COMPARATIVE TOLERANCES OF NON-INDIGENOUS BRIDLED GOBY AND NATIVE EXQUISITE GOBY TO SALINITY, TEMPERATURE AND SEDIMENT

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Why do we love the sea?

It is because it has some potent power to make us think things we like to think...

(Robert Henri)

ABSTRACT

The Australian bridled goby (*Arenigobius bifrenatus)* has been in New Zealand since 1996, and has subsequently spread to thirteen estuaries and the coastal Otara Lake. The native exquisite goby (*Favonigobius exquisitus*) may be displaced or threatened by the bridled goby as they share estuarine habitat. The ecophysiological tolerances and sediment preferences for both goby species were examined to determine the potential for niche overlap and habitat selection.

The results of acute salinity and temperature tolerance tests supported the hypotheses that bridled gobies are more tolerant than exquisite gobies to extremes of salinity and temperature. The incipient ten day LT_{50} values were 6.7°C and 11.8°C for bridled and exquisite gobies, respectively, however, both tolerated temperatures up to 35°C. Both species showed some mortality at low salinities, but mortality did not exceed 50% for either species at the lowest salinity tested (2.2 ppt) after 96 h. Bridled gobies displayed a strong preference for fine sediment (<63 μm), whereas exquisite gobies were less selective, accepting a broad range of sediment grain sizes (63-250 μm).

Bridled gobies have successfully established and dispersed in New Zealand, and their ecophysiological adaptations that allowed them to survive harsh conditions in ballast water, their presumed introduction vector, will likely aid their future spread throughout New Zealand. Their increasing abundance is likely to see them encounter and possibly encroach on the habitat of exquisite gobies. Bridled gobies may displace the smaller exquisite gobies from optimal habitats, as exquisite gobies are unlikely to survive competition from and predation by bridled gobies. However, exquisite gobies should be able to relocate to adjacent habitat with coarse sediment that is unsuitable for burrow construction by bridled gobies. Additionally, acclimatisation to local environmental conditions may extend the tolerance limits determined in this thesis, and may allow bridled gobies to spread to upstream zones in estuaries and occupy freshwater.

New Zealand presently has eight recognised gobiid species of which one is endemic, two are native, two are not recorded in mainland waters and three are proposed as non-indigenous. Additionally, several tropical and subtropical gobies exist in the aquarium trade. Similar ecophysiological tests of tolerance and preference may determine the possibility that these species could establish in the wild following accidental release.

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1.1 GENERAL INTRODUCTION

1.1.1 PROJECT DESCRIPTION

The non-indigenous Australian bridled goby, *Arenigobius bifrenatus* (Kner 1865), was found in New Zealand in 1996 (Gray Jamieson Holdings Ltd, 1996). Research on bridled gobies in New Zealand has so far determined the transport vector (Willis *et al.*, 1999), frequency of introductions (Lavery *et al*., 2006) and ecology (Usmar, 2003). Bridled gobies may pose a threat to the native exquisite goby (*Favonigobius exquisitus,* Whitley 1950), as the two species share habitat (Francis *et al*., 2003). This thesis adds to the current knowledge on bridled gobies by establishing ecophysiological tolerances, sediment preferences and potential impacts on biodiversity, in comparative tests with exquisite gobies.

1.1.2 AIM

The aim of this thesis was to determine the extent of potential niche overlap between non-indigenous bridled gobies and native exquisite gobies. This was achieved in comparative tests on bridled and exquisite gobies by measuring the acute salinity and temperature tolerances, and sediment preferences.

1.2 THESIS OUTLINE

This first chapter discusses relative components of biological invasions, describing non-indigenous marine species (NIMS), transport vectors and the characteristics of New Zealand estuaries that allows for the successful establishment of NIMS. Following this is information on the goboidei suborder, New Zealand's gobies and previous research on bridled and exquisite gobies. This chapter concludes with the hypotheses of my research.

Chapter 2 on the general methods describes study sites, collection methods and laboratory conditions. The salinity and temperature tolerance experiments in Ecophysiological Experiments in Chapter 3 and sediment preference experiments in Preference Experiments in Chapter 4 each contain an introduction, methods, results and discussion.

The conclusion examines the significance of all my findings and reviews what new knowledge has been provided about bridled gobies in New Zealand. Finally, recommendations for future work will discuss research still needed on the bridled goby.

1.3 BIOLOGICAL INVASIONS

1.3.1 NON-INDIGENOUS MARINE SPECIES (NIMS)

> "*Biological invasions are changing the structure and function of the earth's ecosystems*" (Ruiz and Carlton, 2003, p459).

Biological invasions are "an immense, insidious and usually irreversible" (IUCN, 2000) threat to global biodiversity and affect all habitat types in all parts of the world (Wilcove *et al.*, 1998; Stachowicz *et al.*, 1999; Sakai *et al.*, 2001; Williamson *et al.*, 2002; Kolar, 2004). Coastal ecosystems are heavily invaded (Grosholz, 2002) and non-indigenous marine species (NIMS) are one of the top five threats in marine environments (other threats are habitat loss, overharvest, pollution and disease) (Wilcove *et al*, 1998; Hewitt *et al.*, 2004). The impacts of NIMS can range from having no effect to causing serious environmental or economic damage (Simberloff and Von Holle, 1999). NIMS will not harm a new ecosystem if they do not establish, are out-competed, or fill an unoccupied niche (Sakai *et al.*, 2001). However, NIMS can harm biodiversity (by competing with native species for food, habitat and other resources), ecosystem function, industries (e.g. international trade, fisheries and aquaculture), economy (government spending on biosecurity, pest management and human health care) and human health (e.g. shellfish toxins) (Ruiz *et al.*, 1997; Sakai *et al.*, 2001; Williamson *et al.*, 2002; Ruiz and Carlton, 2003; Inglis *et al.*, 2006). For example, the exotic sea squirt, *Ciona intestinalis,* is known to decrease biodiversity (Blum *et al*., 2007; Howes *et al*., 2007) and has damaged the mussel aquaculture industry in the Marlborough Sounds by smothering mussels (Taylor, 2001). This extent of damage is typical of NIMS because of their ecophysiological adaptations. Sakai *et al*. (2001) identified that most invading species possess a greater ability to dominate resources compared with native and non-invading species.

The process of establishment includes subjection to harsh transport conditions, dissimilar habitats, new competitors and new food sources. During dispersal NIMS may be subjected to extreme temperatures, varying salinity, low food availability and desiccation. Strategies to survive transport include behavioural (seeking refugia to remain moist), reproductive (diapausing life stages) and ecophysiological (metabolic depression). These strategies also provide a competitive advantage in a new environment (Sakai *et al*., 2001). Having endured the harsh conditions of transport and establishment, NIMS are generally tolerant to extreme environments (Sakai *et al*., 2001). The extent of NIMS impact is correlated with their tolerance of environmental conditions and determines the way they are categorised and managed.

NIMS and numerous other terms are used inconsistently and interchangably to categorise non-indigenous organisms, although there is some agreement on the following: *pest* describes a harmful organism; *invasive* implies the organism threatens biodiversity; *introduced* organisms have been relocated by human vectors either intentionally or accidentally; *adventive*, *alien* and *exotic* organisms are of foreign origin (Williamson *et al*., 2002; Ruiz and Carlton, 2003). "*Unwanted organism* means any organism that … is capable or potentially capable of causing unwanted harm to any natural resources" (Part I, Section 2, Biosecurity Act 1993, NZ). These categories determine the necessary biomanagement strategy.

To date, bridled gobies have not been officially classified as either a pest, invasive or unwanted organism in New Zealand. However, out of 148 NIMS in New Zealand, only the sea squirt, *Styela clava*, is currently recognised as an unwanted organism (Biosecurity NZ, 2007). Willis *et al.* (1999) described bridled gobies as adventive as they meet at least five of nine criteria to test for Adventism, developed by Chapman and Carlton (1991) and modified by Chapman and Carlton (1994). The first six criteria are applied in a national situation and the final three in a global situation. The following list shows the nine criteria, with * marking the five criteria met by bridled gobies in New Zealand.

1. Appearance in local regions where not found previously. *
2. Initial expansion of local range subsequent to introduction. *
3. Association with human mechanism(s) of dispersal. *
4. Association with (co-exist) or dependency on (prey) other introduced species.
5. Prevalence on or restriction to new or artificial environment(s).
6. Relatively restricted distribution on a continent compared to distributions of native species.
7. Isolated populations on different continents or in isolated oceans.
8. Insufficient active and passive dispersal capabilities to account for the observed distribution of the species. *
9. Exotic evolutionary origin. *

Criterion 3 is assumed to be valid based on three factors. Firstly, the distance that bridled gobies would have travelled independently is excessive, being transferred from Australia on the East Australian Current, then down the eastern coast of New Zealand on the East Auckland Current (EAUC) (Usmar, 2003). Secondly, dispersal by currents suggests that bridled gobies would have been transported past northern New Zealand and the Coromandel Peninsula, such prominent headlands are common establishment points, yet they have not established there despite vast areas of inhabitable mud flats (Francis *et al*, 1999). Finally, in Australian estuaries, bridled gobies occupy mid and upper regions of estuaries, and larvae are unlikely to exit their resident harbours (Neira *et al.*, 1992). It is therefore highly probable that bridled gobies entered the ballast or sea chests of ships within Australian estuaries and were released in New Zealand waters (Willis *et al.*, 1999).

1.3.2 BIOSECURITY

> *"Although coastal estuarine and marine systems are among the most heavily invaded systems in the world, the study of non-indigenous species in these systems has, historically, lagged behind that of terrestrial and freshwater systems"* (Grosholz, 2002, p22).

Biosecurity is a wide field of study and research is increasing (Ruiz and Carlton, 2003). Research in biosecurity fields include pre-empting invasions, controlling the spread of NIMS, protecting important species, monitoring community responses to NIMS, studying changes to biodiversity, the native habitat of NIMS, transport vectors, ecophysiological adaptations, characteristics of the invaded environment, determining if repeated introductions are occurring, what the transport conditions are, and the behaviour of introduced species (Cranfield *et al*., 1998). Biosecurity research provides important information to on population biology, as concentrated evolutionary and genetic changes can occur in both non-indigenous and native species in response to NIMS invasions (Sakai *et al*., 2001). Three crucial stages exist in the process of a successful invasion; introduction into a new habitat, colonisation and establishment, and dispersal to secondary habitats (Sakai *et al*., 2001). Wonham *et al*. (2000) stated that research should concentrate on each stage of invasion to manage biosecurity, so that funding can be appropriately targeted (Kolar, 2004).

The International Maritime Organization (IMO) administers marine biosecurity within the United Nations (Gollasch *et al*., 2007; International Maritime Organization, 2007). New Zealand has additionally ratified or joined a number of international regimes to manage hazards related to NIMS and over 11 government agencies are associated with NIMS remediation (Williamson *et al*., 2002).

The major means of biosecurity for ships is to flush ballast tanks outside New Zealand's Exclusive Economic Zone (EEZ), unless it is unsafe to do so, or to use freshwater ballast (Williamson *et al*., 2002, Coutts and Taylor, 2004). Despite this, 4.4 million mt of ballast water was released within New Zealand's EEZ in 2002 (Hewitt *et al*., 2004). Flushing of ballast tanks with sea water is imperfect for two reasons. Firstly, full flushing is impossible because animals can occupy crevices and support structures in the hull and tanks that are protected from flushing (Ricciardi and Rasmussen, 1998; Rigby, 2001). Also, many invasive organisms are tolerant to extreme environments and may survive short term immersion in sea water or freshwater (Ricciardi and Rasmussen, 1998). Alternative transport vectors are hull fouling and sea chests. Hull fouling is the major vector for NIMS to enter New Zealand (Cranfield *et al*., 1998), despite anti-fouling paint on hulls. Sea chests are compartments that reduce cavitation for water entering the engine and ballast tanks. Sea chests are protected from high water flow by the grate covering (holes are greater than 15 mm) and the interior of sea chests often lack anti-fouling paint. Downward facing sea chests can take in benthic sediment and organisms, which can be released in a new location (Coutts *et al*., 2003).

Biosecurity research is vital to managing biodiversity, fisheries and human health. In New Zealand four key areas are addressed. Firstly, knowledge of existing biodiversity is needed to compare with the biodiversity following an invasion (Lavery *et al*., 2006). Current marine biodiversity is documented but genetic taxonomy is lacking for most species. Secondly, it is important to be aware of the organisms that are likely to invade. International NIMS are well recorded, and the Ministries of Biosecurity, Fisheries, and Agriculture and Forestry advise what organisms should be looked for. The major sources of adventive species are 11% from Japan / East Asia, 11% from tropical Indo-Pacific and 10% from South Australia / Tasmania (Cranfield *et al*., 1998). Thirdly, monitoring is needed to detect new species. New Zealand ports are monitored to detect new NIMS, as part of New Zealand's agreement with the International Maritime Organisation (IMO) (Wotton, 2001). Initially the Ministry of Fisheries conducted port surveys, but now Biosecurity New Zealand employs NIWA to conduct baseline surveys (Inglis *et al*., 2006). Hydrodynamic models to predict larval movement in eight harbours are being created (Inglis, 2002). The susceptibility of ecosystems to invasion varies and no conclusive information is available on what creates a resistant community (Sakai *et al*., 2001; Paavola *et al*., 2005), therefore, all ports need to be monitored. Finally, it is important to have management strategies in place to control NIMS. The Biosecurity Act 1993 advises what is to occur and by who, in the event of a new NIMS encounter. The choice of management strategy is dependent on the NIMS classification (e.g. pest, unwanted organism).

Despite the above research fields, at least 148 exotic marine species exist in New Zealand (Cranfield *et al*., 1998) but the impacts of most of these are unstudied (Ruiz *et al*., 1997; Francis *et al*., 2004b). In addition to non-indigenous gobies (Section 1.5), there are many other non-indigenous teleosts in New Zealand waters. Cranfield *et al*. (1998) reported five non-indigenous marine teleosts in New Zealand waters. The diadromous Chinook salmon (*Oncorhynchus tshawytscha*) was intentionally introduced in 1870 and is now established as a valuable sport and aquaculture species. Four non-indigenous marine fish were unsuccessful at establishing in New Zealand. *Abudefduf vaigiensis* (Indo-Pacific sergeant) was unintentionally introduced in 1975, *Engraulis japonica* (anchovy) and *Sardinops melanostica* (sardine) were released in 1981 and *Scophthalmus maximus* was intentionally introduced in 1913 (Cranfield *et al*., 1998). Australian oyster blennies (*Omobranchus anolius*) were first confirmed in New Zealand in 2003 and appear to either be breeding successfully or have undergone multiple introductions (Francis *et al*., 2004a).

Francis *et al*. (1999) described five non-indigenous fishes new to New Zealand waters, five fishes new to mainland New Zealand and officially confirmed the existence of five non-indigenous fishes in New Zealand (**Table 1.3.1**). He also noted new observations of 20 species that had previously been reported and 20 further fishes have been observed but are not confirmed as established.

Table 1.3.1. Non-indigenous marine fishes in New Zealand. (BoI, Bay of Islands; MI, Mokohinau Island; PKI, Poor Knights Islands; PKT, Poor Knights Terrace).

Species	**Year first observed**	**Location**
First recording in New Zealand		
Abudefduf vaigiensis (Quoy and Gaimard 1825)	1996	BoI
Aluterus scriptus (Osbeck 1765)	1999	Mayor Island
Chromis flavomaculata (Kamohara 1960)	1997	BoI
Coris dorsomacula (Fowler 1908)	1999	BoI
Platax teira (Forsskal 1775)	1999	BoI
First recording in mainland New Zealand		
Chromis vanderbilti (Fowler 1941)	1996	BoI
Chrysiptera rapanui (Greenfield and Hensley 1970)	1984	PKI
Forcipiger flavissimus (Jordan and McGregor 1898)	1998	PKI
Parma kermadecensis (Allen 1987)	1997	PKI
Thalassoma lutescens (Lay and Bennett 1839)	1996	PKI
First official recording of establishment in New Zealand		
Acanthocybium solandri (Cuvier 1831)	1994	BoI, Tutukaka, BoP, PKI and North Cape
Aseraggodes bahamondei (Randall and Lelendez 1987)	1995	PKI
Carcharhinus longimanus (Poey 1861)	1988	PKT
Etelis carbunculus (Cuvier 1828)	1984	Three Kings
Istiophorus platypterus (Shaw and Nodder 1791)	1996	Tutukaka Harbour

Sea surface temperatures that are warmer than average positively correlate with new fish findings in New Zealand, and this occurred most notably during 1969-75, 1988-90 and in the autumns of 1996, 1998 and 1999 (Francis *et al.*, 1999). Non-indigenous pelagic oceanic fishes are usually adults when found in New Zealand, so it is believed they migrate by swimming. Species that occur with floating debris (e.g. *Platax teira* and *Aluterus scritpus*) are capable of dispersing vast distances by drifting. Tropical fish larvae are transported by the East Auckland Current (EAUC), and onshore winds or Ekman transport transfer larvae from the EAUC to land, with the most prominent settlement areas being the Poor Knights Islands and outer Bay of Islands. Norfolk Island shares a large proportion of New Zealand's non-indigenous fish, and is thought to be a likely source of non-indigenous fishes, whereas the introduction of fish larvae from the Kermadec Islands is a rare event. The pattern of invasion in New Zealand, that is invasion from tropical to temperate regions by juvenile or larval fishes, is mimicked around the globe (Francis *et al*., 1999).

It is clear that the abundances of introduced marine fishes are increasing; but it is unknown whether the cause of introduction is human mediated or natural, or even whether an increase in field studies, scuba-diving, and fishing is finding species that have been long established (Francis *et al*., 1999; Francis *et al*., 2004b).

1.3.3 VECTORS

Marine invasions are becoming more common due to frequent international trade and travel (Wonham *et* al., 2000; Ruiz and Carlton, 2003; Coutts and Taylor, 2004; Wotton and Hewitt, 2004; Inglis *et al*., 2006), yet few countries are effectively managing the threat of NIMS (Williamson *et al*., 2002). NIMS transport vectors are well described (**Table 1.3.2**) (Coutts *et al*., 2003; Ruiz and Carlton, 2003; Coutts and Taylor, 2004; Inglis *et al*., 2006). Cranfield *et al*. (1998) reported that the proportions of NIMS occurring on various transport vectors are ship fouling (69%), ballast water (3%), either fouling or ballast water (21%) and sand ballast (3%). Natural introductions by migration are possible but tend to occur at small spatial scales (Willis *et al*., 1999; Ruiz and Carlton, 2003).

Table 1.3.2. Anthropogenic vectors for marine introductions (Williamson et al., 2002 from Carlton, 2001). New: New international introduction. Dom: Domestic (local) spread.

Source	**Vector**	**Target taxa**	**Donor region**
Commercial shipping	Ballast water	Plankton, nekton, benthos in sediment	New / Dom
	Hull fouling	Encrusting, nestling, and some mobile species	New / Dom
	Solid ballast (rocks, sand, etc)	Encrusting, benthos, meiofauna and flora	New / Dom
Aquaculture and fisheries	Intentional release for stock enhancement	Single species (plus associated species)	New / Dom
	Gear, stock or food movement	Various	New / Dom
	Discarded nets, floats, traps, trawls, etc.	Various	New / Dom
	Discarded live packing materials	Various	New / Dom
	Release of transgenic species	Single species	New / Dom
Drilling platforms	Ballast water	Plankton, nekton, benthos in sediment	New / Dom
	Hull fouling	Encrusting, nestling, and some mobile species	New / Dom
Canals	Movement of species through locks due to water motion or active swimming	Various	New
Aquarium Industry	Accidental or intentional release	Aquarium fauna and flora	New / Dom
Recreational boating	Hull fouling	Encrusting, nestling, and some mobile species	Dom
Dive practices	Snorkelling and scuba gear	Algal spores, bacteria, some small mobile species	New / Dom
Floating debris	Discarded plastic debris	Encrusting and some mobile species	New / Dom

1.3.4 ESTUARIES AS ESTABLISHMENT AREAS FOR NIMS

Estuarine ecosystems are gateways between freshwater and fully marine systems (Dame and Allen, 1996; Harrison and Whitfield, 2006). Estuaries are contradictorily described as both stressful environments due to habitat fluctuation, and safe havens for juvenile fishes (Sakai *et al.*, 2001; Atrill and Power, 2002; Atrill and Power, 2004; Lam *et al.*, 2005). The variability of estuarine conditions means few species are resident, and as such, New Zealand estuaries are species depauperate and readily susceptible to the effects of NIMS. For the few resident species (Neira *et al.*, 1992), estuaries offer warm waters, high food availability, many nutrients and protection from predatory fishes in shallow waters or mangrove areas (Mumby *et al.*, 2004; Francis *et al.*, 2005). However, desiccation and terrestrial predation by birds are high risk factors (Laws, 1998).

New Zealand estuaries are well known as nursery habitats for juvenile marine fish (Francis *et al*., 2005), as well as feeding grounds for diadromous fish larvae (e.g. inanga (*Galaxias maculatus*), smelt (*Retropinna retropinna*), common bully (*Gobiomorphus cotidianus*) and banded kokopu (*Galaxias fasciatus*) (McDowall, 1995). Many of these fish are commercially or ecologically important. Bridled gobies have been found in freshwater (Hoese and Larson, 1980; Gee and Gee, 1991; Neira *et al*., 1992) and their invasion has the potential to negatively impact such native freshwater fishes.

Geographic features of New Zealand estuaries that aid larval settlement include harbour bars, barrier islands, spits and breakwaters, which lessen water current and reduce the loss of individuals to the sea. Because of this, new populations are able to settle in high densities (Cranfield *et al*., 1998) and propagule pressure is the most influential factor for a successful invasion to occur (Kolar, 2004). It is likely that the planktonic larval stage of bridled gobies were released from Australian ships and settled in estuaries with low water movement (Willis *et al*., 1999; Usmar, 2003).

Abiotic factors are more likely than biological factors to affect the distribution of estuarine organisms because of the dynamic physical and chemical characteristics of estuaries (Dunson and Travis, 1991; Schofield, 2004; Lam *et al*., 2005). Estuarine variability favours NIMS because they have previously encountered harsh conditions, whereas naturalised species are adapted to local conditions. Estuarine stressors include salinity, temperature, oxygen, pH, wind stress and desiccation (Lam *et al*., 2005). Given the large range of available environments and the ecophysiological adaptations of NIMS, it is likely that NIMS can readily establish in the low diversity of estuaries despite variable conditions (Neira *et al*., 1992). In fact, variable environments may increase the phenotypic plasticity of NIMS populations, allowing them to adapt more effectively in new habitats (Lam *et al*., 2005). NIMS are likely than naturalised species to survive intense environmental change such as storm events that increase salinity, decrease temperatures and erode sediment (including habitat and food sources), remove nutrients, and cause death or displacement of individuals. Following intense environmental change, colonisation requires species to first survive a period with low food and mate availability. The ecophysiological strategies that allowed

NIMS to disperse and establish, also provide a competitive advantage during colonisation. Although naturalised species may have previously encountered environmental change, the presence of NIMS creates new competition for the limited resources (Lam *et al*., 2005).

1.4 HABITAT SELECTION

1.4.1 HABITAT SELECTION

Habitat preference is described as the non-random distribution of organisms in an environment (Underwood *et al*., 2004). Habitat choices affect the physiology and psychology of animals (Bowden *et al*., 2007). In order to demonstrate habitat preference, tests must show a positive preference, by actively selecting a substrate as opposed to disliking the alternative.

Habitats can be defined using a range of variables for estuarine fish, but were limited to salinity, temperature and sediment for this study, as the major factors to influence fish distribution (Usmar, 2003; Harrison and Whitfield, 2006). Additional abiotic and biotic factors that determine environmental preferences and tolerances of animals are age, body size, competition, density, food supply, light intensity/duration, organic matter, oxygen concentration, pH, phenotypic plasticity, plant presence, predation, r- or K- selected strategies, season and substrate type (Wonham *et al*., 2000; Sakai *et al*., 2001; Perez *et al*., 2003; Stoner and Titgen, 2003; Usmar, 2003; Ospina and Mora, 2004; Schofield, 2004; Lam *et al*., 2005; Bowden *et al*., 2007).

Experiments on habitat selection can be affected by an animals multiple preferences, electivity and acceptability. Animals may have multiple preferences for habitat, food and other variables; which are often based on temporal availability (Underwood *et al*., 2004). For example, bridled gobies select habitat based on the seasonal temperature; preferring intertidal mudflats in summer and estuarine channels in winter (Usmar, 2003). It is recommended that multiple habitat preferences be determined prior to experimental work so that separate tests can be conducted if necessary.

Electivity is the preference for an available habitat, taking into account the relative proportion of that selected habitat (Underwood *et al.*, 2004). Selection of a proportionally smaller habitat implies a stronger preference than selection of a proportionally larger habitat. Habitats may be spatially or temporally restricted, so electivity can change as the relative proportions available are altered.

Preferences for desirable habitats are described as acceptability (Underwood *et al.*, 2004). An organism can be forced into an undesirable habitat, creating a non-preferred selection. For example, physical complexity can prevent efficient movement, or the organism may be forced to pass through an undesirable zone to reach preferred habitat (Underwood *et al.*, 2004). In this scenario, laboratory tests would better show preferences than field studies.

1.5 GOBIES

1.5.1 GOBOIDEI

The suborder Goboidei contains the three families Rhyacichthyidae, Odontonbutidae and Gobiidae. Gobiidae is the largest family of marine fishes, with 200 genera and 1500 species worldwide (Hoese, 1994; Kuiter, 2000; Doak, 2003). Gobies are found in tropical and temperate climates; in fresh, brackish or sea water; at depths to 800 m and in freshwater at altitudes of 2000 m (Paulin, 1989; Paul and Moreland, 1993; Hoese, 1994; Kuiter, 2000; Lam *et al.*, 2005). Some freshwater species from the Caribbean and Philippines are diadromous (Hoese, 1994). Gobies are biologically diverse, environmentally tolerant and of a crevicolous nature; making them effective invaders, although some species are endangered (Hoese, 1994; Wonham *et al.*, 2000).

Members of the Gobiidae family were among the most abundant fishes in tropical mangrove ecosystems, intertidal mudflats and tropical tidepools, and occupied the most locations in tropical mangrove and shallow seagrass ecosystems (Lam *et al.*, 2005). Furthermore, Wonham *et al.* (2000) reported gobies as the most abundant fishes found in ballast tanks. However, gobies were relatively uncommon and had low abundances in reef ecosystems (Lam *et al*., 2005).

Gobies are typically benthic or demersal, with varying behaviours of resting on the substrate, digging burrows or burying themselves (Hoese, 1994). The caudal fin is often rounded. A characteristic feature of gobies is fused ventral fins that cling to a surface to prevent displacement (Hoese, 1994). The dorsal fin is in two parts that may be joined by a membrane. The first dorsal fin has six flexible spines and the second part has one spine followed by soft rays. Spines on dorsal and anal fins are flexible (Paulin, 1989; Kuiter, 2000). Gobies have six branchiostegal rays for gill support (Cailliet *et al.*, 1986). The lateral line is absent and sensory cells are concentrated at the anterior end (Hoese, 1994). This may occur because the sedentary nature of benthic and demersal gobies requires anterior sensors to detect prey movement, whereas pelagic fish use laterally positioned canal neuromasts for schooling. Scales may be ctenoid, cycloid or absent. The head is stubby with large dorsally positioned eyes (Paul and Moreland, 1993).

Gobies can live 1-10 years, but most have similar life cycles. Females lay eggs that may be attached to the substrate (Neira *et al*., 1992) and males often guard the eggs (Lam *et al*., 2005). Larvae hatch within a few days and are dispersed in the water column for up to a month, after which they settle in the local habitat (Hoese, 1994). Gobies with short life spans typically produce considerably fewer oocytes than long lived species, and these fish have variable abundances, are recruitment sensitive and vulnerable to disturbances (MacKenzie, 1984).

1.5.2 NEW ZEALAND GOBIES

New Zealand's gobiid fauna is remarkably depauperate by comparison with our nearest neighbour, Australia, which has 350 species (Kuiter, 2000). The taxonomy and origins of New Zealand's gobies have to date been speculative and contradictory (MacKenzie, 1984; Francis *et al*., 2004b), and have only recently been addressed using molecular studies (Lavery *et al*., 2006). Opinions vary over which species are indigenous or introduced, but the black, long-finned and exquisite gobies are probably mainland natives, with *Eviota* sp. and *Priolepis* *psygmophilia* endemic to the Kermadec Islands, whereas the bridled, Asian and Port Hacking gobies are recent introductions. The bridled and exquisite gobies will be discussed in subsequent sections.

The black goby, *Gobiopsis atrata,* is 5-10 cm long and occurs on or near reefs, in shallow waters along the east coast of New Zealand from Cape Reinga to Stewart Island (Paul and Moreland, 1993; Francis, 2001).

Long-finned gobies (*Favonigobius lentiginosus*) are similar to exquisite gobies in morphology, behaviour, range and distribution (MacKenzie, 1986; Laws, 1998). Long-finned gobies were first described from Australian specimens in 1881, and were identified in New Zealand in 1927 (Laws, 1998). New Zealand long-finned gobies were recently found to be conspecific to *Favongobius lateralis* and possibly *Favonigobius tamarensis* in Australia (Lavery *et al*., 2006).

Eviota sp. and *Priolepis psygmophilia* are gobies endemic to the Kermadec Islands (Francis, 1996).

Two forms of Asian goby (*Acentrogobius pflaumii)* exist in Asia. One form has spread to Sydney and then to New Zealand (Lavery *et al*., 2006). Asian gobies were first captured in 2001 at Waitemata and Whangapoua Harbours (Francis *et al*., 2003). This species occupies zostera beds in Asia, and mud- and sandflats in Australia (Morrison *et al*., 2002), thus the potential exists for spread to broad habitat types in New Zealand. Asian gobies presently occupy a small range of locations in New Zealand and are not thought to present the same potential impact as bridled gobies, as they meet only four of the nine criteria to test for adventism (Francis *et al*., 2003).

Port Hacking dart gobies (*Parioglossus marginalis*) were first found in streams at Great Barrier Island and North Cape in 2000 but are known from Australia (McDowall, 2000; Morrison *et al*., 2002; Walsh, 2007). It is tolerant to marine, brackish and fresh waters (McDowall, 2001), suggesting wide tolerance and the potential to disperse. They are captured in bait catchers that are baited with Vegemite.

The aquarium trade poses a significant risk of exotic species invasion (Rixon *et al.*, 2005) yet there is little published information on this risk in New Zealand. There exists the potential for owners of pet fish to inappropriately discard live unwanted fishes. There are marine, brackish and freshwater goby species in the New Zealand aquarium trade and the release of these fishes could potentially harm native species in all aquatic habitats (Table 1.5.1).

Table 1.5.1. Import health standard for ornamental fish and marine invertebrates from all countries. Fishes marked * are reported in New Zealand but not in the Import Health Standard.

Scientific name	Common name	Comments
Amblyeleotris guttata	Sunburst goby	Distributed in the Western Pacific Ocean (Fishbase, 2007)
*Amblyeleotris randalli**	Prawn goby	Western pacific (Fishbase, 2007).
Amblygobius decussatus	Orange-striped goby	
Amblygobius phalaena	Banded goby	
Amblygobius rainfordi	Old glory	
*Brachygobius doriae** *B. nunus** *B. xanthozona**	Bumblebee goby Bumblebee goby Bumblebee goby	From SE Asia, grows to 4.5 cm and prefers 24-30 °C. *B. nunus* lives in brackish and *B. xanthozona* lives in fresh or brackish waters (Age of Aquariums, 2007).
*Cryptocentrus cinctus** (Hollywood fishfarm, pers. comm.).	Yellow prawn goby	Western pacific (Fishbase, 2007).
*Cryptocentrus leptocephalus**	Pink-speckled shrimp goby	Western pacific (Fishbase, 2007).
Nemateleotris decora	Decorated dartfish	Indo-Pacific Ocean (Fishbase, 2007).
Nemateleotris magnifica *Nemateleotris magnifica* *Nemateleotris magnifica*	Fire goby Fire dartfish Magnificant dartfish	Reef fish that grows to 8 cm and lives at 22-26 °C (GrizzlyRun, 2007). Indo-Pacific distribution (Fishbase, 2007).
Ptereleotris evides	Blackfin dartfish	Indo-Pacific distribution (Fishbase, 2007).
Ptereleotris zebra	Chinese zebra goby	Indo-Pacific distribution (Fishbase, 2007).
*Stignatogobius sadanunidio**	Knight goby	Native to Asia, found in estuaries and tidal rivers. Grows to 8 cm and prefers 20-26 °C (Badman's Tropical Fish, 2007)
Valenciennea longipinnis	Long-finned goby	Indo west-Pacific distribution (Fishbase, 2007).
Valenciennea puellaris	Maiden goby	Indo-Pacific distribution (Fishbase, 2007).
Valenciennea sexguttata	Sixspot goby	Indo-Pacific distribution (Fishbase, 2007).
Valenciennea strigata	Blueband goby	Indo-Pacific distribution (Fishbase, 2007).

1.5.3 BRIDLED GOBIES

Bridled gobies (Figure 1.5.1) have been described as *Gobius bifrenatus* (Kner, 1865), *Arenigobius bifrenatus* (Gomon *et al*., 1994) and *Amoya bifrenatus* (Larson and Hoese, 1996) (Eschmeyer, 1998; Eschmeyer *et al*., 1998).

Figure 1.5.1. Bridled goby (B. O'Brian).

In Australia bridled gobies are found in southwest, south and eastern Australia and Tasmania. They are found in seagrass beds, eelgrass meadows (*Zostera meulleri* and *Heterozostera tasmanica*), rocky reefs, coastal lagoons and streams. Neira *et al*. (1992) reported that in the Swan Estuary, Australia, bridled gobies constituted 30% of total fish abundance and 70% of all fish larvae in the upper estuary. Adult bridled gobies are predominantly estuarine, but pelagic larvae have been found occasionally in freshwater (Hoese and Larson, 1980; Gee and Gee, 1991; Neira *et al*., 1992). Adults primarily feed on harpactocoid copepods, gammarid amphipods and ostracods (Robertson and Howard, 1978; Gee and Gee, 1991; Kuiter, 2000; Usmar, 2003). Bridled gobies in Australia co-inhabit areas with exquisite and long-finned gobies (Lavery *et al*., 2006).

Gray Jamieson Holdings Ltd (1996) reported that bridled gobies have been in New Zealand since 1996 and were first located at the coastal Otara Lake, although it is unknown whether this was the original site of introduction. Willis *et al*. (1999) published the first official recording following the capture of bridled gobies in the Whangateau and Waitemata Harbours in 1998. Genetic work by Lavery *et al*. (2006) suggested that either many small or one large and diverse introduction have occurred, but further research is required.

Usmar (2003) stated that abundance of bridled gobies in the Whangateau Harbour doubled between 2002 and 2003, and the species was found in thirteen locations

(Matapouri, Pataua, Whangarai, Mangawhai, Whangateau, Mahurangi, Puhoi, Waiwera, Weiti, Okura, Waitemata, Tamaki and Tauranga).

Intertidal sediment burrows are utilised at low tide, suggesting good hypoxia tolerance (Gee and Gee, 1991; Gee and Gee, 1995). Tolerance to hypoxia is essential in order to maintain a sufficient energy supply to the body (Lam *et al*., 2005). Bridled gobies ventilate the gills in oxygenated water at high tide, but under hypoxic conditions utilise aquatic surface respiration or an air bubble in their buccal cavity, which they use for both respiration and buoyancy (Gee and Gee, 1991; Gee and Gee, 1995). It is common for intraepithelial capillaries to occur in the buccopharyngeal cavity of air-breathing fishes (Lam *et al*., 2005), though this has not been examined in bridled gobies. Risks of air-breathing include an accumulation of carbon dioxide and ammonia (Lam *et al*., 2005). The structure of fish gills that are exposed to air typically differ from fish gills that are permanently submerged (Lam *et al*., 2005).

Usmar (2003) studied the bridled goby's preferences for sediment and habitat type. Silt and clay sediments were preferred as these are likely to form sturdier tunnels than coarse sediment. The preferred habitat types contained mangrove pneumatophores, mud flats and channels, whereas cobbles or sandflats were not selected. Usmar (2003) described bridled gobies as territorial and Francis *et al*. (2003) reported them to dive head first into burrows when disturbed. In captivity, dominant bridled gobies nip the fins of subordinates (pers. obs.).

Bridled gobies use burrows for protection, food gathering and nesting, which may have improved the success of their establishment in New Zealand (Usmar, 2003). Bridled gobies may actively dig burrows or utilise burrows made by crabs and shrimps. The burrows of bridled gobies are complex, often with multiple entrances and convoluted tunnels. Tunnel walls can be smooth or lined with broken shells. The complexity of burrow morphology suggests that bridled gobies can evade predatory shore birds that feed in intertidal zones. Cohabitants of tunnels include crabs, shrimps and eels (Usmar, 2003; pers. obs.). It is likely that at high tide, small fish investigate or utilise the tunnels, although the aggressive and territorial nature of bridled gobies suggests that other fish would not permanently co-inhabit tunnels. Bridled gobies feed by sifting sediment in the mouth and out the gills, only consuming suitable prey (Usmar, 2003).

Bridled gobies are not deemed a threat in New Zealand because they have unique habitat needs compared with the broad habitats of established fishes (Usmar, 2003). However, bridled gobies may expand into the other recorded habitat types as their abundance increases.

1.5.4 EXQUISITE GOBIES

Exquisite gobies (Figure 1.5.2) have been described as *Gobius lateralis* (Macleay, 1881; Hoese, 1986), *Favonigobius exquisitus* (Whitley, 1950), *Acentrogobius lentiginosus* (Ayling and Cox, 1982; Paulin *et al*., 1989), *Favonigobius lateralis* (MacKenzie, 1984) and *Papillogobius exquisitus* (Gill, 1993) (from references: MacKenzie, 1984; Paulin *et al*., 1989; Eschmeyer, 1998a; Eschmeyer, 1998b; Doak, 2003).

Figure 1.5.2. Exquisite goby (B. O'Brian).

In addition to the multitude of name changes, the origin of exquisite gobies is questioned. Doak (2003) reported the exquisite sand goby (*Papillogobius exquisitus*) as a recent invader from Australia, whereas Francis *et al*. (2003) described *Favonigobius exquisitus* as native, which is considered correct in this thesis.

Lavery *et al*. (2006) stated there are large genetic differences between New Zealand and Australian exquisite gobies, as no haplotypes are common between the two countries. There is greater diversity in Australian populations compared with New Zealand populations. Despite this, two major clades occur throughout their current New Zealand range. Exquisite gobies are found in upper North Island estuaries (McKenzie, 1984).

Small exquisite gobies (<20 mm) feed on meiobenthic harpacticoid copepods, whereas adults feed on amphipods, polychaetes and mysids (McKenzie, 1984). The feeding requirements of native gobies and juvenile pleuronectids (e.g. sole and flounder) are similar, though competition is not intense because of differences in recruitment time and spatial separation (McKenzie, 1984).

Fishes living in shallow zones have numerous options to prevent predation and desiccation. Exquisite gobies migrate up the shore with the tide, thus remaining in waters too shallow for predatory fish. At low tide they reside further down the shore, still in shallow waters (Gibson *et al*., 1996). Alternatively, intertidal refuges are occupied at low tide in pools, crevices or empty shells (Doak, 2003). Exquisite gobies also bury themselves in surface sediments for camouflage (pers. obs.). They are commonly captured on sand flats at all tide heights (McKenzie, 1984).

Female exquisite gobies are more abundant than males prior to the recruitment period from November to February. Adults live less than three years and usually breed by the end of the first year. Empty cockleshells are used for shelter and nesting, and fish inflate their abdomen when threatened (Doak, 2003). They grow to 9 cm TL and form aggregations of up to 20 fish (Doak, 2003).

1.6 HYPOTHESIS

Willis *et al*. (1999) surmised that bridled gobies were transported to New Zealand in the ballast tanks of ships, where they would have encountered harsh transport conditions. Bridled gobies also endure inhospitable conditions in the sediment tunnels they inhabit at low tides on mud flats; with highly variable salinities and temperatures, and low dissolved oxygen (pers. obs.). It is therefore hypothesised that bridled gobies will tolerate greater extremes of temperature and salinity than exquisite gobies that primarily occupy the water column.

Bridled and exquisite gobies occur sympatrically in New Zealand and Australia. Both species inhabit the Puhoi Estuary and have been captured together in the Waitemata Harbour (pers. obs.). At Puhoi, bridled gobies occupy fine mud sediments within the estuary, whereas exquisite gobies are found on sand flats near the estuary mouth. In Australia, both species are found in the same local regions (Neira *et al.*, 1992). It is hypothesised that differences in sediment preference may contribute to habitat partitioning between bridled and exquisite gobies.

2 GENERAL METHODS

2.1 STUDY SITE

Bridled gobies were collected from the Puhoi Estuary (Figure 2.1.1). Mud flats on the fringe of mangroves were found to have the densest populations and thus were the primary collection area.

Exquisite gobies were sourced from sandflats at the Puhoi Estuary or Waitemata Harbour (Figure 2.1.2).

Figure 2.1.1: Entrances to bridled goby tunnels, Puhoi Estuary.

Figure 2.1.2. Sulphur Point, Waitemata Harbour.

Gobies were collected between April 2005 and February 2007, with varying levels of capture success. Field conditions were recorded to match with laboratory conditions and to compare with experimental results (Table 2.1.1). YSI meters measured salinity, dissolved oxygen (DO) and temperature and a Eutech pH meter measured pH. Estuarine water conditions were measured by dipping the probe in undisturbed water. Burrow water was siphoned out of the tunnels using a flexible tube attached to a 60 mL syringe.

Table 2.1.1. Field conditions between April 2005 and February 2007 from burrow and estuary water. Samples are independent of each other. Note: ppt is parts per thousand.

	Salinity (ppt)	Temp (°C)	DO (mg/L)	pH
Min	13.5	16	8.33	7.7
Max	36.5	24.7	10.6	8.2

2.2 COLLECTION METHODS

2.2.1 BRIDLED GOBIES

Usmar (2003) found seine netting an inefficient method to capture bridled gobies as they avoid the net or enter burrows when the net passes over. Additionally, their soft mud habitat cannot be effectively seined. The preferred capture method in her study was to dig up burrows at low tide. The greatest abundance in 2003, of 37 fish per 3000 m^{-2}, occurred in the Puhoi Estuary. Inglis *et al*. (2006) captured bridled goby by benthic sled, but the efficiency of this method is unknown.

The morphology of bridled goby burrow entrances was determined during the first field trip by snorkelling at high tide, so subsequent trips did not require snorkelling. Bridled gobies were the only fish observed at high tide, and quickly swam away or dived into burrows. Bamboo stakes were used to mark these burrow entrances. At low tide, burrows were systematically dismantled by probing fingers into an entrance and following the burrow, removing overlying sediment as needed. This usually produced a pit approximately 1 m^2 and up to 0.4 m deep. Bridled gobies are tolerant to handling, so can be picked up and placed in a bucket of estuarine water. To prevent the gobies from overheating in hot weather, ice was added to the water. The gobies were transferred to a large chilli-bin containing aerated, cooled estuarine water. Fish were transported to Waikato University and immediately placed in holding tanks (see Section 2.3).

2.2.2 EXQUISITE GOBIES

Beach seine netting is the most effective way to capture exquisite gobies, as fish occur in shallow waters at all tidal heights. Exquisite gobies are too well camouflaged and swim too quickly to be hand netted, and do not enter minnow traps. A 6 m beach seine with 3 mm mesh and a cod end was dragged about 20 m at approximately 0.5 m/sec. All bycatch was returned; in particular, flounder were immediately returned to the water in accordance with the Ministry of Fisheries Special Permit 281. Exquisite gobies were kept in a bucket of estuarine water while on the beach, and transferred to a chilli-bin with aerated, cooled estuarine water. Exquisite gobies were more sensitive to handling and transport, and survival rates were improved by the addition of 'Stresscoat' (API), which reduced stress from capture and handling.

2.3 FISH HUSBANDRY

All gobies were kept in 50 L tanks with 27 ppt (parts per thousand) recirculating synthetic sea water (RedSea Salt). A UV light and biofilter controlled disease and toxic nitrogenous wastes, respectively. Holding tanks contained pipes and ceramic pots in which fish could hide, and up to six fish were kept in one tank. Diseased or damaged fish (symptoms included weight loss or eroded fins) were removed to separate recovery tanks. Fish were fed frozen blood worms (chironomids) daily. Fish were acclimatised to laboratory conditions for at least two weeks prior to use in any experiments.

2.4 ECOPHYSIOLOGICAL TEST PROCEEDURES

2.4.1 END-POINT OF ECOPHYSIOLOGICAL EXPERIMENTS

The results of the ecophysiological experiments are LC_{50} and LT_{50}, indicating the median lethal concentration and temperature, respectively. In recent times, fatal endpoints have been considered unethical for vertebrates and numerous other endpoints have been introduced.

Mortality provides an exact measurement of tolerance and is easily transformed into LC_{50} information, which is comparable to historical ecotoxicological data. However, the University of Waikato's Animal Ethics Committee raised concerns over using death as an end-point and requested that several non-lethal endpoints be trialled on gobies, but all were unworkable.

Loss of equilibrium (LOE) occurs when a fish is unable to right itself. At this stage, the fish can be transferred to a recovery tank. However, LOE was not appropriate for gobies because they are typically benthic and motionless, and the dorsoventrally flattened bodies and disc-shaped pelvic fins prevented rolling. Furthermore, the time at which a fish looses equilibrium varies at an individual level requiring continuous monitoring which is unfeasible for tests of long duration (e.g. 96 h). LOE was determined by inverting the fish with a blunt seeker and observing whether the fish righted itself. This provided variable results as fish can be distressed by inversion, and swim rapidly around the tank, or show stress induced immobility responses.

Opercular movement was not possible due to the irregular depth and frequency of respiration, and the small size of exquisite gobies. Ford *et al*. (2004) reported that exquisite gobies ventilated at a mean rate of 26±1.4 beats per minute at 20°C, and this increases fivefold in 35°C. Opercular movement is useful for comparison between species, but does not provide concise information of ecophysiological impacts on fish.

Spasms occurred infrequently in both gobies, were individually variable and also required continuous observation. Despite this, spasms were used as an endpoint by Perez *et al*. (2003).

It was concluded that fatal endpoints were the only feasible option for these tolerance tests and produced the least variable results.

2.4.2 REPLICATE SAMPLE SIZE

Use of more than one fish can allow a hierarchy to form and the dominant individual can prevent the subordinate from occupying its preferred sediment (Noakes and Baylis, 1990). Therefore, only individuals were used in this experiment.

2.4.3 FEEDING

Bridled and exquisite gobies were fed blood worms daily when not subjected to experimental trials and were fed an amount that was consumed within an hour.

Various feeding regimes have been recommended for fish, prior to and during experimentation. Feeding fish to satiation prior to sediment tests can eliminate searching behaviour, whereas underfeeding can cause fish to search rather than select preferred habitat (Moles and Norcross, 1995; Stoner and Titgen, 2003).

2.4.4 EXPERIMENTAL CONDITIONS

Both laboratory and in situ conditions can affect the response of organisms to physicochemical variables. Laboratory test results can be influenced from stresses imposed by capture, transport and containment (Rajaguru and Ramachandran, 2001). Contrarily, wild populations are subjected to a combination of fluctuating environmental pressures (Noakes and Baylis, 1990). Seasonal temperature, salinity and oxygen are difficult to control and isolate for study. Laboratory tests are the best way to control an experiment so that only the desired variable is altered and a better predictive relationship between cause and effect is obtained. As such, the following experiments were conducted in a controlled laboratory environment.

Fish are most susceptible to temperature and salinity changes soon after being subjected to treatment conditions. As such, fish were checked often in the first 24 h of treatment, then at 24 h periods thereafter. Over a 96 h period it is clear that proportionally more fish die early on, then adaptation occurs. Rajaguru and Ramachandran (2001) ran temperature tolerance tests for only 24 h. Such data would not provide conclusive evidence of long term tolerance.

3 ECOPHYSIOLOGICAL EXPERIMENTS

3.1 SALINITY TOLERANCE

3.1.1 INTRODUCTION

Estuarine salinities are highly variable due to freshwater inflow from streams and land runoff, and tidal movement of seawater (Dame and Allen, 1996; Lam *et al.*, 2005). Habitat selection, range, dispersal and abundance are partially controlled by salinity (Atrill and Power, 2004; Barletta *et al.*, 2005; Lam *et al.*, 2005; Harrison and Whitfield, 2006). Salinity variation can cause physiological changes in fish (e.g. euryhaline fish) and localised migration (e.g. stenohaline fish). Salinity tolerance experiments indicate the conditions that fish can survive for defined periods of time (Nordlie, 1985).

Estuarine fishes that are exposed to increased salinity multiply the number of chloride cells (Cioni *et al.*, 1991), and increase plasma sodium and chloride ions (Lam *et al.*, 2005). In low salinities, plasma becomes hypertonic to the environment, so water is taken in through the gills (Bridges, 1993). Euryhaline fish have adaptive osmoregulatory systems to accommodate salt fluctuations. Estuarine or intertidal fishes that experience tidal salinity changes (e.g. bridled gobies) must adapt or avoid the salinity changes, whereas stenohaline fish must leave the area or suffer reduced fitness (Lam *et al.*, 2005). Salinity can negatively affect growth due to increased energy use in gill functioning (Schofield, 2004).

This study aimed to determine the level of salinity tolerance in bridled and exquisite gobies, using acute tests with salinities ranging from 2.2 to 35 ppt. Differences in low salinity tolerance may allow one species to occupy habitat zones within estuaries that exclude the other. It is further hypothesised that both species are tolerant of full strength seawater to allow dispersal between estuaries.

3.1.2 METHODS

Salinity tolerance was tested by acute exposure to a geometric series of salinities (2.2, 4.4, 8.8, 17.5, 27.0 (control) and 35.0 ppt). Treatments were conducted in 25 L tanks containing 10 L of synthetic sea water. The water was aerated between experiments to saturation, but aeration was removed during experiments to prevent disturbance and to allow closer inspection of fish. Preliminary trials indicated that dissolved oxygen did not fall significantly below full saturation during the course of the experiments (96 h). The experiments were conducted between July and October 2006. The room temperature during the experiment was 10-16.0°C and the water temperature 10.1-14.7°C. Fish were not fed for the duration of the experiment.

Fish were randomly allocated to treatments at 8 am on the first morning. Observations occurred at 0, 2, 4, 8, 12, 24, 48, 72 and 96 h. Dead fish were removed from the tank at the time of observation and survivors at 96 h were moved to a recovery holding tank.

ANALYSIS

Raw data were plotted in Microsoft Excel to show the relative trends in mortality due to temperature over time.

Statistica 7 was used to create contour plots for salinity.

3.1.3 RESULTS

Bridled gobies were more tolerant of low salinities than exquisite gobies (Figure 3.1.1 and Figure 3.1.2). Although there was a clear response to saline concentration in the exquisite goby, LC_{50} values could not be ascertained because mortality did not exceed 50% for either species at any salinity.

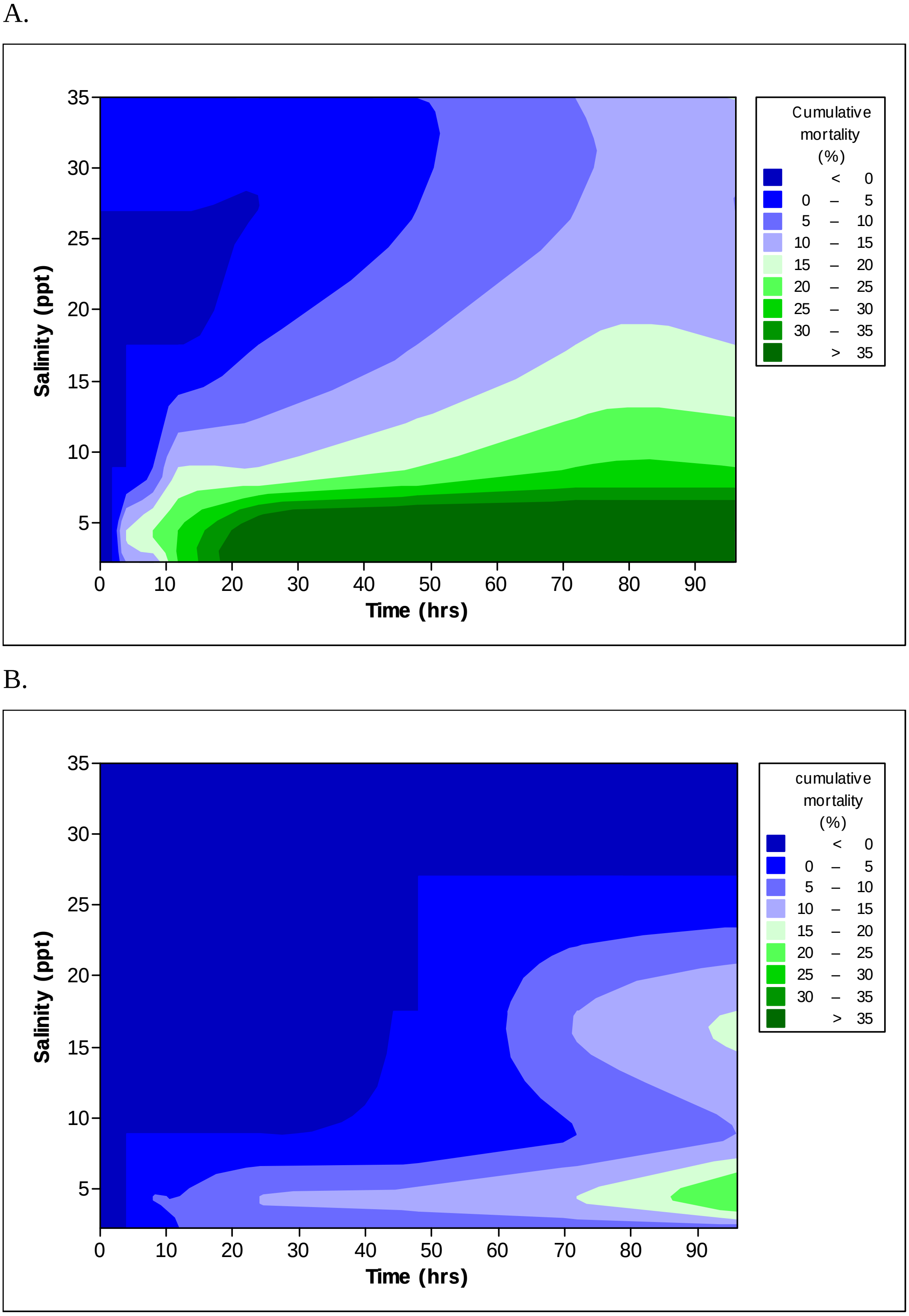
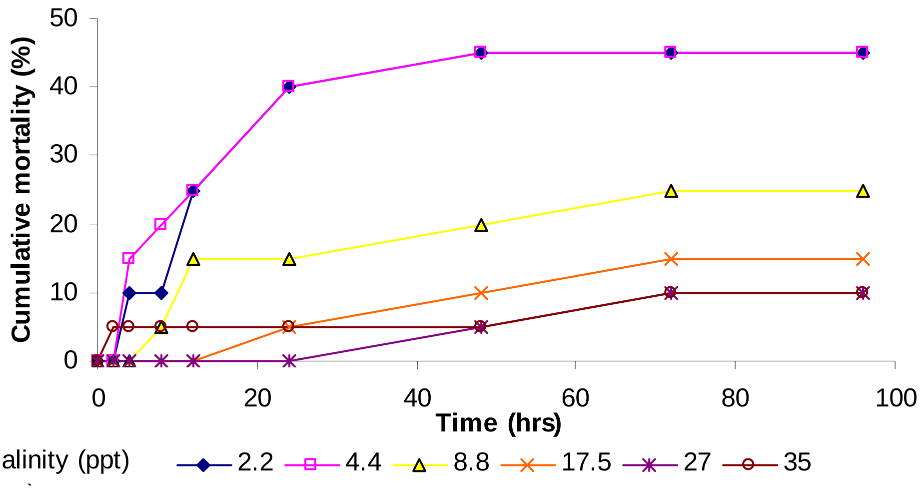


Figure 3.1.1. Contour plots of salinity tolerance for A. exquisite goby and B. bridled goby.

A.



B.

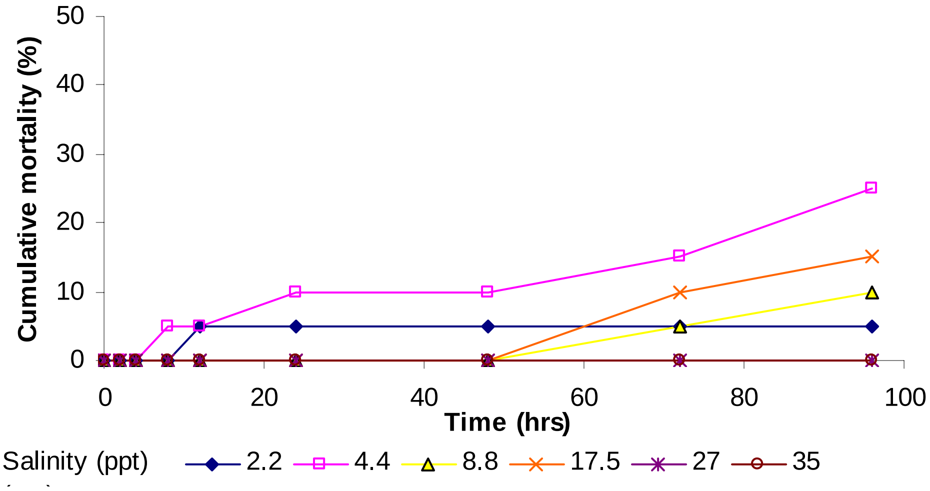


Figure 3.1.2. Salinity tolerances for A. exquisite goby and B. bridled goby.

3.1.4 DISCUSSION

The results demonstrate that both species are euryhaline, but exquisite gobies are less tolerant of conditions prevailing in upstream zones of estuaries influenced by riverine flows.

Bridled and exquisite gobies can tolerant a range of salinities, with the majority of individuals surviving for at least 96 h. When either species translocates they are likely to survive coastal salinities. Bridled gobies are more tolerant than exquisite gobies to low salinities, which could explain why bridled gobies in Puhoi Estuary were captured in the upper reaches and exquisite gobies were only found closer to the estuary mouth.

Bridled gobies could potentially spread further upstream to reaches that encounter greater freshwater input. In wild situations, acclimation may allow bridled gobies to increase their tolerance to low salinities and potentially become a greater risk to fishes that inhabit the upper reaches of estuaries, such as native freshwater migrants (e.g. inanga (*Galaxias maculatus*), smelt (*Retropinna retropinna*), common bully (*Gobiomorphus cotidianus*) and banded kokopu (*Galaxias fasciatus*) (McDowall, 1995). Australian bridled gobies have been recorded in freshwaters (Hoese and Larson, 1980; Gee and Gee, 1991; Neira *et al.*, 1992).

The bridled gobies wide tolerance to extreme salinities is likely to contribute to their successful invasion and spread in New Zealand. Exquisite gobies are less tolerant to the range of salinities and therefore are less likely to voluntarily migrate or survive sudden salinity changes. During dispersal exquisite gobies will likely avoid undesirable salinities, whereas the bridled gobies may quickly pass through less desirable areas to invade new habitats.

Mortalities that occurred in control experiments (27 ppt) are thought to be due to stress from handling, movement to a novel environment (treatment tank) or the low overnight water temperature. This experiment was conducted in a minimum water temperature of 10.1°C, although this low temperature only occurred briefly overnight as was equivalent to holding tanks, and fish in holding tanks did not suffer mortality during the experiment duration. The temperature tolerance experiment (Section 3.2) found the incipient ten day LT_{50} to be 11.8°C for exquisite gobies. However, some deaths that occurred in this experiment may be due to the temporary low temperatures. Despite the occurrence of deaths that are not from treatments (e.g. mortality from low temperature and handling stress), a clear pattern is seen in salinity tolerance by exquisite gobies.

Parkyn *et al*. (2002) found the salinity that juvenile red drum (*Siaenops ocellatus*) are reared at, determines the preferred salinity of adults. This demonstrates that by controlling rearing conditions, adult fish dispersal could be restricted. This concept could be used to restrict the escape of aquaculture species and the spread of non-indigenous fishes. Salinity is unlikely to be able to be used to control bridled gobies, but this method may be useful for other fishes.

3.2 TEMPERATURE TOLERANCE

3.2.1 INTRODUCTION

Daily and seasonal temperatures vary greatly in estuaries and water temperature plays a key role in fish physiology and habitat selection (Lam *et al*., 2005). Fish living in optimal thermal habitats will have the greatest health and success, and typically these fish possess ecophysiological adaptations and competitive behavioural strategies. Temperature tolerance experiments can predict the distribution and dispersal of fishes, and provide information on optimal conditions for native and pest species (Perez *et al*., 2003).

The immune systems of fish work most efficiently at a temperature optimal for that species (Bowden *et al*., 2007). Fish are subjected to varying environmental temperatures that can impact abundance, activity, distribution, growth, fecundity, feeding, fitness, maturation, metabolic rate, range and reproduction (Portner *et al*., 2001; Mallekh and Lagardere, 2002; Atrill and Power, 2004; Harrison and Whitfield, 2006). Atrill and Power (2004) showed that fish distribution was determined by temperature rather than food supply, as a variety of estuarine fish will enter warm waters for the duration of feeding, and subsequently return to their preferred temperatures. Poikilotherms including fish use thermal cues to initiate reproduction and migration (Bowden *et al*., 2007). Fish that are forced to live outside their thermal limits suffer stress and reduced growth, and subordinate fish that remain within optimal thermal regions have an impaired ability to avoid predators due to over-crowding (Carveth *et al*., 2007).

Global temperature change impacts fish fitness, and temperature tolerance experiments provide essential information about responses to climate change (Ospina and Mora, 2004). Global temperature change and the North Atlantic Oscillation (NAO) control fish abundance and growth in estuaries, particularly for vulnerable juvenile fish (Atrill and Power, 2002). The NAO Index and population abundance can be negatively correlated (e.g. for flatfish and herring), positively correlated (e.g. for bass and sprat) or show no correlation (e.g. for gadoids and dover sole). Fish are ectotherms and so unable to physiologically regulate body temperature, therefore, global climate changes will affect them more than endothermic animals (Bowden *et al.*, 2007).

Tropical South African estuaries are undergoing cooling and most fish are expected to be negatively affected in some way (Figure 3.2.1; Harrison and Whitfield, 2006). Group 2 are the only eurythermal fishes in this system, so will be least affected by temperature changes. The highly endemic fauna of warm tropical estuaries (group 3) have restricted dispersal capabilities and are likely to suffer.

Climates have greater variation further from the equator, so temperate animals are more susceptible to poor health and growth (Bowden *et al.*, 2007), however, Lam *et al.* (2005) reported the expected 2 to 4 °C temperature increase over the next half-century is likely to severely impact tropical shallow environments, as fish in these regions are living almost at their thermal limits. When sea surface temperatures increased in New Zealand over the autumn months of 1996, 1998 and 1999, new fish species were observed (Francis *et al.*, 1999).

Competition for thermal resources occurs to optimise ecophysiological success, but can result in spatial separation. Atrill and Power (2004) found three methods that fish use to prevent competition for thermal resources in the Thames Estuary. Firstly, *partitioning of the thermal resource* occurs when fish have similar temperature preferences, but form aggregations at distinct temperatures (e.g. the average temperature difference between any two fish species was 2.9 °C; the minimum was 1.7 °C between herring and sprat; and pogge and plaice had the maximum temperature difference of 5.8 °C). Secondly, *discrete temperature preferences* of fish species prevent habitat overlap: the greatest difference occurred between flounder and Poor cod. Thirdly, fish can *timeshare* a temperature resource, by migrating into a thermal region only when it is not occupied by another species.

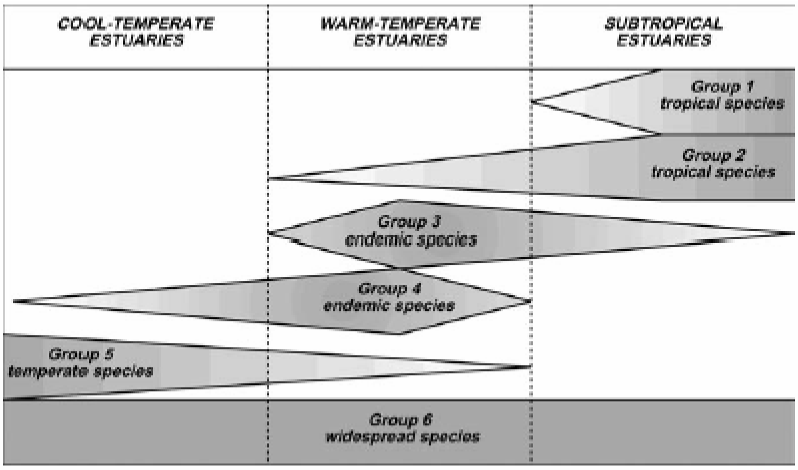


Figure 3.2.1. South African estuarine fish groupings are based on saline and thermal conditions. The relative sizes of each group are shown diagramatically. From Harrison and Whitfield (2006). Group 1 are tropical species restricted to warm, brackish, subtropical estuaries. **Group 2** are as for group 1, but species can extend into temperate waters. **Group 3** fishes are endemic and found sparsely in temperate and subtropical estuaries. **Group 4** species are endemic in temperate estuaries. **Group 5** comprises fishes that prefer cool estuaries. Fishes of **group 6** are tolerant to all environmental characteristics and include the globally abundant mullet, *Mugil cephalus*.

Temperature tolerance data can provide strategic information for the management of native fishes, aquaculture species and pest fish (Tsuchida, 1995; Bevelhimer and Bennet, 2000; Rajaguru and Ramachandran, 2001; Carveth *et al.*, 2007). This study aimed to compare the temperature tolerances of bridled and exquisite gobies, in order to find how susceptible these fishes are to temperature change and to provide predictive information about the possible geographic limits to colonisation by the bridled goby.

3.2.2 METHODS

Temperature tolerance was tested in acute tests at temperatures of 5, 10, 15, 20, 25 and 30 °C. Treatments were conducted in 25 L tanks containing 10 L of 27 ppt synthetic sea water. The water was areated between experiments. All treatments remained fully saturated for the duration of tests. The experiments were conducted in a 5°C temperature controlled room, with heaters in all the tanks adjusted to the appropriate temperature. Tests were conducted over the period from July 2006 to January 2007. Fish were not fed for the duration of the experiment.

Fish were randomly allocated to treatments at 8 am on the first morning. Observations occurred at 0, 1, 2, 4, 8, 12, 24, 48, 72 and 96 h. Dead fish were removed from the tank at the time of observation and survivors at 96 h were moved to a recovery holding tank.

ANALYSIS

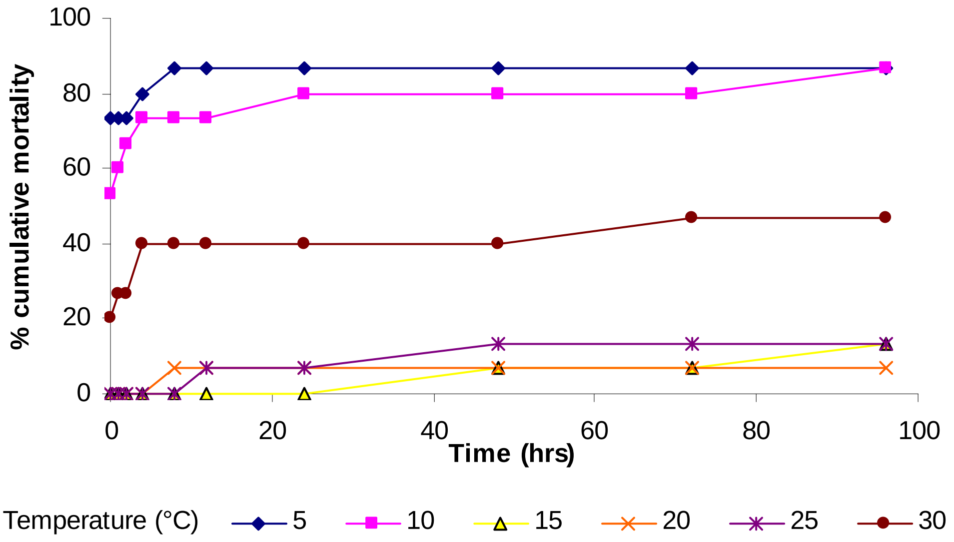
Raw data were plotted in Microsoft Excel to show the relative trends in mortality due to temperature over time.
Data were analysed in ToxCalc (Tidepool Scientific Software). LT_{50} values could be obtained for data with partial mortalities above 50%. LT_{50} values for each species were then graphed and incipient ten day LC_{50} values were determined by graphical extrapolation using non-linear regression.

3.2.3 RESULTS

Both exquisite and bridled gobies were most tolerant to temperatures between 15 and 25°C, experiencing up to only 15% mortality over 96 h (Figure 3.2.2). Tolerances to 5, 10 and 30°C were lower, with exquisite gobies sucumbing to the lower temperatures immediately. Bridled gobies were more tolerant of low temperatures than exquisite gobies.

LT_{50} values were established (Table 3.2.1) and incipient ten day (240 h) LC_{50} values were graphically extrapolated (Figure 3.2.3). Ten day median lethal temperatures for bridled and exquisite gobies were 6.7°C and 11.8°C, respectively.

A.



B.

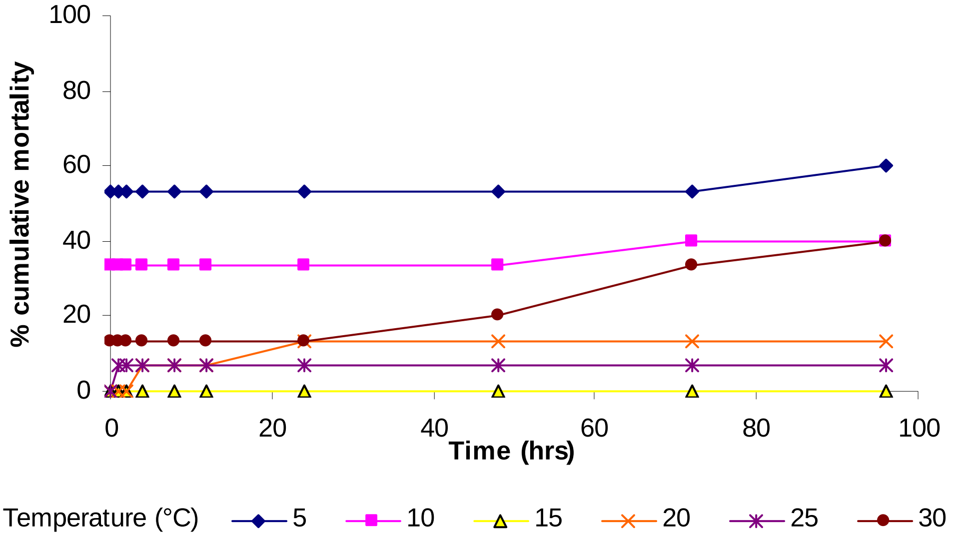


Figure 3.2.2. Temperature tolerances for A. Exquiste gobies and B. Bridled gobies.

Table 3.2.1. LT_{50} values from temperature tolerance experiments. LC_{50} values for 24-96 h data determined in ToxCalc. Ten day (240 h) LC_{50} values were determined by graphical extrapolation.

Species	Time (hrs)	LT_{50} (°C)
Bridled	24	5.5
	48	5.5
	72	5.8
	96	6.4
	240	6.7
Exquisite	24	10.2
	48	10.7
	72	10.7
	96	11.3
	240	11.8

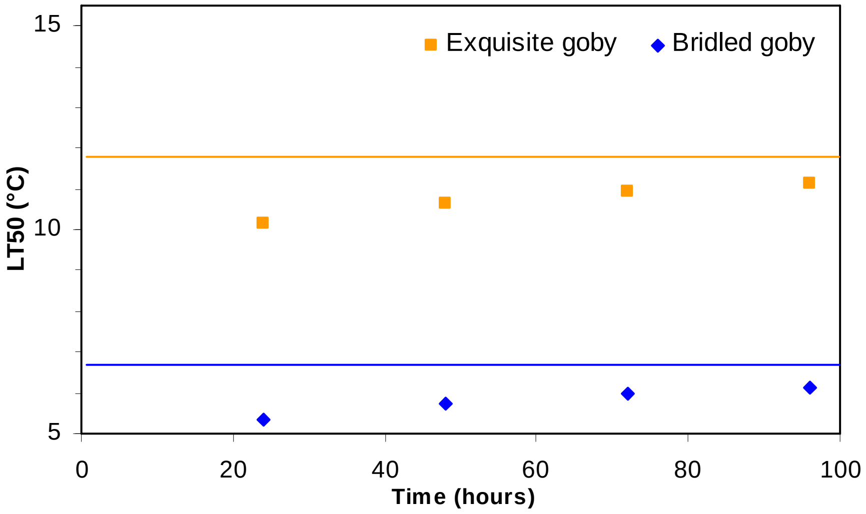


Figure 3.2.3. Temperature tolerance. Symbols indicate LT_{50} from temperature tolerance experiments. Lines indicate the 10 day LT_{50} determined by graphical extrapolation.

3.2.4 DISCUSSION

This study has determined the temperature tolerances for bridled and exquisite gobies. Bridled gobies are more tolerant than exquisite gobies to low temperatures, but both species survive well in 15-25°C, which was expected as sea surface temperatures in the upper North Island range from 18-23°C in summer and 14-16°C in winter (Chiswell, 1994; Ta Ara New Zealand Online Encyclopedia, 2007).

Exquisite gobies are more susceptible than bridled gobies to acute temperature changes; however, abrupt temperature changes rarely occur in nature. The most rapid changes are caused by intense freshwater input during elevated winter rainfall. Daily temperature changes in winter can have a large range, usually between 5–15°C (Chiswell, 1994).

The incipient ten day LT_{50} of 6.7°C for bridled gobies indicates they are likely to survive in the regular temperatures found in North Island estuaries. Exquisite gobies are likely to have a restricted range due to their LT_{50} of 11.8°C. However, exquisite gobies have been found throughout New Zealand's length.

A portion of deaths in treatments could be attributed to handling stress or pathogens, although no visible signs of disease were observed in deceased fish.

Differences in methods exist for temperature tolerance temperature. Acclimatised acute temperature experiments and preference tests were both trialled. Bevelhimer and Bennet (2000) stated that acclimatised temperature tolerance tests represent true environmental conditions and chronic exposure can provide non-lethal results that are not easily observed in acute tests. Acclimation to extreme conditions can improve organism survival as the thermal limits are extended and acclimation to cold temperatures improves aerobic performance by increasing mitochondrial density (Rajaguru and Ramachandran, 2001; Perez *et al.*, 2003). Hesthagen (1979) found acclimation temperature not to affect the lower thermal limit; however, Currie *et al.* (1998) determined that acclimation affects low temperature tolerances rather than high tolerances. Additionally, acclimation did not influence in juvenile angelfish test results (Perez *et al.*, 2003).

The death of exquisite gobies at low temperatures may be due to thermal shock, rather than intolerance to thermal conditions. To reduce temperature shock, fish were placed in ambient water (15°C) at 5pm, and put in a 2°C temperature controlled room. At 8am the following morning, water had reached 5°C and from this time fish were monitored for 96 h. Five exquisite gobies did not survive at 5°C after 12 h of acclimation.

Temperature preference tests have lower precision than temperature tolerance tests because seasonal temperatures can alter temperature preference, but not tolerance (Hesthagen, 1979; Currie *et al*., 1998). Temperature preferences can be affected by the thermal variation that occurred in previous days or seasons (Perez *et al.*, 2003). A temperature preference experiment was trialled on bridled gobies using a circular tank that had a continuous gradient. Warm and cold water entered from opposite ends and was able to drain. A temperature gradient proved unachievable due to flow rate and the formation of a vertical gradient. Static tests were also unable to be set up.

4 PREFERENCE EXPERIMENTS

4.1 SEDIMENT PREFERENCE

4.1.1 INTRODUCTION

Sediment can provide benthic fishes with a means of camouflage and a source of food (Underwood *et al*., 2004). Sediment is integral in dictating species distribution at regional and local scales (Moles and Norcross, 1995). Sediment preference for fish can be determined using a variety sediment characteristics including grain size (Underwood *et al*., 2004), organic content (Usmar, 2003) and colour (Laws, 1998). Grain size was deemed most suitable in this study, due to the lifestyle of both gobiid species; bridled gobies utilise sediment for creating burrows and feeding, and exquisite gobies bury into surface sediment. Numerous methods are available to test sediment grain size preference, and these are described below.

Usmar (2003) hypothesised that sediment grain size was an important habitat characteristic for bridled gobies, and that they would not establish in an area if sediment suitable for burrowing was unavailable. Burrows are used for protection, accessing prey and nesting (Usmar, 2003). Burrowing is advantageous for bridled gobies. They can avoid aquatic predation at low tide whereas exquisite gobies must chance predation by remaining exposed within the water column. In adverse conditions burrows provide shelter and prevent displacement.

In preference experiments it is possible to mistake a positive preference for responses that are negative (dislike of the alternative) or neutral (no preference for either substrate) (Underwood *et al*., 2004; Perez *et al.,* 2003). Additionally, external variables (e.g. light or temperature) can influence the position of an animal, independently of the treatment condition (Underwood *et al.,* 1994). The use of sufficient replicates should reduce the impact of false positives and external variables.

Previous sediment preference studies have used variable experimental conditions. Usmar (2003) found no difference between the results of multiple (four sediments) or paired (two sediments) preference tests for bridled gobies. Stoner and Ottmar (2003) suggested starving fish prior to experimentation and adding Alaskan flatfish to coarse sediment. Conversely, Moles and Norcross (1995) fed Alaskan flatfish prior to testing and placed them in the centre of the tank. Variations also exist in conditions of holding tanks, sediment preparation, sediment grain size and sediment distribution (see Moles and Norcross, 1995; Stoner and Ottmar, 2003; Stoner and Titgen, 2003). Stoner and Titgen (2003) found habitat selectiveness to be dependant on light level for Pacific halibut, with affinity for structured habitat being 60% in darkness and 90% in light.

Moles and Norcross (1995) placed eight sediments in a carousel and recorded choices after 20 h, using both one and two flatfish. This method is problematic for two reasons. Firstly, it is erroneous to assume that the position of the fish at 20 h is the preferred selection; as fish can continue to search once the preferred sediment is located (e.g. bridled gobies). This thesis tested for preference using the greatest proportion of time spent on any sediment. Secondly, using more than one fish in a behaviour experiment can allow a hierarchy to form, where a dominant fish can displace a subordinate from its preferred sediment. This thesis avoided that problem by using only one fish in each treatment.

This study aimed to determine the sediment grain size preferences of bridled and exquisite gobies, using paired preference tests. If the sediment preferences of both species are similar, then one species may exclude the other from its preferred habitat.

4.1.2 METHODS

Sediment was collected from Waitemata and Puhoi, and then divided into four fractions by wet sieving: A. silt and clay (< 63 μm), B. fine sand (63–125 μm), C. medium sand (125–250 μm) and D. coarse sand (250–500 μm). Six combinations were possible for paired tests (AB, AC, AD, BC, BD, and CD). Dry 50 L tanks were divided into two equal sections and each section was filled to a depth of 15 mm with appropriate sediment (Figure 4.1.2). The tank was filled with 27 ppt synthetic sea water by siphoning onto a watchglass, to prevent disruption of the sediment. The tanks were surrounded with black polythene and placed side by side on a bench in a random order. The experiment was conducted with one goby in each tank. The fish were randomly selected for each experiment, measured and placed in numbered holding tanks. Each day at 7.30am, fish were placed in allocated tanks (Table 4.1.1). Observations were taken each hour from 8am-8pm under ambient laboratory illumination. The experiment was conducted from October to December 2006. The room temperature was 10-13.8°C and water temperature was 11°C.

Preliminary studies on bridled and exquisite gobies showed that both species searched with equal regularity in light and dark situations, so the experiment was solely conducted in light, hourly from 8 am to 8 pm.

250-500 µm	63-125 µm	125-250 µm	125-250 µm	250-500 µm	250-500 µm
<63 µm	<63 µm	63-125 µm	<63 µm	125-250 µm	63-125 µm

Figure 4.1.1. Position of sediment grain sizes in tanks.

Table 4.1.1. Example of treatment conditions for each fish. Six bridled and six exquisite gobies were randomly numbered 1-12 (in italics). Each fish was allocated to each of six possible treatments over 12 days.

	TREATMENT					
DAY	DA	BA	CB	CA	DC	DB
1	*10*	*3*	*1*	*5*	*6*	*7*
2	*9*	*12*	*12*	*6*	*10*	*2*
3	*12*	*6*	*7*	*2*	*4*	*8*
4	*2*	*4*	*5*	*3*	*11*	*9*
5	*3*	*8*	*10*	*9*	*12*	*1*
6	*5*	*5*	*2*	*1*	*8*	*10*
7	*8*	*9*	*12*	*4*	*1*	*11*
8	*6*	*11*	*3*	*7*	*2*	*4*
9	*1*	*10*	*8*	*11*	*7*	*12*
10	*11*	*2*	*6*	*10*	*5*	*3*
11	*4*	*7*	*9*	*12*	*3*	*6*
12	*7*	*1*	*11*	*8*	*9*	*5*

Figure 4.1.2. Sediment preference tanks. (2 of 6 tanks).

ANALYSIS

The 12 observations for each individual of each species in each sediment type were combined (Figure 4.1.3), and tested by chi-squared analysis in Microsoft Excel, to determine significant ($p<0.05$) sediment preference.

4.1.3 RESULTS

Bridled gobies consistently preferred the finest grain size (<63 μm) when available. Exquisite gobies significantly distinguished between sediment combinations in only three cases, each time preferring a different grain size (<63 μm, 63-125 μm and 125-250 μm), however, in each case the finer grain size was preferred (Figure 4.1.3, Table 4.1.2).

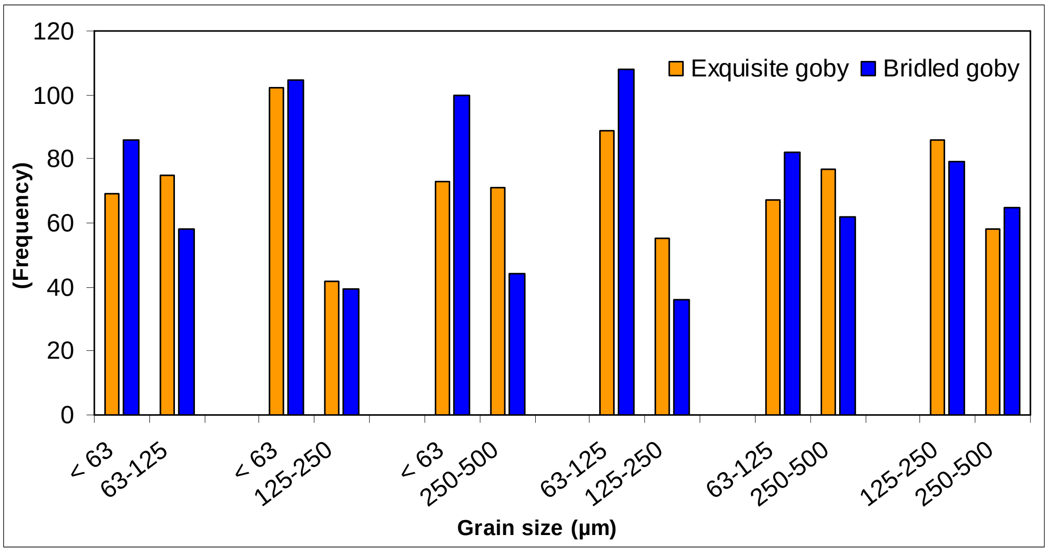


Figure 4.1.3. Sediment preferences for exquisite and bridled gobies.

Table 4.1.2. Sediment preferences for exquisite and bridled gobies. Significant observations are in bold.

Sediment combination		Exquisite goby p-value from χ^2	Exquisite goby Preferred sediment (μm)	Bridled goby p-value from χ^2	Bridled goby Preferred sediment (μm)
< 63	63-125	0.6171		**0.0196**	< 63
< 63	125-250	**0.0000**	< 63	**0.0000**	< 63
< 63	250-500	0.8676		**0.0000**	< 63
63-125	125-250	**0.0046**	63-125	**0.0000**	63-125
63-125	250-500	0.4047		0.0956	
125-250	250-500	**0.0196**	125-250	0.2433	

4.1.4 DISCUSSION

The sediment preferences of bridled and exquisite gobies were tested in paired sediment experiments, using four grain sizes (<63, 63–125, 125–250 and 250-500 μm). Bridled gobies consistently preferred fine sediment, which is consistent with

Usmar's (2003) study, in which bridled gobies preferred fine (consisting of 60-70% <63 μm) and medium (consisting of 30-40% <63 μm) sediment. Exquisite gobies did not significantly prefer any one sediment type, although, showed evidence of preference for finer grained sediment.

It was hypothesised that bridled gobies significantly prefer fine grained sediment because coarse sediment is less suitable for constructing burrows or for feeding on meiofauna (Usmar, 2003). Bridled gobies that remain fully subtidal may encounter coarse sediments that cannot be burrowed into; however, these individuals avoid predation with cryptic colouration and behaviour. Therefore, bridled gobies that are partially or fully subtidal may show different sediment preferences to the intertidal gobies collected for this study.

Subordinate bridled gobies may be forced to the edge of preferred sediment types, possibly encroaching on the habitat of exquisite gobies. Despite subordinate fish typically suffering from reduced fitness (Schofield, 2004), bridled gobies may prey on exquisite gobies (pers. obs.).

The results show that exquisite gobies have weak preferences for sediment grain size, and are thus likely to have broad habitat preferences, which may contribute to their widespread distribution. Prey type and abundance varies with grain sizes, so it is likely that the diet is more varied than currently recorded (McKenzie, 1984). Exquisite gobies have long been known to inhabit both fine and sandy sediments (McKenzie, 1984), but it is unknown whether they are now displaced from fine sediments in areas occupied by bridled gobies.

The results for both gobies are consistent with their wild habitat, with bridled gobies found on mud flats and exquisite gobies typically captured on sandy sediments (63-500 μm) (pers. obs.).

Estuarine sediment grades from coarse near the mouth to muddy upstream, though sediment type is mainly determined by catchment geology. Whangarei, Mangawhai, Whangateau and Tauranga are barrier enclosed tidal inlets, making them sandy at the mouth. Mahurangi, Puhoi, Waiwera, Weiti, Okura and Waitemata are drowned river valleys and more muddy (deLange, pers. comm.).

Fish are known to select placement within tanks based on factors other than treatment, such as light and structure (e.g. corner or exposed section of tank). Observations during the experiment included recording the position of fish within tanks and between tanks. No pattern was observed in the fish's position in tanks, so it is most likely that the position was based on sediment grain size, rather than an external factor such as light or structure.

Underwood *et al*. (2004) stated that sediment grain size should be measured in narrow classes and be repeated for fish of different ages and sizes. This experiment used narrow sediment size classes, but was only able to use adults, as juveniles were not captured.

Preference experiments require that an organism is aware of every option available (Noakes and Baylis, 1990). This was achieved by placing the fish in the centre of the tank and observing fish hourly to ensure that both sediment types were encountered. In 58% of cases fish moved between the sediments more than once, indicating they had experienced both sediment types. In 30% of cases the fish remained on one sediment type for all observations. However, as observations occurred hourly it is possible that the fish encountered the alternative sediment during unobserved periods.

Behaviour experiments generally do not reuse individuals; however, in this case it was more favourable to do so. Each individual was put in all sediment combinations so its preference for all sediment types could be ranked.

5 CONCLUSIONS

5.1 CONCLUSIONS

Bridled gobies have been in New Zealand since 1996 (Gray Jamieson Holdings Ltd, 1996), and it is believed that several invasions have occurred (Lavery *et al.*, 2006). This research aimed to determine the extent of niche overlap between bridled gobies and exquisite gobies. This chapter will draw together results and information from previous chapters to show what has been learnt and what research needs to occur in the future, in order to fully understand the impact of the invasive bridled goby in New Zealand.

Bridled gobies were widely tolerant to water salinity and temperature, but have specific fine sediment preferences. Their current survival and breadth of dispersal was likely aided by these tolerances. They are most likely to establish in the upstream zones of estuaries with fine sediment, rather than the mouth of estuaries or coastal regions. Dispersal between estuaries is most likely to involve the larval stages because adults would be required to migrate through regions of unsuitable habitat. Exquisite gobies currently occupy diverse sediment types within estuaries, and the increasing spread of bridled gobies may displace exquisite gobies from fine sediments. It is unlikely that the small exquisite gobies will withstand competition with and predation by bridled gobies in fine sediment zones, but the exquisite goby's broad sediment preferences will allow them to colonise sandy habitat, where the potential threat from bridled gobies is low. Bridled gobies have spread around north-eastern estuaries in the North Island, from one known site in 1996 to 13 sites in 2003, and are likely to continue dispersing given the range of suitable environments available.

Other small estuarine fishes may be susceptible to bridled gobies, including commercially (e.g. juvenile flatfish, *Rhombosolea* spp. and *Peltorhamphus* spp., and snapper, *Pagrus auratus*) and ecologically (e.g. diadromous) important species. The tolerance to low salinities by bridled gobies may allow them to encroach upstream in estuaries to occupy zones important for the spawning of diadromous species such as inanga (*Galaxias maculatus*). Environmental tolerances of the bridled goby are likely to exceed the tolerance limits determined in this thesis due to long term acclimatisation to local environmental conditions.

Although spatially distant from likely sources of non-indigenous coastal marine species, New Zealand estuaries have a high likelihood of colonisation by introduced organisms because of the typical estuarine morphology, and low biodiversity which reduces competitive exclusion of invaders. Current information suggests that bridled gobies could potentially invade any estuary with muddy sediments in the North Island.

5.2 RECOMMENDATIONS FOR FUTURE WORK

5.2.1 MONITORING

Monitoring is needed to track the spread of bridled gobies and to establish niche overlap or exclusion with native estuarine species in estuaries already colonised, since the current biosecurity measure of port monitoring is limited only to commercial harbours, it will not ascertain spread of the species to non-monitored harbours and estuaries. Monitoring will only determine whether bridled gobies are expanding their distribution if efficient methods for capture or observation are employed (such as those used for this thesis), although there is a low probability that seine (Willis *et al*., 1999) or fyke (Gray Jamieson Holdings Ltd, 1996) nets and benthic sleds (Inglis *et al*., 2006) will capture bridled gobies. Excavation of burrows or snorkel surveys are effective but time consuming and tidally dependent.

It remains uncertain whether bridled gobies have spread around New Zealand via the ballast tanks of ships or by swimming (Usmar, 2003). Hoese and Larson, (1980); Gee and Gee, (1991) and Neira *et al*., (1992) reported that bridled gobies occur in Australian freshwaters, and this affinity was confirmed in the salinity tolerance experiment. In New Zealand, bridled gobies have yet to be captured in freshwater, yet monitoring of their dispersal is required to demonstrate whether this is occurring, and if so, study of their effect on native freshwater fishes is essential.

5.2.2 ECOLOGY AND PHYSIOLOGY

Usmar (2003) studied the diet of bridled gobies; however, further work could determine the extent of prey overlap with other estuarine fishes. Additional research on reproductive strategies and further ecophysiological tolerances (e.g. hypoxia) will assist predictions of the further spread and competitive impacts of this invasive fish species.

5.2.3 GENERAL

New Zealand presently has eight recognised gobiid species of which one is endemic, two are native, two are not recorded in mainland waters and three are proposed as non-indigenous. Improved taxonomic identification of all these species would confirm the existing gobiid biodiversity. Detailed molecular studies could also identify the source populations of bridled gobies in Australia, and confirm the most likely method of introduction.

There are a large number of tropical and subtropical gobiid species in the aquarium trade and some of these species have the potential to survive in the wild in northern New Zealand. Public education may be needed at the time of purchase, to ensure owners know the correct means to dispose of unwanted fish.

It would be ideal to prevent future bioinvasions from occurring. Currently, the predominant strategies to reduce international transfer of marine organisms are ballast flushing and antifouling paint, which are both imperfect. It is recommended that more effective treatment of ballast water occur, and water deoxygenation is a favoured method, however, although this may kill larvae, it would be ineffective on the hypoxia tolerant adults of the bridled goby. Sea chests remain a possible vector for the transfer of this and other species, and it is likely that only physical inspection of ships in port will detect the presence of alien species.

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