). An important finding was that fish collected from NSW waters of the Snowy River, 'suspected as being stocked fish from the Narooma hatchery', were found to have significantly different Sr:Ca and Ba:Ca ratios compared to fish collected elsewhere. This finding gives confidence that this approach of stock identification has merit if further stock identification is required in the upper Snowy River.

Mean Ba:Ca ratios suggested that the juveniles from Snowy (VIC) and juveniles Snowy (NSW) were most similar, however, a significant difference was detected between these groups. Therefore, the application of Ba:Ca ratios may have great potential use for separating sources of Australian bass in the Snowy River. However, achieving this would be less difficult if all stockings were occurring from one single hatchery facility and more information was known about the success of natural recruitment in the Snowy River. Previous studies have found that Ba could be reliably used to determine if fish were reared in freshwater or saltwater (Pender and Griffin, 1996; Gillanders, 2005). Findings from this study indicate mature lower Snowy fish have increased levels of Ba:Ca ratios at the otolith core compared to fish collected elsewhere in the Snowy, suggesting this group is from a different source. This finding will be useful for further comparisons of otolith core ratios from the Snowy River as it may be used as a benchmark.

Otolith transitional analysis confirmed juvenile Australian bass collected from the upper Snowy River (NSW waters) appear to be originally from an environment high in salinity (high Sr:Ca ratios and low Ba:Ca ratios) and later in life in a more freshwater environment (increased Ba:Ca ratios and reduced Sr:Ca ratios) (Figure 6.4). This finding is consistent with previous assumptions that any Australian bass collected in the NSW waters of the Snowy River would most likely have originated from a hatchery environment (i.e. stocked fish) and not be wild individuals (based on NSW DPI, unpublished data). Among the samples examined, this finding is consistent (Figure 6.4) as fish that originally reside in saline water then move to freshwater environments will consistently demonstrate increased Ba:Ca ratios concurrently with decreased Sr:Ca levels (Gillanders, 2005).

There was evidence to suggest frequent movement between freshwater and marine environments in mature-sized fish collected from the lower Snowy River. Considering the mature-size of the fish examined, these observations were typical for this catadromous fish species (Harris, 1988). The migratory patterns have also recently been examined in other Australian riverine fishes in a study which confirmed undescribed lifecycles for five species (Miles *et al.*, 2009).

Examination of juvenile-sized Australian bass collected from the lower Snowy River (VIC waters) suggested that movements between fresh and marine environments occurred throughout the life of these fish (Figure 6.6). Downstream movement from freshwater reaches

to the estuary occurs prior to spawning which takes place in brackish estuarine waters for Australian bass (Harris, 1986). More movement between freshwater and marine environments were observed in the larger fish (Figure 6.6 a-c) compared to the smaller fish (Figure 6.6 d) among the juveniles collected from the lower Snowy River. These observations correspond with the size at maturity when downstream migration may occur at 3+ years for males and 5+ or 6+ years for females. This means that migration patterns would be more expected in larger fish (e.g. 400-500 mm) compared to smaller fish given that maturity would not be expected until at least 200-300 mm (Harris, 1987), assuming spawning activity is the motivation for this movement.

## **6.5. Conclusion**

Otolith microchemistry proved to be a useful tool for inferring potential sources of Australian bass throughout the Snowy River by examining chemical ratios at otolith cores in this current study. Based on the current study, it would be possible to use otolith microchemistry to determine wild from stocked Australian bass from the Snowy River, by the use of otolith core Sr:Ca and Ba:Ca ratios.

Examination of otolith chemistry at core-to-edge demonstrated the ability to investigate life-history of Australian bass collected from the upper Snowy River (i.e. fish originating from the Narooma hatchery). Additionally, this analysis showed evidence of Australian bass movement throughout the lower Snowy River based on elemental ratios. Furthermore, these results indicate that the Australian bass population in the lower Snowy River are somehow 'triggered' to perform these movements, suggesting environmental conditions in the Snowy River promote migration in and out of freshwater and saltwater areas.

New information presented here is essential for future fisheries management of the Australian bass population in the lower Snowy River as Ba:Ca and Sr:Ca ratios suggests that the population of Australian bass in the lower Snowy River may not be essentially reliant on stocking as some natural recruitment may be occurring. However, evidence suggests that the upper Snowy River is heavily reliant on stocking.

## 7. CONCLUSIONS

### 7.1. Survival of Australian Bass

Short-term survival following each Australian bass stocking event in 2007, 2008 and 2009 were evident. Sampling recovered fish from both high and low stocking density reaches and also from non-stocked reaches suggesting possible dispersal from stocking sites. Fish surveys indicate that residency of stocked Australian bass within the two stocked reaches appeared limited as fish were only collected until the following winter (up to nine to 10 month post-stocking). These observations either suggest low survival or dispersal from the stocking sites. Mean growth of Australian bass until winter compare with growth in other areas (Harris, 1987) and fish were also of sufficient condition but decreased during winter.

To investigate the low residency times or possibly low survival, a series of experimental tests were performed to determine thermal tolerances of Australian bass to sudden temperature changes. Upland reaches of the Snowy River experience a wide thermal range over the diel period, particularly at high altitudes (fish were stocked at altitudes of between 252 and 760 m). A series of experiments confirmed hatchery-reared juvenile Australian bass have a precise critical thermal range. Findings from these experiments indicated that irrespective of acclimation temperatures and the rates of which water temperature declines, temperatures below 6°C are likely to result in loss of equilibrium (LOE) which may ultimately lead to death within hours and most certainly within several days. Of the four treatments used during experiments, one sought to replicate thermal regimes in the upper Snowy River (8°C acclimation followed by a 1°C.d<sup>-1</sup> decline in water temperature). All experimental fish experienced either instant or delayed mortality under these conditions.

Little evidence was found of predation on stocked Australian bass by other fish species. Considering the size at stocking of Australian bass was quite small and sampling showed that there are populations of particularly large eels (over 900 mm) and brown trout (over 400 mm) in the Snowy River, it may have been expected that larger quantities of stocked fish would be consumed by these large potential predators. Other fish such as Australian smelt and brown trout were positively identified from the stomachs of a number of fish but only three Australian bass were identified from all samples examined suggesting that predation may not be a significant factor influencing these stocking efforts.

### 7.2. Natal origin and movement of Australian bass

Examination of Australian bass otoliths at the core collected from lower Snowy River (VIC waters) confirmed that the natal origin of juvenile sized and mature sized fish from this region were statistically similar based on Sr:Ca ratios suggesting that the juvenile sized fish examined are possibly wild recruits. Ba:Ca ratios (and Sr:Ca ratios to a lesser extent) proved useful in separating fish from different natal origins from the Snowy River. Fish collected from NSW waters of the Snowy River, 'suspected as being stocked fish from the Narooma hatchery', had significantly different Sr:Ca and Ba:Ca ratios compared to fish collected at other locations suggesting that this approach of stock identification is reliable and could be used for further stock identification is required in the upper Snowy River if required.

Otolith microchemistry was also performed to assess Australian bass movement between freshwater and marine environments. Analysis of otoliths showed fluctuating Sr:Ca and



Ba:Ca ratios, Australian bass throughout the lower Snowy River move between freshwater and saltwater environments frequently. These findings are consistent with the catadromous lifecycle for this species. Results also confirmed that Australian bass collected from the upper Snowy River (NSW waters) originated from an environment much higher in salinity. These findings support the prior assumption that any individuals collected from this section of the river of the Snowy River would have originated from a hatchery environment where they would have been held in saline water prior to release.

### **7.3. Impacts on resident fish fauna**

Differences in fish community structure were identified between high and low density stocking reaches. More Australian bass were collected from the high density stocking zone. However, the abundance of Australian bass did not account for a large majority of observed differences. The high density reach actually contained lower relative abundances of Australian smelt and Congolli, and higher abundances of salmonids and eels. The high and low density reach was separated by Snowy Falls, a substantial barrier to fish migration and likely prevented the colonisation of upstream reaches by the two smaller species. It appeared that the presence of the Snowy Falls played a more substantial role in structuring fish communities than the stocking activities. Collection of before data, and comparison with data collected after stocking would have been useful to confirm this observation.

Dietary analysis of six co-existing fish species of the upper Snowy River revealed that there were considerable differences in the overall diet among species. Most fish species were found to possess feeding strategies similar to those previously reported in other areas. Dietary overlap between stocked Australian bass and other fish species was low in general suggesting that competition with other fish species which occur in the upper Snowy River is unlikely to impact on the success of Australian bass stockings in the Snowy River. Australian bass were found to have one of the least varied diets by number of different prey items found, however common prey items (aquatic insects and crustaceans) were similar to previous studies for this species (Harris, 1985).

In order to determine the effectiveness of stocking programs, the ability to distinguish between wild and stocked fish is essential (Mohler, 1997). Understanding this ratio can help provide information on whether stocking is required at all. If high numbers of recovered fish are wild, this suggests that fish may be spawning and recruiting adequately. Recovery of large numbers of stocked fish could indicate poor wild recruitment or high competition from stocked fish. Determining impacts of stocked fish on resident populations therefore requires a robust technique to distinguish between the two groups but there are few methods which have been validated for Australian bass. During this study, experiments were undertaken to assess the suitability of calcein to chemically-mark juvenile Australian bass. Experiments showed that Australian bass could be chemically-marked without increased mortality or a reduction in growth rates. These findings are important for future stocking programs. Along with survival and growth rates post-marking, detection of marked from unmarked fish were assessed. Results indicated that this chemical could be used to mark fish prior to stocking and with the use of a handheld detection unit, accurately distinguishing marked (stocked) fish from non-stocked fish (non-stocked) would be possible.

### **7.4. Recommendations for future stocking activities**

Results from the CTMin study suggest that environmental conditions may not support the establishment of an Australian bass 0+ population in the upper Snowy River. Based on the results of these experiments and historical temperature data for the upper Snowy River which suggests every winter is somewhat similar in water temperature, further stockings of fry

should be undertaken in areas where this species has the ability to withstand winter water temperatures, possibly in the lower Snowy River at lower altitudes and higher minimum water temperatures. As a precaution, stocking of 0+ Australian bass should not occur in areas where water temperatures fall to below 6°C.

Fish stocked from the initial event would now be approaching maturity at four years of age. These fish may be activity dispersing into upper freshwater reaches and also preparing to migrate into estuarine reaches to spawn. Anglers are beginning to provide anecdotal reports of larger fish captures from downstream reaches. It would be useful to perform additional collections, combined with fish ageing, to determine age and growth information on the population to determine the time the time post-stocking that these fish enter the fishery at a catchable size. Furthermore, additional sampling efforts in the upper Snowy (i.e. Snowy Falls upstream to below Jindabyne Dam) would be useful to determine the population size and abundance throughout this high-altitude zone where Australian bass never naturally existed. This information would assist decision makers involved in further stockings whether or not to attempt further stockings throughout this reach identified as being too cold from the results of the CTMin experiments.

Further investigations are necessary to determine growth and survival of calcein-marked fish over a longer time period than several months. Additionally, it would be beneficial if the GFP meter could positively distinguish marked from unmarked fish for a much longer period of time, such as several years. Unfortunately a longer-term study was outside the scope of this project, but is highly recommended that future experiments are undertaken under field (natural) conditions as marking techniques are now developed. If further studies result in similar findings for growth and survival and the GFP meter is found to accurately distinguish calcein-marked fish over a period of years, future stockings of Australian bass into the Snowy River should include calcein-marking of fish if monitoring of these stocks is intended. This would make it easier to answer research questions which aim to study migration within the Snowy River and provide the ability to determine the amount of natural recruitment in the Snowy River.

Australian bass were stocked into the upstream migration reach during the 2008 stockings (and a gain in 2009) which confounded part of the experimental design of this study. As a result, it is not possible to positively report on the presence of upstream migration of any stocked fish from either of the two stocked reaches. If the proposed experimental design was followed it would have been possible to report on any upstream migration and this information would have added a significant amount of value to the current findings of the sampling program as stocked fish which “disappeared” during the winter months may have moved upstream in search for more suitable conditions. It is recommended that if further stockings are to occur, and determining movement patterns of stocked fish is of interest, that suggested stocking strategies are strictly followed to maintain the integrity of subsequent experimental designs.

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## 9. APPENDIX 1: FUTURE RESEARCH SUGGESTIONS

### **PROJECT: Field trial of calcein for marking hatchery-reared fish and the GFP meter for detection.**

#### *Current knowledge of calcein and its potential for use*

A number of previous studies have assessed the suitability of calcein for producing a distinguishable mark on fish to allow for post-stocking detection. However, published literature which has assessed the use of calcein for marking Australian native fish species is lacking. Millions of hatchery-reared Australian native (and exotic) fish are stocked annually in south-eastern Australia. Currently, few programs exist which assess the success of these stockings. Therefore, the success of the majority of stockings remains unknown. One reason for this is a lack of trialled and developed methods to allow mass-marking of large numbers of hatchery-reared fry and fingerlings simultaneously prior to stocking. Without such methods, differentiating between wild and stocked fish post-stocking is difficult via non-lethal methods.

This study found that calcein could be used to chemically-mark Australian bass fry and fingerlings without any increased mortality or reduction in growth rates compared to unmarked fish. Additionally, marking methods used during this study allowed for detection of marked fish for a period of up to four months. However, Australian bass are a long-lived species of at least 20 years and many studies which aim to gauge the success of stocking will include monitoring of stocked populations over a period of at least several years. Therefore, a marking method which allows detection of stocked fish for a period of several years would have endless benefit.

#### *Suggested project*

In order to fully assess calcein and its ability to be a reliable marking method to detect stocked from wild fish, a long-term field trial is required. Any such trial should assess if detection of calcein marked fish in the wild is possible over a period of several years and determine if the detection of calcein marked fish is reduced overtime with the use of the GFP meter.

The Snowy River would be an ideal location to stock calcein-marked Australian bass and undertake any such study, as currently, no calcein-marked Australian bass exist within the system. Furthermore, if future stockings of Australian bass are completed in the Snowy, a proposed study such as this one, could augment any monitoring efforts of the stocked population which would ultimately reduce the cost of this work. With a known-age population of calcein-marked Australian bass stocked into the Snowy River, it becomes less complicated to study Australian bas migration and movement throughout the Snowy River.

#### *Opportunities for linkage or collaboration*

As stocking of Australian bass is undertaken in NSW and VIC reaches of the Snowy River, there are opportunities to link any such study with stockings in either state. Regardless of the stocking locations of calcein-marked fish, it would be possible to answer key research questions and provide scientific data on fish migration upstream or downstream of the stocking sites.

*Project objectives*

- To stock calcein-marked Australian bass into the wild (Snowy River or elsewhere).
- To regularly monitor these stocks using standard fisheries sampling techniques.
- To determine whether calcein-marked fish are detectable via non-lethal methods for a period of at least 2-3 years.
- To assess if detection capabilities (mean ticks of fluorescence per fish) reduces significantly over time.

*Key tasks*

- Hatchery-rear Australian bass fry or fingerlings (suggest collaboration with Narooma Aquaculture for the supply of any such fish).
- Calcein mark these fish using the methods optimised during this study.
- Stock fish in a pre-selected location.
- Collect calcein-marked fish post-stocking and use the GFP meter to measure ticks of fluorescence.
- Report these findings

*Anticipated outcomes*

This proposed project would augment the work previously completed to assess the suitability of calcein for chemically marking hatchery-reared Australian bass fry and fingerlings under laboratory conditions. A field study using a catadromous Australian native fish species would be a pioneer study and highly original work. Ideally, this proposed work would be completed in conjunction with a future stocking event of Australian bass in the Snowy River.