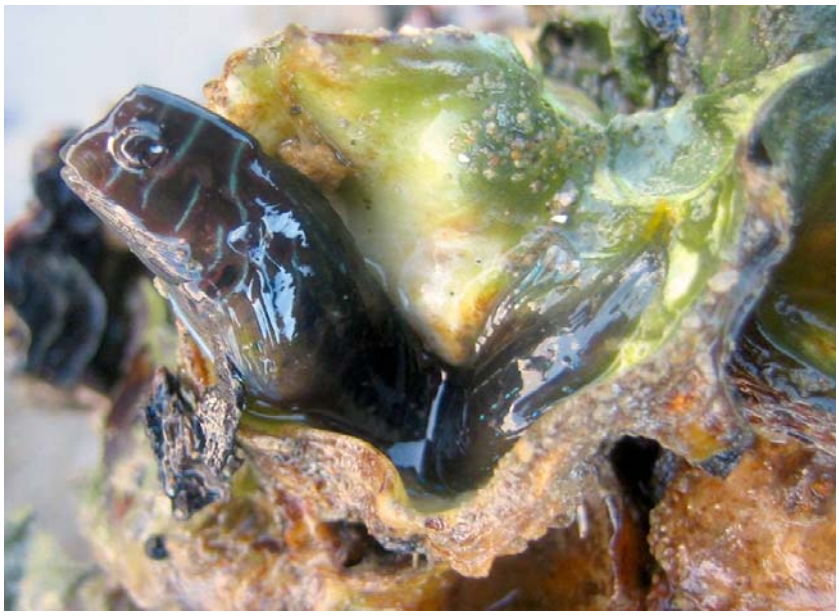
The introduced Australian oyster blenny, *Omobranchus anolius*



A thesis submitted to Auckland University of Technology in partial fulfilment of the degree of

Master of Applied Science

Jeremy J. Barker

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Abstract

In 2003, an intertidal fish, the Australian oyster blenny (*Omobranchus anolius*) was discovered in Auckland, New Zealand. Subsequent surveys of the inner Hauraki Gulf found only 24 specimens, the majority of which (n=20) occurred at a single location within Tamaki River. Most non-indigenous species do not establish viable populations, few of those that do spread; even fewer become abundant and widespread and have negative ecological impacts. Little was known about the ecology of this fish, it was unclear whether it would establish and spread in New Zealand, and in the event it did, what effect it would have on native flora and fauna.

This study had two main components. First the distribution and habitat utilisation of *O. anolius* was determined. Physical surveys were undertaken from the east coast of Coromandel Peninsula to Whangarei Harbour, while questionnaires were sent to oyster farmers from the Waitemata Harbour to Houhora Harbour. Thirty minute counts were used to establish abundance. The second main part of this study was on the life history characteristics of *O. anolius*. Monthly samples were collected and gonad development was determined over the course of the year. Nests were also collected, and the number of eggs in each determined.

Results from this research show that *O. anolius* is now widespread and abundant throughout the Waitemata Harbour and Tamaki River region, and that it has spread as far north as Whangateau Harbour and as far east as Coromandel Peninsula. Its preferred habitat is in the shells of the invasive oyster *Crassostrea gigas*, especially when the oysters have grown into clumped assemblages. It was also found on hull fouling and in oyster farms.

Omobranchus anolius is successfully breeding in New Zealand. The spawning season is from October through to March, based on histological examination of gonads, presence of nests, and gonosomatic index. It is a batch spawner, releasing eggs throughout the spawning season.

Based on these findings *O. anolius* is likely to increase its range in New Zealand and this spread is likely to be further facilitated by the invasive oyster *C. gigas*.

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Attestation of Authorship

I hereby declare that this submission is my own work and that to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Jeremy J. Barker

Chapter 1: General Introduction

Project description

The Australian oyster blenny, *Omobranchus anolius* (Valenciennes, in Cuvier & Valenciennes, 1836) was first reported in New Zealand in 2003 (Francis *et al*. 2004). This prompted surveys of the inner Hauraki Gulf in 2003 which found 24 specimens, 20 of which occurred at a single location (Francis *et al*. 2004). Little was known about the biology or ecology of *O. anolius* at this time. It had not been found outside of Australia before and it was unclear whether it would establish a viable population. If it did, it was uncertain whether it would expand its geographic distribution and become widespread. This study focuses on three important aspects of the biology and ecology of this introduced fish. 1) A determination of the spread of *O. anolius* in Hauraki Gulf. 2) An investigation of its preferred habitat in New Zealand. 3) An investigation of its life history characteristics. This information is needed to answer the question, is the introduced oyster blenny, *O. anolius* likely to become widespread and have negative environmental impacts in New Zealand waters?

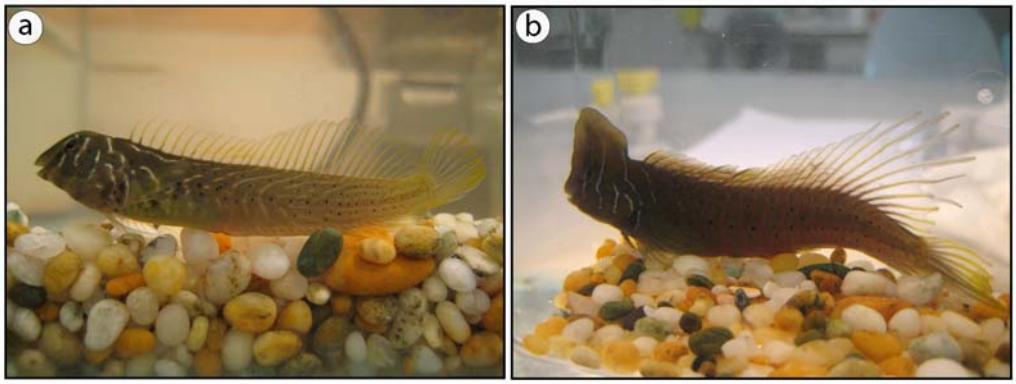

Figure 1. *Omobranchus anolius*: a=female, b=male.

Omobranchus anolius

Omobranchus anolius is a small (<82 mm TL) greenish-brown fish with small black spots scattered along the trunk (Figure 1). They can also exhibit vertical blue-white lines along the head and cheeks, blue-white spots along the dorsal and anal fins, and chevron-like white bands along the trunk. The colour patterns are more blue and distinctive in males. This species possess a single row of comb-like teeth, with an enlarged canine tooth located posteriorly on each side of both upper and lower jaws (Springer 1972, Kuiter 1993). Males can be distinguished from females by elongated posterior dorsal fin rays and a prominent, medial blade-like head crest, a feature absent

or poorly developed in females (Springer & Gomon 1975). *Omobranchus anolius* commonly occur inside dead oyster shells on tidal mudflats, which they use for refuge and nesting sites (Smith-Vaniz 2008); males also show parental care (Thomson & Bennett 1953).

Blenniidae

Omobranchus anolius belongs to the family Blenniidae (suborder Blennioidei, order Perciformes), a large family currently recognised to contain 57 genera, five tribes, and 387 species (Hastings & Springer 2009). Fish within the suborder Blenniodei are referred to as blennioid fish and are commonly given the ambiguous common name 'blenny', with those in family Blenniidae called combtooth blennies, but sometimes simply referred to as blennies. To avoid confusion the term blenny or blenniid fish will be used throughout this thesis to denote those members of the family Blenniidae only; fish belonging to the suborder Blennioidei will be referred to as blennioid fish.

Blennies are distributed worldwide but most commonly are found in the tropics, with a few species in temperate waters; species diversity reduces in higher latitudes (Kuiter 1993). They occur in a variety habitats including: coral reef, mangrove, mollusc beds and brackish waters, and rocky reef (Hastings & Springer 2009), where they normally inhabit crevices, burrows or the empty valves of bivalves.

Blennies are small elongate fish with adults ranging in size from 15 to 532 mm standard length (SL); most do not exceed 150 mm (TL) (Springer 1982). They have blunt heads, and continuous dorsal fins, and are characterised by teeth arranged in a comb-like row and the absence of scales (Smith-Vaniz 2008, Patzner *et al*. 2009). Some genera have enlarged canines which can be used in combat or defense (Smith-Vaniz 2008, Patzner *et al*. 2009). Blennies are usually near-shore benthic fish that lack a swim bladder, although there are exceptions in species that occur in the pelagic zone (Smith-Vaniz 2008). Benthic species feed on a mixed diet of algae and invertebrates, with intertidal-dwelling species having a higher proportion of algae in their diet (Kuiter 1993). Pelagic species are planktivores or are specialised to feed on scales and fins of larger fish by mimicking cleaner fish (Kuiter 1993).

All known blennies are nest builders and exhibit parental care (Springer 1982, Almada & Santos 1995). Blennies generally are sexually dimorphic, with males usually attaining a larger size than females, and they often exhibit secondary sex characteristics (Oliveira *et al*. 2001). Blennies follow a generalised pattern of reproductive behaviour which consists of a male preparing a nest in a cavity e.g. crevices, holes or empty bivalve shells, then attracting a female to the nest; the female then deposits a layer of demersal eggs in the nest which are guarded and sometimes fanned by the male (Fishelson 1975, Gibson 1993, Almada & Santos 1995, Oliveira *et al*. 2001). Courtship interactions for most blennies are initiated exclusively by males (Almada & Santos 1995, Kraak 1996, Shibata & Kohda 2007). Males often mate with multiple females, and can even mate with several females at the same time (Shibata & Kohda 2007).

Seven species of blenny occur in New Zealand, five of which are common (Francis 2001), but only two occur in coastal waters of mainland New Zealand; the others are restricted to the subtropical Kermadec Islands (Paulin & Roberts 1992). New Zealand's blennioid fish are well represented by triplefins (family Triptergiidae) with 26 species, all endemic to New Zealand, apart from three species that have been accidentally introduced to Australia (Clements 2003).

Omobranchini

Within the family Blenniidae *O. anolius* belongs to the tribe Omobranchini which contains seven genera and 34 species (Hastings & Springer 2009). They are native to the Indo-Pacific with one species *Omobranchus punctatus* introduced to the Caribbean (Springer & Gomon, 1975). All Omobrachini are benthic and occur at depths of less than 5 m; those in the genus *Omobranchus* (21 species) rarely occur deeper than 0.5 m (Springer 1972, Springer & Gomon 1975). They normally occur on corals, rocks and shell rubble substrates (Springer 1972, Springer & Gomon 1975). Most Omobrachini attain a length not exceeding 75 mm SL; a few species reach 100 mm (SL) (Springer 1972). Sexual dimorphism is significant in many species, with most differences (e.g. larger size, coloration and a head crest) making males more visible; perhaps the result of sex recognition or territory maintenance (Springer & Gomon 1975).

Biological introductions

Human-mediated introductions of non-indigenous species into regions where they did not exist in evolutionary and ecological time are one of the most immediate threats to biological diversity (Vitousek *et al*. 1997, Sala *et al*. 2000, Bax *et al*. 2003). Non-indigenous species (NIS) can predate native species, compete with them for food, habitats and other resources, and transmit pathogens and hybridise with natives (Mack *et al*. 2000, Sakai *et al*. 2001). These negative interactions can result in displacement or extinctions of native species (Clavero & Garcı´a-Berthou 2005, Sax & Gaines 2008) and can disrupt entire ecosystems (Mack *et al*. 2000, Crooks 2002). As well as severe ecological impacts they also can have significant economic and social impacts (Parker *et al*. 1999, Pimentel *et al.* 2000, Hewitt *et al*. 2004).

Although introductions in marine systems have received less attention than freshwater and terrestrial systems (Carlton 1999), they are one of the most heavily invaded systems in the world (Grosholz 2002) and these introductions are a major threat to marine biodiversity (Sala & Knowlton 2006). Most non-indigenous marine species (NIMS) are molluscs or crustaceans (Ruiz *et al*. 2000); relatively few are fish introductions (Baltz 1991). Few NIMS become invasive and thus result in negative ecological impacts, however most NIMS are poorly studied and it remains unknown which will or will not have an impact (Ruiz *et al*. 1997, Hewitt *et al*. 2004).

The introduction of NIMS has been occurring since humans started to traverse the oceans (Carlton 1999, Hewitt *et al*. 2004). Our lack of knowledge on the original state of environments prior to 1900 means the full extent of these invasions remain unknown and probably underestimated; many NIMS are now fully incorporated into native biota and cannot be distinguished as introductions (Carlton 1989, Baltz 1991, Cranfield *et al* 1998). Shipping via hull fouling, sea chests and ballast water are the largest vector of NIMS, but deliberate introductions, aquaculture transfer, aquarium trade and connection of waterways through canals are also important vectors (Ruiz *et al*. 1997, Cranfield *et al* 1998, Ruiz *et al*. 2000, Coutts *et al*. 2003, Coutts & Dodgshun 2007). The rate of invasion is increasing as reliance on international shipping increases (Ruiz *et al*. 1999, Ruiz *et al*. 2000). Historically, hull fouling was considered the largest vector but ballast water has now become the most prominent transport mechanism for NIMS (Ruiz *et al*. 1997), with over 7000 species estimated to be transported globally each day (Carlton 1999). At a regional scale, fouling on the hull and other parts of the vessel is an

important vector because in can occur on vessels of all sizes; as opposed to ballast water which occurs only in large vessels (Wasson *et al*. 2001, Floerl & Inglis 2005, Dodgshun *et al*. 2007, Acosta & Forrest 2009).

The successful establishment and spread of NIMS is a process which involves a series of sequential transitions (Williamson 1996; Kolar & Lodge 2001, 2002). The prospective NIMS must: a) be entrained by a vector, b) survive the transport to a novel environment, c) establish a self sustaining population and then d) possibly increase in abundance and spread over a wider geographical range. Only when a non-indigenous population becomes widespread and abundant will it cause ecological or economic harm and receive the title of invasive (Lockwood *et al*. 2007). These stages can be viewed as barriers with each having a high probability of failure, so few species ever establish populations and even fewer become invasive (Williamson 1996; Kolar & Lodge 2001, 2002).

NIMS must be in the vicinity of a potential vector and have characteristics that allow it to be entrained by that vector before it can be transported. This often means they must have populations near a port or marina (Floerl & Inglis 2005) and they must be able to either attach as hull fouling, or be up taken into ballast water (Wonham *et al*. 2000). They must then survive the journey which may involve difficult conditions. For example ballast water will usually have extremes of salinity, temperature and of course light regimes (Lockwood *et al*. 2007).

Those species that survive transport and are released into the novel community must find a recipient environment that meets their physiological requirements. The potential for mismatch is high and is the main reason many species fail to establish (Lodge 1993, Lockwood *et al*. 2007). If the basic environmental factors are met, species establishment and success is governed by a complex suite of interacting biotic and abiotic factors.

One barrier a new organism must face is the biotic resistance of the community, that is predation by, and competition with, native species or NIMS that have previously established (Baltz & Moyle 1993, Moyle & Light 1996, Mack *et al*. 2000). However, sometimes native species can actually facilitate the establishment of a NIMS (Wonham 2005) while previously established NIMS can do the same (Simberloff & Von Holle 1999). This cycle can be repeated again and again, leading to a process of "invasion meltdown", but as of yet there is no conclusive evidence for this (Simberloff 2006).

As NIMS founding populations are generally assumed to be small, another barrier to establishment are the associated genetic bottlenecks (Sakai *et al*. 2001, Allendorf & Lundquist 2003). Reduced genetic diversity can have two consequences: first it can result in inbreeding depression which can limit population growth and lower the probability that a population will persist; second it can reduce the ability of the population to evolve (Sakai *et al*. 2001, Allendorf & Lundquist 2003). In contrast, recent research suggests that genetic bottlenecks are not as common as first thought due to the occurrence of multiple introductions and high propagule numbers in vectors such as ballast water (Roman 2006, Roman & Darling 2007). The likelihood of establishment is substantially increased if more individuals are released, if the individuals are healthy, and if there are multiple release events (Wonham *et al*. 2000, Ruiz *et al*. 2000, Verling *et al*. 2005, Colautti *et al*. 2006).

The life history of the NIMS will also play a role in determining whether they will establish a population and spread (Sakai *et al*. 2001, Lockwood *et al*. 2007). Attempts to identify the specific life-history characteristics that increase the probability of establishment has been the focus of many studies across a range of taxa (e.g. Lodge 1993; Wonham *et al*. 2000; Kolar & Lodge 2001, 2002; Marchetti *et al*. 2004; Olden *et al*. 2006; Garcia-Berthou 2007). However, while characteristics can be identified in specific situations, no generalised patterns have been found because of confounding factors (Colautti *et al*. 2006, Lockwood *et al*. 2007). For example particular life-history characteristics may be important at one stage of an invasion but may not be relevant or even detrimental at another stage (Wonham *et al*. 2000, Kolar & Lodge 2001).

The properties of the recipient community also play a role in whether a species will establish and become invasive. It has been hypothesised that communities with high species richness have fewer niches for fish to go into and this has been described as the vacant niche theory. For example it has been shown that NIMS are most abundant in brackish waters with low species richness (Paavola *et al*. 2005). Also the enemy release theory hypothesises that NIMS in a novel environment may find that they are free from predators, and because of this they may flourish (Colautti *et al*. 2004).

Non-indigenous marine species in New Zealand

The introduction of NIMS into New Zealand pose serious threats to New Zealand's marine environment. Due to its geographic isolation New Zealand is particularly susceptible to marine invasions because over 90% of trade is transported via shipping, these waters receive a disproportionately large amount of ballast water relative to their size (Hewitt *et al*. 2004, Wotton & Hewitt 2004).

It is likely that Maori introduced the first NIMS into New Zealand (Hewitt *et al*. 2004), but since European colonisation many more species have been introduced; the majority (69%) of these are believed to be due to hull fouling. Ballast water is the largest concern currently (Hewitt *et al*. 2004), however Hewitt *et al*. (2004) list a variety of vectors and identify 43 pathways for NIMS to arrive in New Zealand. Cranfield *et al*. (1998) reviewed the literature and using the criteria of Chapman & Carlton (1994) identified 159 adventive marine species in New Zealand, 148 of which were introduced accidentally. Nineteen percent of the species failed to become established (many more would have failed before being recognised), and 21% only occur at one locality; 66% have established at more than one locality (Cranfield *et al*. 1998). The majority of these introductions were algal or invertebrate species (Cranfield *et al*. 1998). Until recently the only introduced marine fish in New Zealand that was recorded as established was the deliberately introduced Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum in Artedi, 1792) (Francis *et al*. 2004). Since 1998 four suspected introductions of small marine fish to northeastern New Zealand have been discovered (Francis *et al.* 2004): in 1999 the Australian estuarine goby *Arenigobius bifrenatus* (Kner, 1865) was discovered (Willis *et al.* 1999); in 2000 a brackish-water microdesmid *Parioglossus marginalis* (Rennis & Hoese, 1985) (McDowall 2001); in 2003 another estuarine goby *Acentrogobius pflaumii* (Bleeker, 1853) (Francis *et al.* 2003); and in 2003 the Australian oyster blenny, *Omobranchus anolius*. Each invasion is suspected to have resulted from transport through shipping via hull fouling, sea chests or ballast water (Francis *et al*. 2004).

Aims

This study focuses on three important aspects of the biology and ecology of the introduced *O. anolius*. 1) determination of the spread of *O. anolius* throughout Hauraki Gulf. 2) An investigation of its preferred habitat in New Zealand. 3) An investigation of its life-history characteristics.

This thesis has been written as two main chapters that each will form the basis of papers destined for peer-reviewed journals; the first on the spread, distribution and habitat utilisation of *O. anolius*, and the second on the life-history characteristics of *O. anolius*. Each Chapter is thus self-contained while the General Discussion brings together the findings of the two chapters to address the underlying questions of this thesis: is the introduced oyster blenny *O. anolius* likely to become spread beyond its current range, and is it likely to have negative environmental impacts in New Zealand waters?

Chapter 2: Habitat and distribution

Introduction

The distribution and habitats that are utilised by non-indigenous species (NIS) is critical data to determine their potential impact (Grosholz & Ruiz 1996). Abundant and widespread NIS's are more likely to impact on native species (Ruiz *et al*. 1997). The wider the range of potential habitats a NIS may occupy the more species it is likely to impact upon (Parker *et al*. 1999). In its introduced range the variety of habitats a NIS occupies may be greater or lesser than its native range because of varying biotic and abiotic conditions (Grosholz & Ruiz 1996). Therefore knowledge on the habitats that the NIS occupies in the introduced range must be determined and not be entirely derived from knowledge of its native habitat.

In Australia *Omobranchus anolius* has a wide latitudinal distribution, ranging from Spencer Gulf, South Australia, to the southern Gulf of Carpentaria (Springer & Gomon 1975, Francis *et al*. 2004). However *O. anolius* has only once been reported north of Gladstone on the eastern coast of Queensland, based on a single specimen from Norman River in the southern Gulf of Carpentaria in 1914 (Francis *et al*. 2004). The habitat of *O. anolius* in Australia has not been described in detail, but *O. anolius* has been found in shallow sheltered in-shore waters, is common on tidal mud flats in bays and estuaries in association with oyster beds, and is usually found inside the valves of dead oyster shells (Thompson & Bennett 1953, Grant 1987, Kuiter 1993, Smith-Vaniz 2008), though it also has been found in tubeworm encrustations (Kuiter 1993, Smith-Vaniz 2008), pools of water (Thompson & Bennett 1953), and a rock crevice (Ogilby 1911).

In 2003 the distribution of *O. anolius* in New Zealand was determined from a rapid survey of 36 intertidal sites within the inner Hauraki Gulf (Francis *et al*. 2004) (Figure 2). *Omobranchus anolius* was present at Okahu Bay and at three sites along the north-western side of Tamaki River estuary. However the distribution of *O. anolius* was largely limited to one site in Tamaki River estuary, *Tahuna Torea* Reserve, where 20 of the 24 then-known specimens were collected. All specimens were collected on mudflats beneath boulders with pools of trapped seawater. About half of the specimens were found on the underside of rocks either between the articulated valves of dead oyster (*Crassostrea gigas*) shells or amongst accretions of live oysters and tubeworms (*Pomatoceros caeruleus*). The other half were found in pools of water beneath boulders. No specimens were found on exposed intertidal oyster clumps or on wharf pilings. Not all sites surveyed had suitable habitat but some sites at which *O. anolius* was absent had

habitat which appeared to be suitable. Francis *et al*. (2004) suggested that the large numbers of blennies found at *Tahuna Torea* might partly reflect a preferred microhabitat. The tidal flats at *Tahuna Torea* have a sandy mud substratum and shallow gradient, allowing shallow pools to form around the base of boulders at low tide; it also has boulders with dead oysters on the underside around the high-intertidal zone.

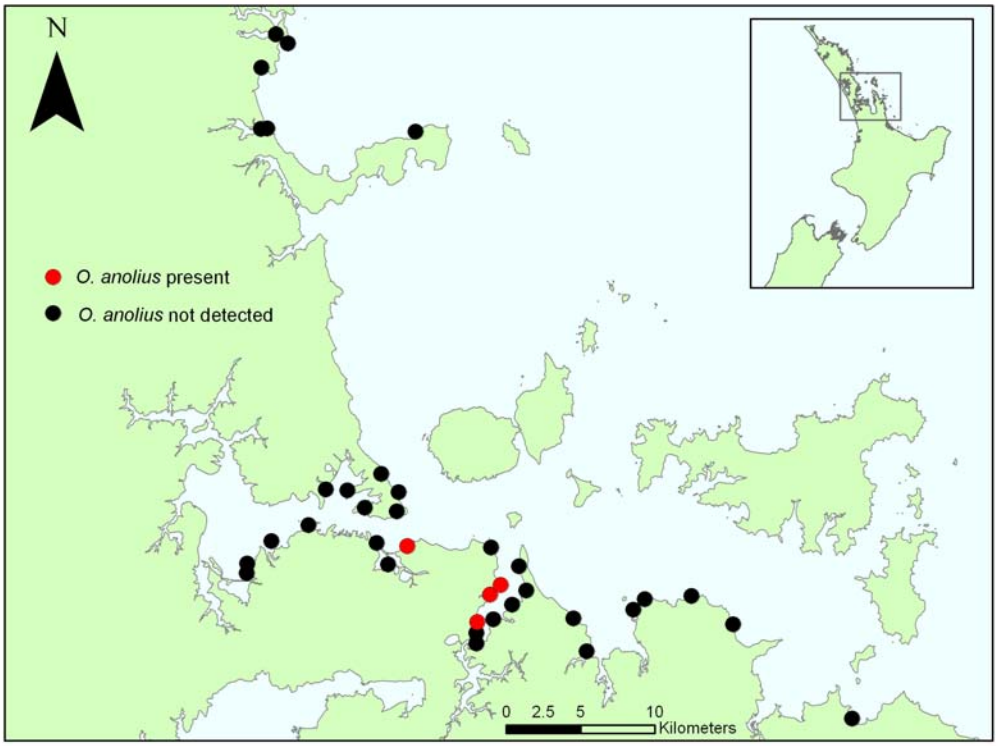

Figure 2. Sampling sites and records of *O. anolius* from Francis *et al.* (2004).

Prior to this study it was unknown whether *O. anolius* had established a self-sustaining population in New Zealand, and if so whether it had successfully spread. Knowledge on the habitat of this species was limited and it was unknown whether it would occupy a limited range of habitats affecting a small number of sites or whether it would be widespread. Therefore the aim of this study was to: 1) establish the geographic range of *O. anolius*, 2) determine the abundance of *O. anolius* within its range, and 3) determine the habitat of *O. anolius* at different spatial scales.

Methods

Survey areas and site selection

Between April 2008 and March 2009 128 sites were physically sampled on the east coast of North Island from Whitianga Harbour (36°50′S, 175°43′E) in the south to

Whangarei Harbour (35°44′S, 174°20′E) in the north. The locations sampled were: Whangarei Harbour, Whangateau Harbour, Waiwera estuary, Orewa estuary, Okura River estuary, Rangitoto Island, Waiheke Island, Waitemata Harbour, Tamaki River estuary, Firth of Thames, Coromandel Harbour, Colville Bay, Whangapoua Harbour and Whitianga Harbour. To determine how widespread *O. anolius* was in the area where it was discovered, 51 sites were sampled within Waitemata Harbour and Tamaki River region. Additional sites were surveyed by questionnaire as outlined below.

The major selection criteria for initial surveys of Waitemata Harbour and Tamaki River were based on the descriptions of *O. anolius* habitat in Australia and New Zealand. Tidal mud and sandy mudflats were initially sampled, however an explorative approach was taken; if *O. anolius* was present at a site that had been selected due to the presence of mud or sandy mudflats, then adjacent broad-scale habitat was sampled. Potential sites were ascertained from aerial photographs, but the final selection of sampling sites was conducted by pedestrian reconnaissance to identify oysters and tubeworm encrustations that could possibly provide shelter for *O. anolius* during low tide periods. In the event no oysters or tubeworm encrustations were identified then the site was still sampled using the protocol below with any possible habitat that could provide shelter carefully examined, e.g. isopod burrows, boring-bivalve holes, under rocks, cracks and crevices etc. Geographic coordinates of the sites sampled in 2003 were obtained from Francis *et al*. (2004) and these sites were sampled again, including those sites that did not meet the selection criteria used in this study (e.g. an exposed sandy beach).

Sampling

Sampling was undertaken 2 hours either side of low tide, with an effort made to sample on tides of 0.3 m or less when possible. One to six sites could be searched during one tidal cycle depending on the distance of the final site selected from the vehicle and the proximity between sites. At each site, position was recorded using a hand-held global positioning system (GPS) (Garmin eTrex®) and an intensive and systematic investigation was conducted for 30 minutes. The top and side of each three dimensional structure was examined, and if present the underside (as in the case of boulders). If a pool of water was present on the underside this was allowed to clear before searching for fish. The focus was on oyster shells but if other shelter (e.g. cracks, crevices, burrows, underneath boulders without oysters) were present these were examined also. Tweezers were used to open dead oyster shells and examine cracks and crevices, in

cases where there was a dense layer of oysters, these were removed by hand (using gloves) and examined. For each fish found, habitat variables at broad to fine spatial scales were recorded (Table 1). Each fish was euthanized by pithing (*sensu* Mountfort *et al*. 2002) and placed into pre-labelled containers in 10% formalin. The abundance and habitat variables of each native fish encountered were also recorded. In cases where the identification of native fish was uncertain these were euthanized and returned to the laboratory for identification. When sampling was conducted within a marine reserve, fish (native or introduced) were not collected, only the number of fish found within 30 minutes recorded. Habitat variables were also recorded for fish collected for reproductive studies (Chapter 3) that were not included in the timed 30 minute count. All sampling was conducted by the same person to minimise sampling bias. Voucher specimens of *O. anolius* collected during sampling were accessioned into the Auckland War Memorial Museum *Tamaki Paenga Hira* and Museum of New Zealand *Te Papa Tongarewa*.

Habitat description

After extensive sampling of intertidal habitats around estuaries and bays along the northeast coast of the upper North Island, habitat variables relevant to *O. anolius* were established. For each site, habitat was categorised into seven broad-scale categories: mudflat, sandy mudflat, reef platform, artificial, mangrove, sand and cobble (Figures 3 and 4). Within each of these broad habitats it was possible for small sections of habitat meeting the description of another broad-scale habitat to be present because the habitat was generalised at a broad-scale e.g. a small section of reef could be within an expansive mudflat. However at a location where more than one distinct broad-scale habitat was present each of these habitats were treated as separate sites, e.g. one location may have a mudflat site, mangrove site and reef platform site. The divisions between each broad-scale habitat were obvious, and sandy mudflats were distinguished from mudflats in that they were firm under foot and had a high proportion of coarse sand grains present in the sediment. The tidal zone of each broad-scale habitat was defined into three categories: low, medium or high.

Table 1. Description of habitat variables see figure 3 &4 for corresponding photographs.

Habitat Variable	Definition
Broad-scale	
Mudflat	Soft sediment with fine grains.
Sandy mudflat	Presence of coarse grains than mudflat. Sediment is much firmer and may contain ripples.
Reef platform	Bedrock that may or may not be covered with a layer of sediment.
Artificial	Highly modified habitat, consisting only of man-made structures
Mangrove	Mangrove trees and their pneumatophores
Oyster species	*Saccostrea glomerata* was distinguished from *Crassostrea gigas* by the presence of denticles in *S. glomerata* surrounding the valves (these are absent in *S. glomerata*).
Oyster growth form	
Encrusted	Lower valve of oyster grows flat against attachment. Sides may touch forming a layer but valves do not grow on top of one another.
Clumped	Valves may be upright and may grow on top of one another. Forms a relatively more complex structure compared to the encrusted growth form.
Other	Tube worm
Attachment	
Boulder	Size >260 mm
Mangrove	Mangrove trunk or pneumatophores
Artificial	Human-developed artificial structures
Rock Reef	Bedrock that may or may not be covered with a layer of sediment.
Loose	Oyster not attached to a permanent hard substratum. May be a single shell or a clump of shells growing on one another.
Cobble	57 – 260mm
Other	Other hard substrata, e.g. Logs, metal and plastic debris
Shelter found	
Shell	Inside the intact articulated valves of an oyster shell
Between shells	In crevices between a clump of oyster shells
Pool	The moist sediment on the underside of a structure that may or may not form a pool depth enough to cover the animal.
Position	
Top	Upper horizontal or convex surface
Side	Vertical surface
Bottom	Underside surface
Pool	Moist habitat underneath a hard substratum which may or may not actually form a pool
Available habitat	To investigate if the fish preferred top, side or underneath structures, when a fish was encountered the adjacent “available” habitat was recorded

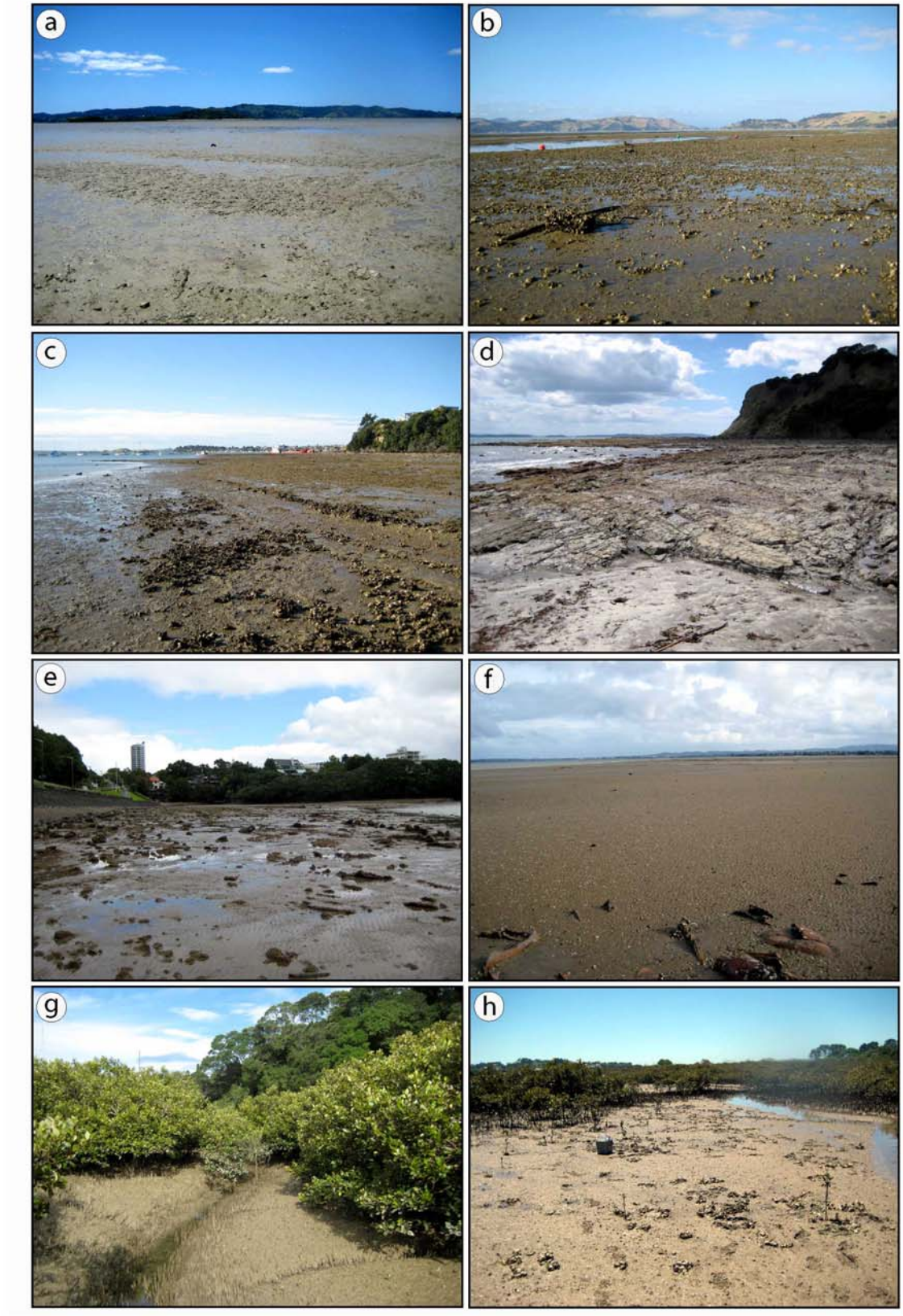

Figure 3. Examples of broad-scale habitats sampled: a & b = mudflat, c & d = reef platform, e & f = sandy mud, g & h = mangrove.

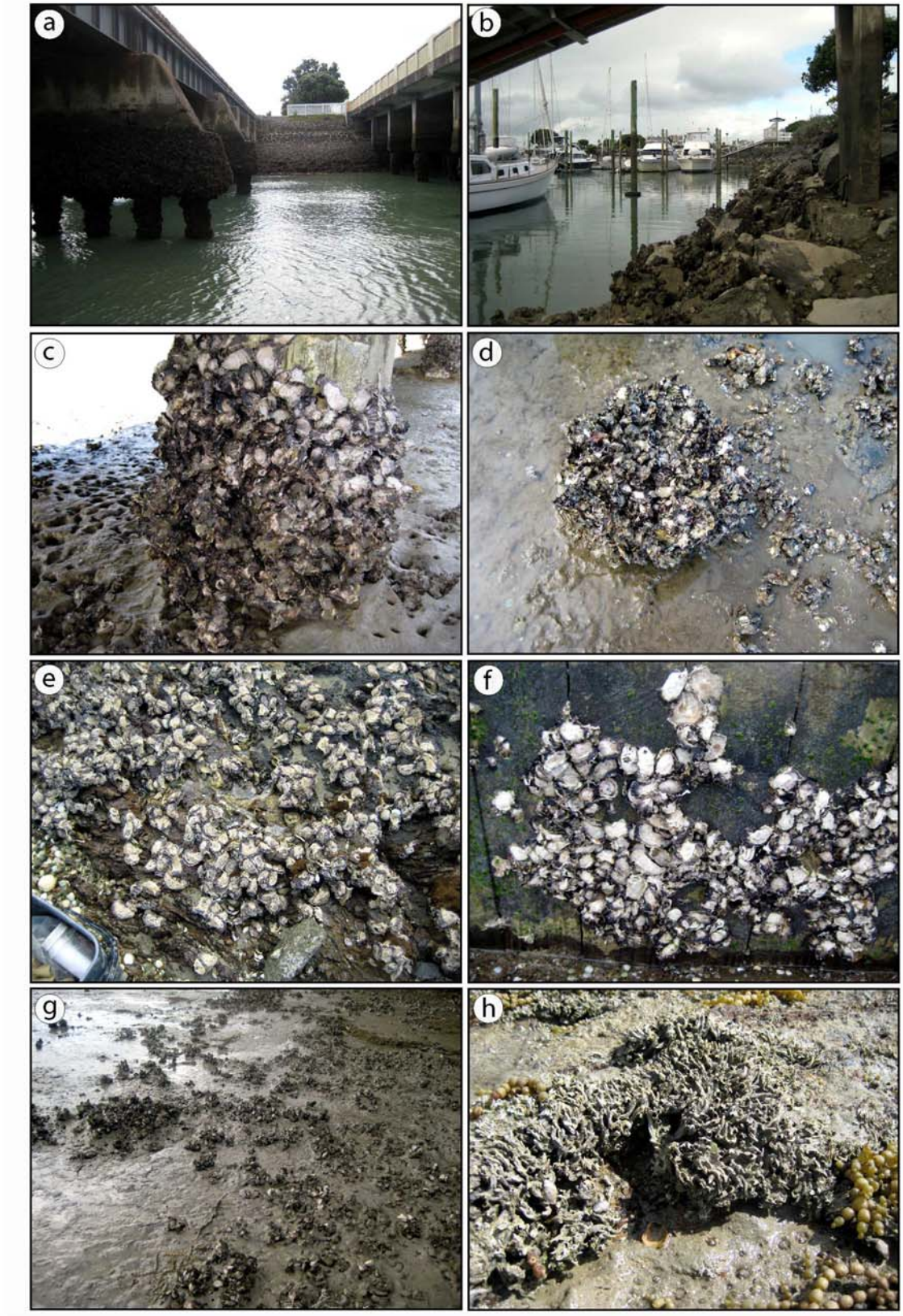

Figure 4. Examples of broad-scale habitat and examples of shelter: a & b = artificial habitat, c & d = clumped oysters, e & f = encrusted oysters, g = loose oysters, h = tubeworm encrustation.

At each site the presence of tubeworm encrustations and oysters was recorded. Two species of oysters were encountered which, based on external characters, can be difficult to distinguish. Internally, denticles are present on the articular edges of the native rock oyster *Saccrostrea glomerata* but not in the invasive oyster *Crassostrea gigas*, therefore at each site a selection of shells were examined internally (Figure 5). There was also two oyster growth forms, "encrusting" and "clumping", that are typical of each species but not limited to either. Encrusting oysters grow flat against a hard substratum and may grow singly or form a crust over the substratum, with the sides of each shell touching but not overlapping or growing on top of one another (Figure 4e and 4f). Clumping oysters may grow on top of one another forming a more complex habitat and are typical of *C. gigas* (Figure 4d and c).

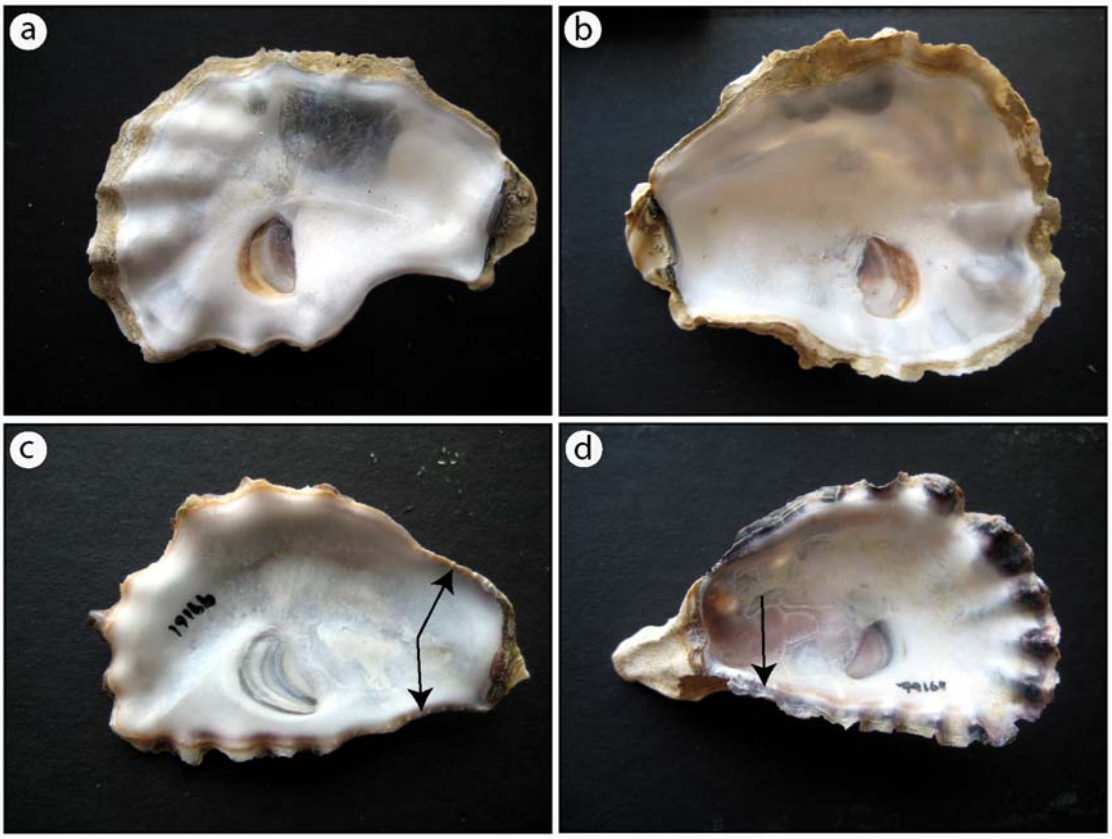

Figure 5. Oyster species: a & b = *Crassostrea gigas*, c & d = *Saccostrea glomerata* (arrows point to characteristic denticles on valves).

When a fish was found the immediate habitat was described in detail as in Table 1. Whether they were found inside an oyster shell or within the spaces between oyster shells, in cracks or crevices, or under a boulder in the moist sediment which may or may not form a pool of water (in this study we define this as "pool"). If a fish was found associated with oysters or tubeworms it was recorded what hard substratum they were

attached to, if any. These categories were: boulder, mangrove trunk or pneumatophore, artificial substratum, small section of reef platform, cobbles, 'other' which included logs, metal and plastic debris, or 'loose' which was where oysters were not attached to any fixed substratum other than a small stone, cockle shell or another oyster which can then form conglomerations of oysters. It was also recorded whether the fish were found in tube worms or oysters on the top, side or underside of the structure they were attached to. For example, a boulder fully coated with oysters provides potential habitat on its top, side and underside. In many cases not all of these options were available, so which if these were available was also recorded. For example on a wharf pile blennies could only be found on the side as there were no other options, or on a reef platform only the top or perhaps a side may be available, but not underneath.

Marine Farm survey

Information provided by an oyster farmer suggested that *O. anolius* was present in some oyster farms and that they had been observed while shucking oysters in the processing plant. To confirm whether *O. anolius* was associated with this potential vector and to enable sites to be sampled outside of the study area, questionnaires were sent to oyster farmers (Appendix II). An address list for oyster farmers was obtained from the New Zealand Oyster Farmers Association, and questionnaires were sent to 50 oyster farmers with postal addresses in the North Island. The questionnaire was designed to find out whether *O. anolius* was present in their farm(s) and if present when they were first noticed. To aid farmers in the identification of *O. anolius* a poster was designed with photographs and a simple description of each fish, including fish that it could be confused with. Prototype posters and fish samples were given to people who were inexperienced in fish identification and they were asked to identify the distinguishing characteristics they found helpful in identifying *O. anolius*. From their comments the slender nature of *O. anolius* and presence of head crest were considered the most obvious features; descriptions of dorsal fin rays were considered confusing and were removed from the poster. Each questionnaire was accompanied with a self-addressed stamped envelope and an introductory letter and consent form to make sure the oyster farmers were fully informed of the study and that their consent was obtained. The questionnaire and all accompanying documents were submitted to Auckland University of Technology Ethics Committee (AUTEC) and modified according to their requests. Final approval was received on 2 December 2008 AUTEC Reference number 08/241.

Results

Distribution and abundance

Of the 128 sites examined, *O. anolius* was present at 61 sites; a total of 486 *O. anolius* collected during the timed 30 minute counts. Including fish collected for reproductive studies a total of 734 *O. anolius* were collected. The northernmost site *O. anolius* was present was Whangateau Harbour (36º20′S, 174º45′E), the eastern most sites were on the western side of the Coromandel peninsular at Colville Bay (36º37′S, 175º27′E) in the north and Tarau (37º70′S 175º31′E) in the south of the peninsula (Figure 6). Outside of this range *O. anolius* was not detected at 14 sites in Whangarei Harbour or at 8 sites on the eastern side of the Coromandel Peninsula. The maximum number of *O. anolius* captured during the timed 30 minute counts was 28, with a mean number of 7.8 (SE 0.88) in the 61 sites where they were present. On one occasion, not included within the timed count, 31 fish were found amongst the oysters of one boulder (Figure 10a).

Within Waitemata Harbour and Tamaki River region, *O. anolius* was distributed widely, being present at 49 of the 58 sites sampled with a total of 407 specimens collected (Figure 7). During the distribution survey, 35 of the 36 sites surveyed by Francis *et al*. (2004) in 2003 were resurveyed and 233 *O. anolius* were found at 22 of these sites (Figure 8). The native rockfish *Acanthoclinus fuscus* and triplefin *Grahamina* sp. occurred throughout the entire sampling range. A total of 52 *A. fuscus* were recorded from 12 sites with the maximum number recorded during the timed 30 minute count was 15, with a mean of number of 4.3 (SE 1.3). A total of 23 *Grahamina* sp. were recorded from 10 sites, the maximum number recorded during the timed 30 minute count was 7, and the mean number recorded was 2.30 (SE 0.58).

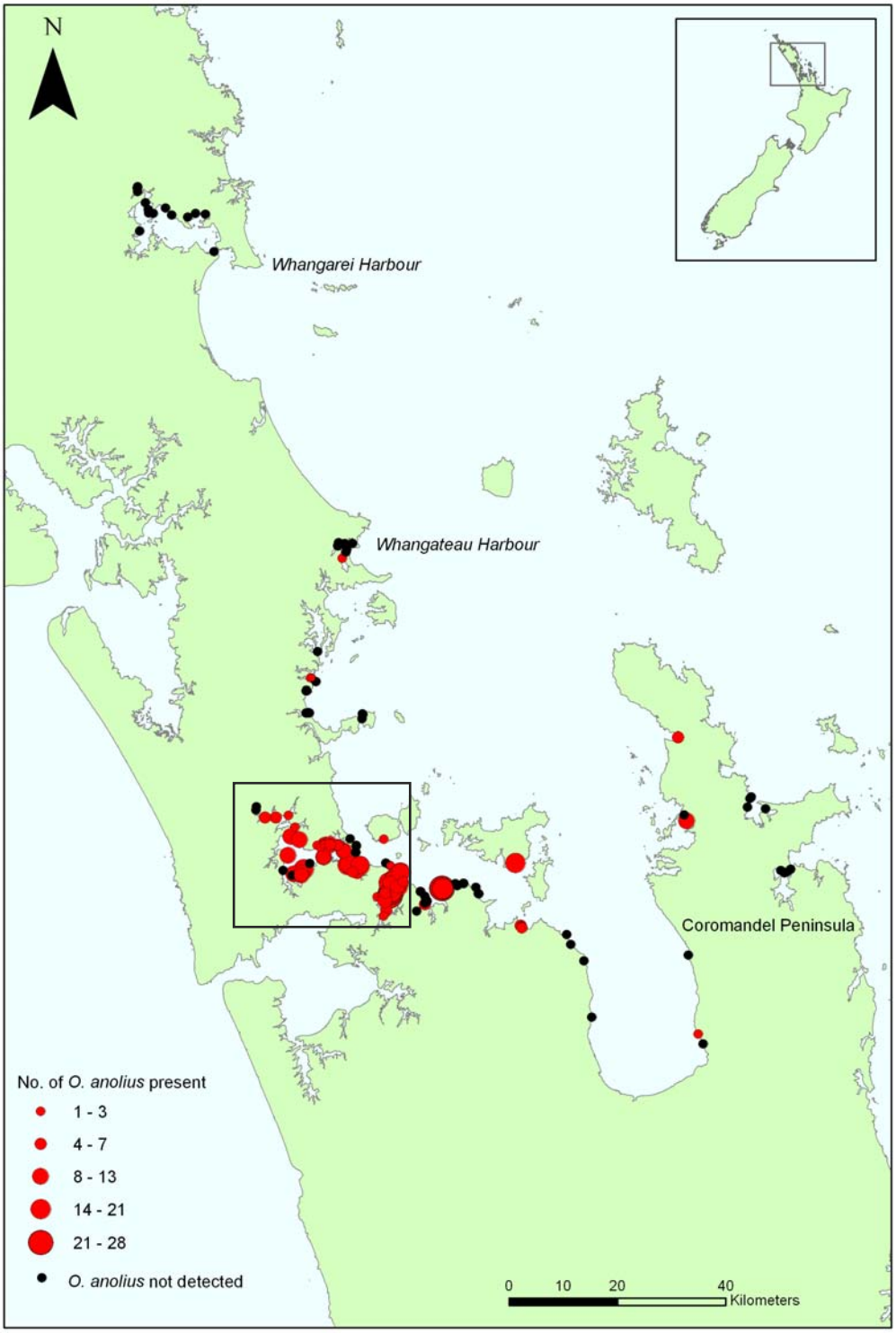

Figure 6. Upper North Island, New Zealand showing sites sampled for *O. anolius* in 2008/2009, their presence/absence and number found in a 30 minute timed count. Area inside the box is enlarged in Figure 7.

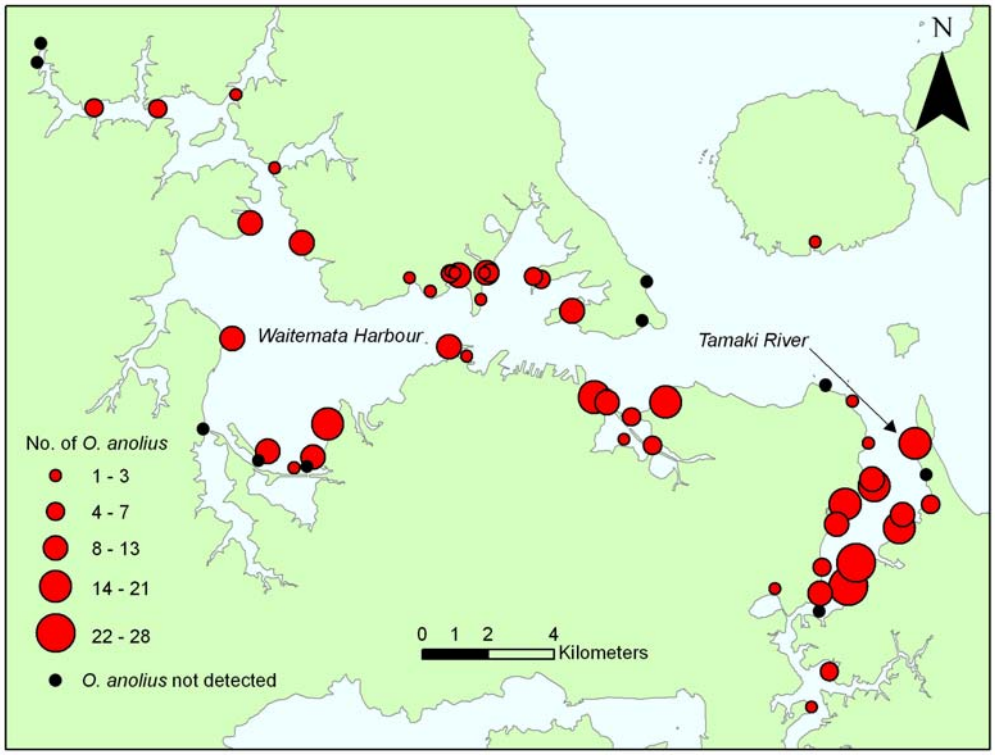

Figure 7 Sites sampled for *O. anolius* within Waitemata Harbour and Tamaki River region 2008/2009, with presence/absence and number found at each site in a 30 minute timed count.

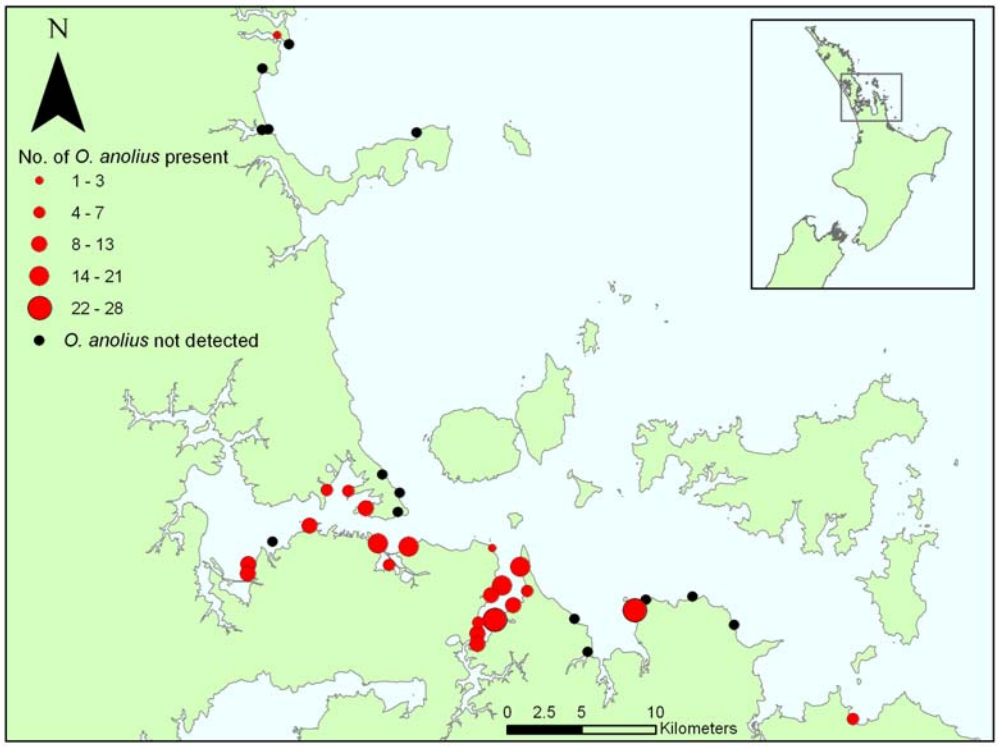

Figure 8 Francis *et al.*'s (2004) 2003 sites resampled in 2008/2008 with, presence/absence and number found in a 30 minute timed count at each site.

Marine farm survey

Responses were received from 11 farm owners representing 18 oyster farms from as far north as Houhora Harbour (34°45′S, 173°7′E) and as far southeast as Coromandel Harbour (36°45′S, 175°28′E). These locations were (on the east coast): Houhora Harbour, Whangaroa Harbour, northern and southern Bay of Islands, Mahurangi Harbour, Waiheke Island and Coromandel Harbour; and on the west coast, Kaipara Harbour. Seven farms reported the presence of *O. anolius* from three locations: Mahurangi Harbour (36°26′S, 174°42′E) the most northern location, Waiheke Island, and Coromandel Harbour (36°45′S, 175°28′E) the most eastern location (Figure 9). All farms within the geographic limits of recognised O. *anolius* distribution established from the physical sampling that responded returned positive results; all farms outside this range returned negative results.

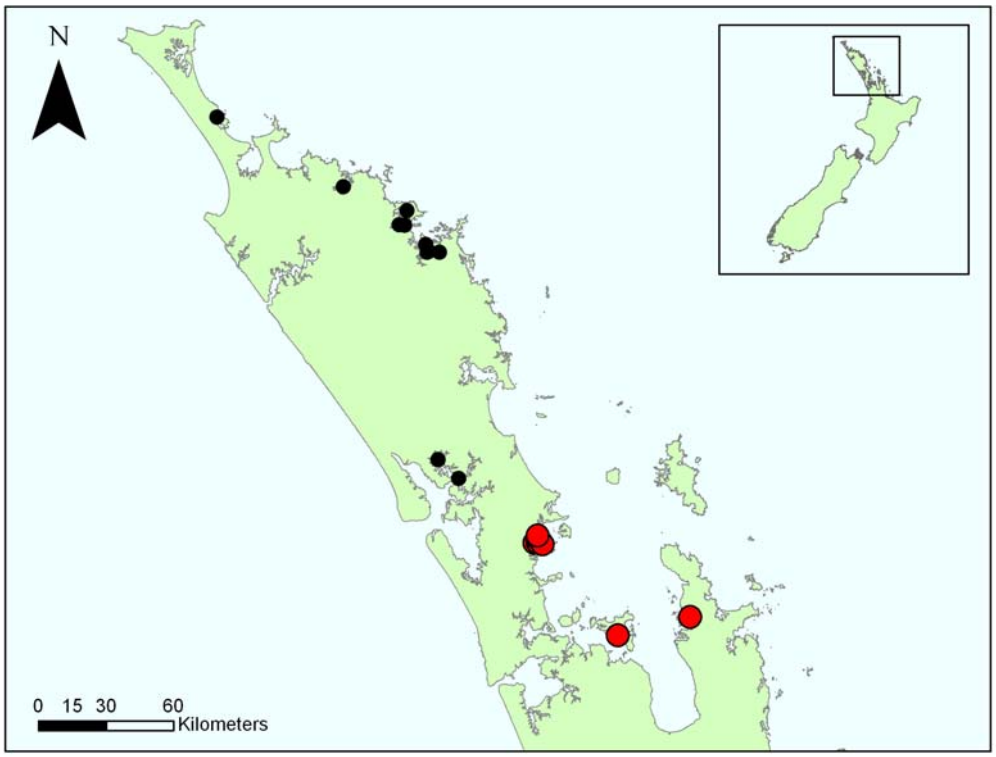

Figure 9. Locations of marine farms that responded to the questionnaire regarding the presence of *O. anolius* in their farm (red dot = present; black dots absent).

The Waiheke Island oyster farmer first noticed *O. anolius* occurring amongst the oysters during winter 2005 and as they were so unusual he made a record of it. Two of the Mahurangi Harbour oyster farmers estimate arrival as 3 – 5 years ago with the other Mahurangi farmer estimating their arrival as two years ago. The Coromandel Harbour oyster farmer believed they had been in their farm longer than five years. Additional

comments offered were that they had been found alive after being out of water for four days, while one of the Mahurangi oyster farmers noted that they see *O. anolius* nearly every day.

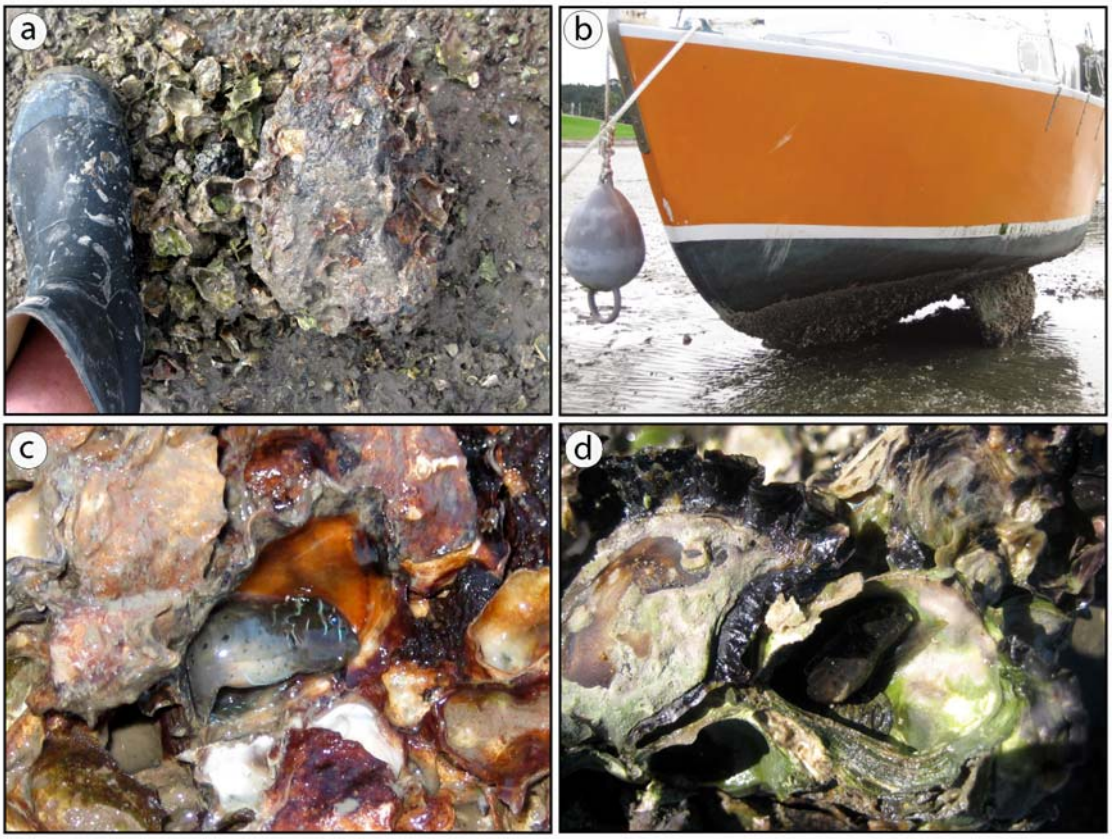

Figure 10. Examples of oyster habitat: a = boulder from which 31 *O. anolius* were found amongst the oysters removed, b = oyster fouling on vessel hull, from which an *O. anolius* specimen was collected, c & d = upper valve of oyster removed revealing *O. anolius*.

Habitat

Omobranchus anolius was present inside oysters in the following broad-scale habitats: artificial, mangrove, mudflat, reef platform, sandy mud and cobble but was not present in sand Most (91.7%) *O. anolius* occurred within the intact valves of dead oyster shells or within the spaces between oyster shells, with the greatest majority (82%) of these inside the valves of an oyster shell (Figure 10c and 10d). Other shelter that *O. anolius* utilised was the moist sediment or pools on the underside of a boulder with, 7.3% of *O. anolius* occurring here. Two fish were found inside tubeworm (*P. caeruleus*) encrustations, four fish were found in crevices and cracks within a reef platform and two fish were found inside holes within a reef platform, created by boring bivalves (family Pholadidae). One site in Tamaki River, *Tahuna Torea* was unusual in that

22.8% of fish collected from were found on the underside of a boulder in a pool. A large (184) number of fish were collected from this location and if it is excluded from the statistics, 97.6% of blennies were found amongst oysters and only 0.87% were found in pools.

O. anolius prefers *Crassostrea gigas* in the clumping growth form. On just four occasions *O. anolius* were found within the native oyster *S. glomerata*, and in three of these occasions *S. glomerata* exhibited the clumping growth form. All other fish occurring in oysters were found within the Pacific oyster *C. gigas*, with only two fish found within its encrusted growth form.

Omobranchus anolius seems to prefer shells with no sediment. Although often in a muddy habitat in which empty oyster shells could contain sediment *O. anolius* was only in found in oysters with sediment on three occasions. On only four occasions were fish found under a boulder with no oysters and on each of these occasions they were less than 2 m from boulders with oysters. On no occasion were *O. anolius* found outside of any shelter. *O. anolius* was often found sharing an oyster shell with invertebrate species, particularly the half crab, *Petrolisthes elongatus,* and chiton *Sypharochiton pelliserpentis*. One small recreational vessel that had oysters growing on the hull was examined and one *O. anolius* was found (Figure 10b).

As data did not meet the assumptions of ANOVA, a non-parametric Kruskal-Wallis test was employed to examine differences in abundance between broad-scale habitat types (Figure 11). There was no significance difference in abundance between the broad-scale habitats (H = 3.17 DF = 4 P = 0.529) (Figure 11). When the analysis was conducted using data from all results again the differences were insignificant (H = 6.59 DF = 6 P = 0.361)

Statistical tests were carried out to examine the relationship between fish length and habitat variables and tidal height. Unfortunately, the lack of balanced sampling between habitat variables meant that a nested ANOVA was unable to be used. There was a significant (p=0.000) difference in fish length between broad-scale habitats (Table 2, Figure 12), and between the tidal height at which the fish were found (Table 2, Figure 13). However, the larger fish found in artificial structures and mudflats may simply reflect the fact that these two habitat types were not found in the higher tidal zones (Figure 14).

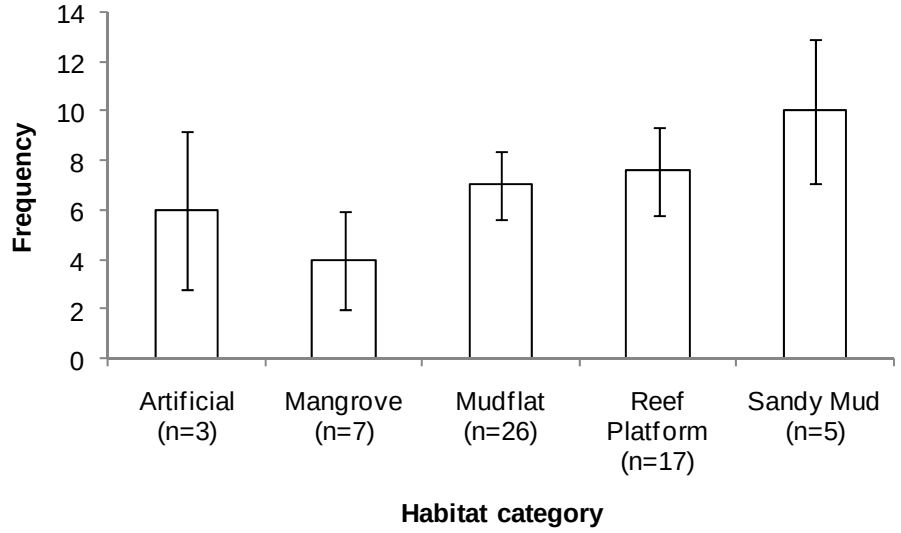

Figure 11. Mean number of *O. anolius* found in different broad-scale habitats within Waitemata Harbour and Tamaki River region during 30 minute timed counts. Error bars = SE.

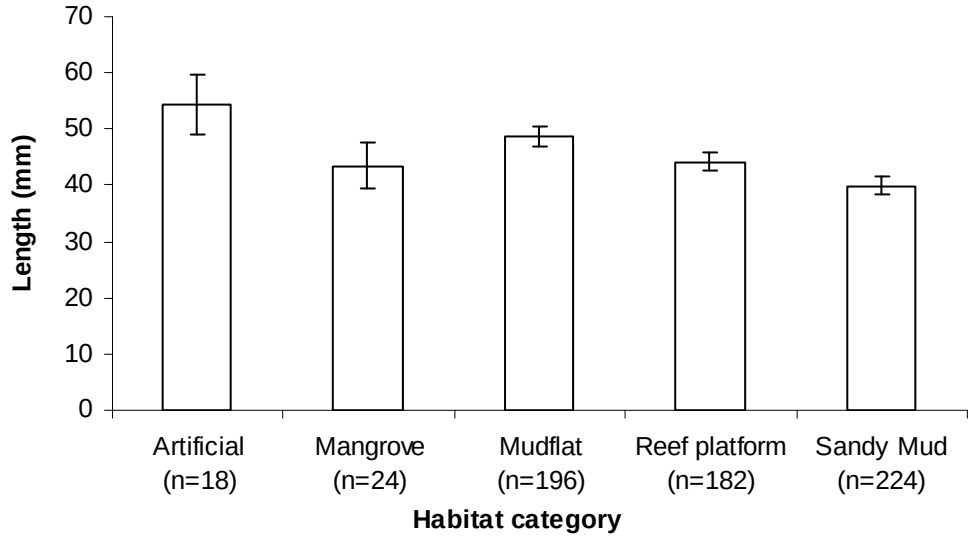

Figure 12. Mean length of *O. anolius* found in different habitats. Error bars=SE.

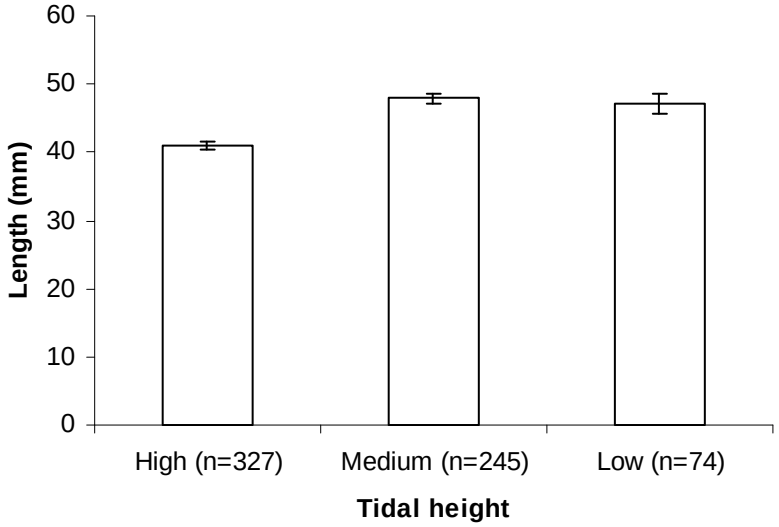

Figure 13. Mean length of *O. anolius* found in tidal zones. Error bars=SE.

Table 2. ANOVA of differences in size of *O. anolius* in habitats (broad) and tidal height (tide).

	SS	df	MS	f	P
Broad Scale					
Intercept	473547.6	1	473547.6	3500.054	0.000000
Broad	10353.1	4	2588.3	19.130	0.000000
Error	86590.2	640	135.3		
By Tide					
Intercept	895361.2	1	895361.2	6399.293	0.000000
Tide	7285.2	2	3642.6	26.034	0.000000
Error	89965.8	643	139.9		

To investigate the preferred micro-position of *O. anolius* the position the fish was found in was analysed against the position available. When the top, side, underside or the moist sediment under the rock was available 74.5% were found on the underside with 12. 3% found on the side and 12.6% found in pool; only 2 out of 341 fish were found on the top (Figure 15). When there was no underside available 80% of fish were found on the side and 20% on the top (Figure 16); when there was no side and just the top and bottom of a rock available, 97.4% of fish were found on the underside (Figure 17).

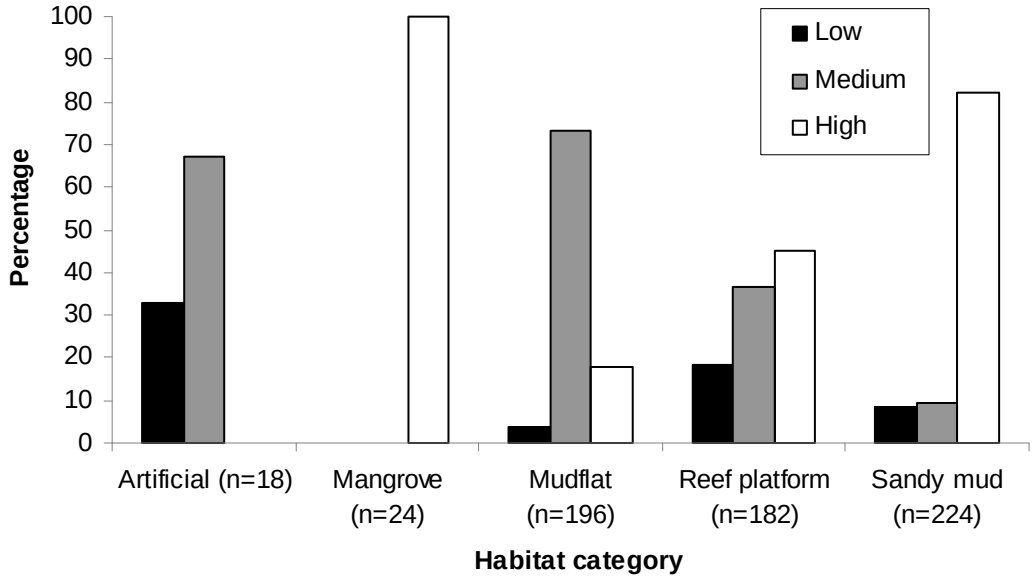

Figure 14. The proportions of *Omobranchus anolius* found within each tidal zone in each habitat.

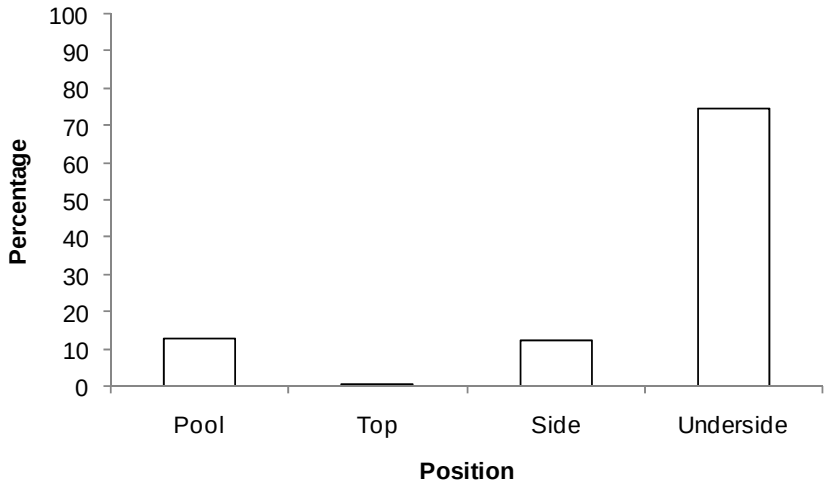

Figure 15. Position *Omobranchus anolius* was found when top, side, underside and pool were available (n=341)

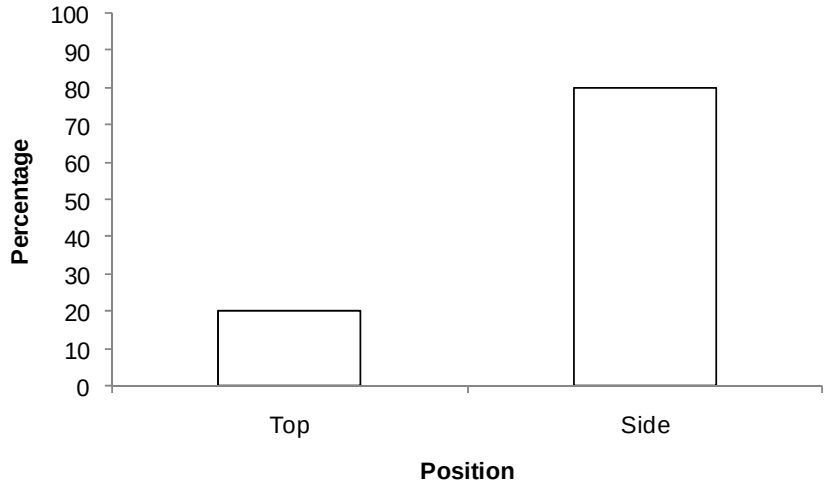

Figure 16 Position *Omobranchus anolius* was found when top and side positions were available only (n=115)

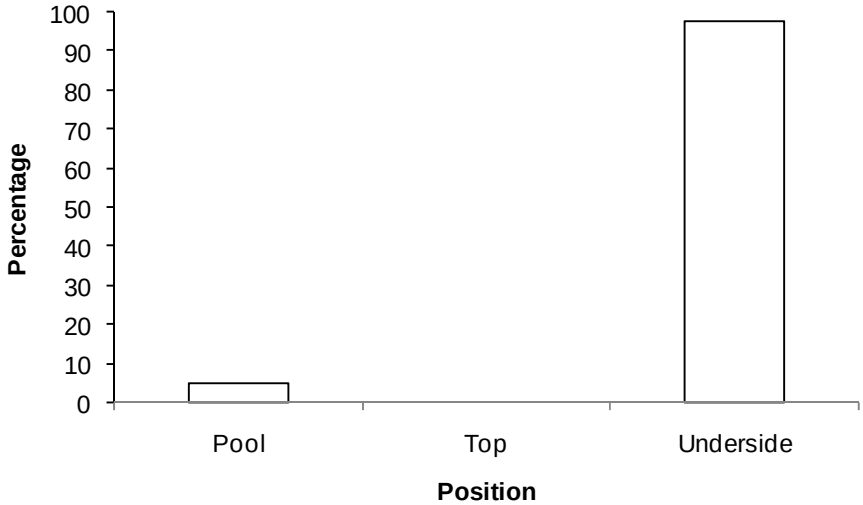

Figure 17 Position *Omobranchus anolius* was found when only top and underside were available (n=78)

All *Grahamina* sp. were found at low and mid-tidal heights under boulders with pools of water deep enough to fully immerse the fish, with 47% found on the underside of a boulder in the crevices created by oysters (but not in the shell); remaining fish occurred within the pool. The high proportion of *Grahamina* sp. found amongst the oysters during the 30 minute count was not what was observed generally in the habitat searched and the result was slightly skewed with seven fish found amongst the oysters under the same rock. Of those *Grahamina* sp. collected during the 30 minute count that were returned to the laboratory to confirm identification, all were *G. gymnota* (Scott 1977). However the estuarine triplefin *G. nigripene* was also identified outside of a 30 minute count and because of difficulties distinguishing these two species in the field, the identity of the individual species cannot be reported in the results.

All *A. fuscus* were found on the underside of a boulder and on one occasion one was found on the inside of an oyster shell; the rest were found in a pool. The pools of water in which they were found could be shallow and not immerse the fish, they also could be found at all tidal heights, but most commonly at lower tidal heights.

At all sites native fish occurred, *O. anolius* occurred also (except outside of its known range). *Omobranchus anolius* was regularly observed under the same boulder as native fish, although when native fish were in a pool *O. anolius* always occurred in oyster shells.

Discussion

Habitat

The place a particular species is found in an environment depends on the species' physiological capabilities that allow it to take advantage of the habitat (Bond 1996). Intertidal fish encounter extreme environmental conditions and they must seek shelter from desiccation and predation when the tide recedes (Gibson 1993). The preferred shelter for *O. anolius* is within dead oyster shells and as long as this shelter was present there were no significant differences in abundance between broad-scale habitats. Within this habitat they were very cryptic. When available there was a preference for oyster shells under or on the side of a structure as these positions offered greater protection from temperature extremes and desiccation. The choice of these positions can also be partly explained by the generally larger number of dead oysters present on the underside of boulders.

Habitat can be viewed at different spatial scales. Blennioid fish can utilise different microhabitats, resulting in the occupation of different habitats within the same location (Syms 1995, Wellenreuther *et al*. 2007). Under the same boulder native fish species were generally found in the pools while *O. anolius* was generally found inside the oysters on the underside of the boulder. *Grahamina* sp. occurred in the cracks between oysters but was never found inside them. By utilising smaller scale habitat there is potentially more habitat available to *O. anolius* and this partly explains how they could reach high densities when there was an abundance of dead oyster shell. Utilising dead oyster shells for shelter ensures *O. anolius* is not restricted to living under boulders to avoid desiccation, as oysters grow on a wide range of substrates.

O. anolius occurred throughout the tidal range and could even be found well into the high intertidal zone. Although they were able to survive in the high tide zone, fish were significantly smaller than those at lower tidal heights. Fish found higher up the tide zone have less time to feed than those lower down (Zander *et al*. 1999), therefore this difference in size maybe due to slower growth rates due to a restriction of food. This theory could be tested using otoliths to construct von Bertalanffy growth curves for different tidal heights. Occurring high up in the tide can also reduce the risk of predation (Gibson 1993); an alternate explanation for this pattern is that larger fish migrate

towards low tide as their size increases for some reason (e.g. better able to defend territories).

Omobranchus anolius was not restricted to oyster shell for habitat. They occurred in crevices and holes within the reef platform and between the intertwining calcium carbonate tubes of tubeworm encrustations. However, very few *O. anolius* were found in this type of shelter and there was a strong preference for oyster shells with intact valves as the majority of *O. anolius* did not utilise the spaces between oyster shells but were found inside dead oysters. In Australia *O. anolius* occurs within tubeworm encrustations (Smith-Vaniz 2008) but it is unknown whether the New Zealand tube worm *P. caeruleus* form similar habitat to the Australian species. Tubeworm encrustations, once common within the Auckland area, are now a rare habitat (Dromgoole & Foster 1983) and only occurred at a few sites sampled in this study. If this habitat had been present at more locations, greater numbers of *O. anolius* might have been found in this habitat. Because this habitat was rare, destructive sampling was not used; it was only examined with tweezers and it is possible *O. anolius* specimens were missed. Reef platforms were not excavated to get into deeper holes and cracks and it is possible some specimens were missed here also.

O. anolius rarely occurred in the native oyster *S. glomerata*, with the vast majority of specimens occurring in the invasive oyster *C. gigas*. *C. gigas* was the most common habitat available with the structural complexity necessary to provide protection from desiccation. This oyster species was first reported in New Zealand in 1970 from Mahurangi harbour (Dinamani 1971) after being accidentally, or perhaps deliberately introduced (Dromgoole & Foster 1983). It has since spread and has extensively modified much intertidal habitat in the north of North Island, particularly Waitemata Harbour, Kaipara and Manukau Harbours (Dromgoole & Foster 1983; Hayward 1997; Hayward *et al*.1999; Morton 2004 & Lohrer *et al*. 2008). It has a faster growth rate and out-competes *S. glomerata* on hard substrata, especially at lower tidal heights (Dromgoole & Foster 1983). It can also attach to cockle shells or other oyster shells on soft sediments which *S. glomerata* can not. By colonising cockle shells and subsequently attaching to other oyster shells it has created extensive intertidal banks of oysters where bare sediment previously existed (Hayward 1997, Morton 2004). *C. gigas* form complex structures as the entire lower valve doesn't need to attach to a hard substratum and they can grow vertically (Morley 2004). Because they can grow on top

of one another they form complex structures and retain dead oysters (Hayward *et al*. 1999) that can be used as nests for *O. anolius*.

C. gigas has invaded many countries and its ability to create structurally complex habitat, particularly in soft sediments, has drastically altered food webs and communities by creating habitat for a suite of different organisms (Crooks 2002) including blennioid fish (Ruesink *et al*. 2005). Other types of invasive biogenic reefs have facilitated the invasion of introduced species and although this has not been examined in New Zealand, this could certainly be the case with *C. gigas* in New Zealand (Ruesink *et al*. 2005).

Distribution in New Zealand

The northernmost range of *O. anolius* in this study was Whangateau Harbour, where two fish were collected. Habitat similar to that found in Waitemata Harbour occurred in the more northern Whangarei Harbour but *O. anolius* was not found at 14 surveyed sites there, suggesting that it has yet to reach this far north, or that it has but in very low numbers. This is further supported from responses from marine farm surveys. On the west coast of Coromandel Peninsula *O. anolius* was present at Coromandel Harbour and Colville Bay, but was not found from the east coast of the Coromandel Peninsula. It could be that it has not spread this far as yet, or it may just be that there was no suitable habitat in the places searched. Whitianga Harbour had no *C. gigas* and Whangapoua only had one site where *C. gigas* formed clumps similar to the western side of the Coromandel Peninsula or Waitemata Harbour. The marine farmer (who responded to the survey) on the western side of Coromandel Peninsula believed *O. anolius* had been present in his farm longer than five years; if this is correct then it would be expected that *O. anolius* will spread further. Therefore further sampling of other estuaries and harbours in western Coromandel and further south in Tauranga Harbour is required to more fully characterise the recognised distributional limits of *O. anolius*.

In addition to *O. anolius*, there have been three other recent accidental introductions of marine fish into New Zealand that have established (Francis *et al*. 2004). The bridled goby, *Arenigobius bifrenatus* (Kner, 1865) was officially recorded in New Zealand in 1999 and is well established in at least nine harbours or estuaries from Matapouri Bay north of Whangarei, down to Tauranga Harbour (Willis *et al.* 1999, Francis *et al*. 2003, Usmar 2003, Francis *et al*. 2004). A cryptogenic goby, probably *Parioglossus*

marginalis (Rennis & Hoese, 1985), was first recorded from North Cape and Great Barrier Island in 2000 and was common at both localities suggesting it may be more widespread than this (McDowall 2001). The Asian goby, *Acentrogobius pflaumii* (Bleeker, 1853), discovered in New Zealand in 2001 and was reported from Waitemata and Whangapoua Harbours (Francis *et al*. 2003). *A. pflaumii* was reported again in Waitemata Harbour in 2007 (Morrisey *et al*. 2007) suggesting it is established but it is unknown if it has spread and its occurrence at two locations is a result of separate introduction events (Francis *et al*. 2003).

Spread and dispersal

Based on the latitudinal distribution of *O. anolius* in Australia, *O. anolius* is capable of colonising estuaries and harbours in North Island of New Zealand, and possibly the top of South Island (Francis *et al*. 2004). However Francis *et al*. (2004) point out that this is only speculation without better knowledge of *O. anolius* habitat preference and environmental tolerances.

This study identified *C. gigas* as preferred habitat within the inner Hauraki Gulf. This oyster species is common in harbours in the upper north North Island and is particularly common within the Manukau and Kaipara Harbours on the west coast where it has extensively modified the habitat (Hayward 1997, Morton 2004). The southernmost record of *C. gigas* is Okari Lagoon, South of Westport. It has colonised extensive areas of the top of South Island and its distribution appears to be increasing (NABIS, retrieved December 2008). To identify whether *C. gigas* exhibited the clumping growth in its southern range the Nelson/Marlborough Department of Conservation conservancy was contacted and clumping habitat described; they confirmed it was present.

In its southernmost range in Australia *O. anolius* occurs mostly in tubeworms encrustations (Smith-Vaniz 2008), and they have also been found in this habitat in New Zealand. *P. caeruleus* encrustations occur throughout South Island and Banks Peninsula, where they dominate the middle eulittoral zone (Morton 2004). Based on these observations, what appears to be *O. anolius* preferred habitat occurs as far south as the top of South Island, while potentially suitable habitat occurs further south. As we know little of the environmental tolerances of *O. anolius* we cannot be sure if it can survive in the colder southern New Zealand waters.

Dispersal of *O. anolius* around New Zealand is most likely through larval dispersal as most blennies have limited adult movement and disperse through planktonic larvae (Almada & Santos 1995, Watson 2009). In one study off the coast of New South Wales, plankton trawls were conducted at a variety of depths and 97% (n=47) of *O. anolius* occurred in surface trawls (Gray & Miskiwiz 2000). This suggests that as well as being driven by tidal currents, wind-driven currents could play an important role in the dispersal of this species. How far *O. anolius* larvae may travel is unknown although marine organisms with planktonic larvae have the potential to travel large distances (Grosholz 1996). In a review of the literature of ten marine invasions (Grosholz 1996) found that rates of expansion were surprisingly low; and that length of time spent in the plankton was not related to the rate of expansion. Therefore it is not known how far *O. anolius* could spread and the possible rate of spread.

There is little that can be done to stop the spread of NIMS through larval dispersal but many NIMS are unable to spread to new regions naturally (Cranfield *et al*. 1998). At an interregional scale anthropogenic transport can be an important way of spread (Wasson *et al*. 2001). In one study examining the sea chests of vessels in New Zealand waters, four native species of fish (including two blennioid species) were found showing that sea chests could be an important vector for fish (including *O. anolius*) (Coutts & Dodgshun 2007). At a regional scale hull fouling is an important vector for transporting NIMS (Dodgshun *et al.* 2007) and considering that this study found *O. anolius* on the hull of one boat, this is a potentially significant vector for this species.

The transport of oysters during aquaculture is an important pathway for NIMS (Elton 1958, Wasson *et al*. 2001, Ruesink *et al*. 2005). Within the oyster farming industry in New Zealand there are weekly movements of adult oysters from the Bay of Islands to sites in the Coromandel and transfers from east coast sites including Mahurangi Harbour to Kaipara Harbour on the west coast (Dodgshun *et al*. 2007). McCulloch (1917) reported that a live *O. anolius* was discovered in an oyster shell at the fish markets, and New Zealand oyster farmers reported seeing *O. anolius* alive in an oyster after four days out of the water. This demonstrates that there is real potential for *O. anolius* to be transported in this manner. All of New Zealand's four introduced fish species have thus far only been reported from the east coast (Francis *et al*. 2004). To reach the west coast larvae must travel some distance to reach suitable habitat and overcome strong currents so it may take some time for *O. anolius* to reach the west

coast. If *O. anolius* is (or perhaps has already been) translocated to the west coast it is likely to establish as there is ideal habitat in Kaipara and Manukau Harbours (Morton 2004).

Impact

All invasions will have some impact (Ruiz *et al*. 1999), but the more widespread and abundant a species is the more likely it is to have a large impact (Ruiz *et al*. 1997, Parker *et al*. 1999, Wotton & Hewitt 2004, Lockwood *et al*. 2007). Within the Waitemata Harbour and Tamaki River region where *O. anolius* was discovered, it occurred at 86% (49 of 58) of the sites sampled; at five sites 20 or more specimens were collected within 30 minutes. The sampling method used in this study was designed to detect rare, cryptic and patchily distributed organisms and this method does not allow density to be measured because a defined area is not searched (Murray *et al*. 2006, Hill *et al*. 2006). However the density of *O. anolius* was high at some sites (31 fish found on just one boulder). These results indicate that *O. anolius* can reach high abundance relative to native intertidal fish and are likely to be having a negative impact within some of the communities they have invaded. NIMS have been shown to cause a decline on native species they predate which can lead to trophic cascades (Grosholz 2002). Trophic cascades have been observed in blenniod fish where they reduce the abundance of herbivorous invertebrates, causing a subsequent increase in biomass and diversity of algae (Bruno & O'Connor 2005).

What impacts *O. anolius* is having are difficult to determine. But the two most likely impacts are 1) predation of native species and 2) competition with them for food and/or habitat (Mills *et al*. 2004). In freshwater systems, introduced fishes have been observed to displace native fishes from preferred habitat and force them to have nesting sites in deeper water, increasing predation of their young (French & Jude 2001). In determining the outcome of interactions between NIMS and native species, size can be an important factor, with larger species usually dominating (Grosholz & Ruiz 1996). However, the interactions between native and introduced species can be complex and it is often a combination of factors that cause a negative impact (Mills *et al*. 2004).

Chapter 3: Life history

Introduction

Understanding the life history of an individual is essential to understanding its ecology (Hutchings 2002). Life history encompasses such aspects as: growth rate, size at maturity, reproductive strategies, fecundity; ultimately life history determines the success of a population (King & McFarlane 2003). Differences in life history are most pronounced at the Family and Order level, but important differences occur at the species level (Hutchings 2002). The success of an invader in a new environment often depends on its life history (Sakai *et al*. 2001, Lockwood *et al*. 2007) with different aspects of life history being important at different stages of an invasion (Kolar & Lodge 2001, 2002). Therefore knowing the life history of an introduced organism such as *Omobranchus anolius* is one of the first steps in risk analysis to determine the potential impact of an NIS (Wotton & Hewitt 2004).

To date, the published literature on the life history of *O. anolius* is limited to observations and taxonomic descriptions. Early authors noted that *O. anolius* attached eggs in batches to the inside of oyster shells, exhibited parental care and that a head crest was present in male specimens (Ogilby 1911, McCulloch 1917). The most detailed work to date on the life history *O. anolius* was conducted by Thomson & Bennett (1953). They collected three oyster shell nests (with one nest having an accompanying male inside) and returned them to the laboratory to observe the behaviour of the male and development of the eggs. The male only left the oyster shell nest for brief periods and created a water current over the eggs using small movements of his tail for prolonged periods. When the eggs hatched the male took each larva into its mouth and expelled it from the nest with a spitting motion. It seemed to Thomson and Bennett that the eggs had been laid in batches, and they classified the development of the eggs into four stages. They reared the larvae until 5 days old and this early larval development was also described. In their synopsis of the tribe Omobranchini and revision of the genus *Omobranchus* (Springer 1972, Springer & Gomon 1975) reviewed the literature on *O. Anolius*, in addition to examining 41 specimens. They described *O. anolius* as sexually dimorphic, with males obtaining a larger size, having a more striking colour pattern, elongated posterior dorsal-fin rays and a prominent head crest, that was absent or poorly developed in females. They suggest that this confers a greater visibility on the males which may be important in courtship and territorial behaviour. Neira *et al*. (1998) analysed larvae of *O. anolius* caught in plankton trawls and further described the larval development of this species. Apart from these literature sources, only brief descriptions

of *O. anolius* have occurred in fish identification books (for example Grant 1987, Kuiter 1993).

The spawning period of this fish has not been studied, however a review of the literature on larval fish found in plankton trawls in Australia indicates that the *O. anolius* may spawn over an extended period during the warmer months of the year. *Omobranchus anolius* larvae have occurred in plankton trawls from October to May, with peak abundance between December and April in New South Wales (Miskiewicz 1987, Gray *et al*. 1992, Druce & Kingsford 1995, Gray & Miskiewicz 2000), and have occurred in plankton trawls in January in Victoria (Jenkins 1986). Thomson & Bennett (1953) collected nests in October from New South Wales. The length of time larvae spend in the pelagic phase is unknown but they hatch at approximately 3.2 mm (Body length) and settle between 12.7 mm and 17.2 mm (SL) (Neira *et al*. 1998).

Omobranchus anolius is one of many small native Australian fish that has received little attention in the scientific literature. However, now that it has been introduced to New Zealand, an understanding of its reproduction and life history has gained importance. The aim of this study was to provide some information of the life history of this species in New Zealand. Specifically to: 1) describe the gonad development, 2) determine the spawning season, 3) determine its fecundity, 4) assess age at maturity, and 5) determine sex ratios.

Methods

Fish and nest collection

A total of 734 fish were collected; 733 were weighed and measured, and, where possible, sex determined from external morphology (one fish was unable to be weighed and measured due to damage sustained during collection). For seasonal data on gonosomatic analysis and histology, 362 blennies were collected monthly from locations in Tamaki River (36°52′S, 174°53′E) and Little Shoal Bay (36°49′S, 174°44′E) between April 2008 and March 2009. Sampling was conducted two hours either side of low tide. Upon capture all fish were immediately euthanized by pithing (*sensu* Mountfort *et al*. 2005) and fixed in 10% buffered formalin. Once in the laboratory the fish were weighed to the nearest 0.0001g, and total length measured (standard length was also measured for most fish). Sex was externally assessed by the

presence of a prominent head crest in males (Springer & Gomon 1975). Nests were collected along with any guarding males and fixed in 10% buffered formalin. Eggs were counted in nests where the male could be positively identified as being associated with the nest, and where there was a high proportion of fertile eggs still present (i.e. there were few empty egg cases).

Gonad and liver preparation

Gonads and liver were excised under a dissecting microscope, damp dried and weighed to the nearest 0.0001g and stored in 10% buffered formalin. To examine potential seasonal variation in liver weight a hepatosomatic index (HSI) was calculated by measuring the relationship between liver weight (LW) and total weight (TW) by the equation HSI = LW/TW x 100. Gonads used in histological analysis were dehydrated in a graded ethanol series, cleared with xylol, infiltrated and embedded with paraffin wax (*sensu* Bancroft & Stevens 2008). Using a rotary microtome (Leica RM2235) 5–7 μm sections were cut and stained with Harris's haematoxylin and eosin (see Appendix I for detailed histological protocols). Gonads of fish smaller than 30 mm TL were too small to embed in wax so sections of entire decalcified fish trunks were made (*sensu* Roberts & Smail 2001). The head and tail were removed and the remaining trunk bathed for a minimum of 48hr in 10% (w/v) ethylenediamine-tetra-acetic acid (EDTA), adjusted to pH 7.4 with 1M sodium hydroxide (NaOH). Prior to dehydration, sections were rinsed in tap water to prevent the precipitation of alcohol. Multiple sections were made of each trunk to ensure the gonad was included.

A pilot study on the differences between the two gonads of individual fish was undertaken to determine the ability to use only one gonad and then extrapolate to both gonads. Both gonads from thirty randomly selected fish were examined histologically to look for differences in maturity. This pilot study showed no differences in maturity between the gonads of individuals, so only one randomly selected gonad was used for the remainder of the study; the other female gonad was set aside for fecundity analysis.

Annual Spawning Season

The annual spawning season was assessed in three ways. First, by the presence of collected nests. Second, a gonosomatic index (GSI) was calculated by measuring the relationship between gonad weight (GW) and total weight (TW) using the equation

GSI = GW/TW x 100. The third and most precise measurement was determined from microscopic examination of female gonad development. Ovaries were classified into four stages of development using the most advanced oocyte stage present (Wallace & Selman 1981, West 1990). Briefly these stages were primary growth, cortical alveoli stage, vitellogenic stage and the ripe stage. The spawning season was defined as the first and last day the females were collected with oocytes staged as ripe.

Batch Fecundity

Batch fecundity was assessed using the hydrated oocyte method (*sensu* Hunter *et al*. 1985), where hydrated oocytes are considered to be the batch of oocytes about to be spawned. For this part of the study, females with high GSI values were selected to increase the likelihood that hydrated oocytes would be present in ovaries. One ovary from each of 15 fish was broken open and all hydrated oocytes were counted. Hydrated oocytes were identified by their large size and translucence (Hunter *et al*. 1985, West 1990).

To ensure that the fish had not recently released hydrated oocytes, the other gonad from each fish was prepared for histological examination (as above) and screened for the presence of post-ovulatory follicles. If these follicles were present these gonads were not used as these indicate recently released hydrated oocytes (Murua *et al*. 2003). To confirm that hydrated oocytes represented the most advanced batch of oocytes, the size frequency method (Hunter *et al.* 1985) was used on the gonads of two fish. In these fish all oocytes in the gonad greater than 250 μm diameter were measured on the basis of random orientation (West 1990). The largest modal group corresponds to the size of the hydrated oocytes observed (Hunter *et al.* 1985).

Length at Maturity

This was accessed for fish collected from December to March (when histological examination revealed all ovaries were ripe) to avoid classifying resting mature fish as immature. Females were considered mature if their ovaries contained vitellogenic oocytes while males were considered mature if there was spermatids or spermatozoa in the gonad (*sensu* Carrassón & Bau 2003, Longeneckera & Langston 2005). Length at maturity was based on 80 males (total length 23.5–76.7 and 68 females (total length 26.3 mm–57.0 mm). A logistic regression curve was fitted to the data, to estimate length at 50% maturity (L_{50}) and 95% maturity (L_{95}).

Statistical analysis

All statistical analyses were performed using specialised software, Minitab 15. Chi-square analysis was used to test for significant deviations from a 1:1 sex ratio. Data for GSI and HIS was graphed by month and analysed for differences using One-Way Analysis of Variance (ANOVA) with Tukey's post-hoc test to analyse multiple comparisons. In cases where these assumptions of ANOVA were not met the data were log transformed. General linear regression was used to analyse the relationship between batch fecundity and total length, and between the number of eggs in a nest and the accompanying male's total length.

Results

Size distribution and sex ratios

A total of 733 fish were weighed and measured (Table 3) with sizes ranging from 81.6 mm total length (TL), 4.1 g, to 16.1 mm TL, 0.04 g. The 37 largest fish were all males; the largest female was 65.5 mm TL (2.6 g) (Figure 18). Males were significantly ($p<0.000$) larger than females, with mean TL 49.9 mm ± 0.70SE, and mean weight (WT)

1.01 g ± 0.04SE, and mean TL 42.8 mm ± 0.45SE and mean WT 0.743 g ± 0.02SE for females.

Table 3. Length and weight measurements of *O. anolius* used in this study

Variable	sex	n	Mean ± SE	Minimum	Maximum
TL	M	319	49.9 ± 0.70	20.5	81.6
	F	360	42.8 ± 0.45	17.1	64.9
SL	M	300	42.2 ± 0.57	18.5	66.2
	F	340	37.0 ± 0.39	14.8	56.2
WT	M	319	1.089 ± 0.04	0.089	4.063
	F	360	0.743 ± 0.02	0.072	2.735

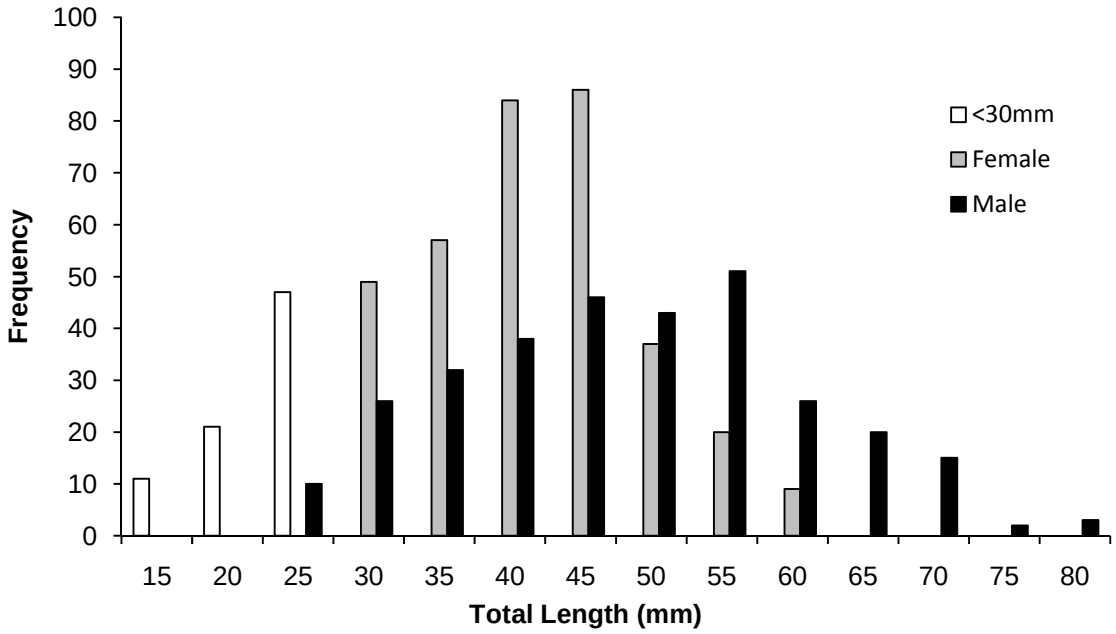

Figure 18. Size frequency distribution of *Omobranchus anolius*.

The allometric relationship between weight and total length was best represented by a power curve. The equation $Y=2E\text{-}05x^{2.7606}$ gives the best fit for males ($r^2 = 0.9609$, n = 293) and the equation $Y = 2E\text{-}05x^{2.8196}$ gives the best fit for females ($r^2 = 0.9438$, n=361) (Figure 19).

Table 4. Sex ratio of *O. anolius* by 10 mm length intervals and chi-square values of tests for a 1:1 ratio. Chi squared values with asterisk differ significantly from a 1:1 sex ratio ($p<0.05$).

Total Length (mm)	Total (n)	Histology used for sexing	Males (n)	Females (n)	Males (%)	Females (%)	Sex ratio (M:F)	Chi sq
>30	36	36	10	26	27.77	72.22	1:2.6	7.11*
30–39.99	160	39	58	102	36.25	63.75	1:1.75	12.10*
40–49.99	249	88	83	166	33.33	66.66	1:2	27.67*
50–59.99	146	48	91	55	63.33	37.67	1:0.60	8.88*
60–69.99	54	14	45	9	83.33	16.66	1:0.2	24.00*
70–79.99	20	2	20	0	100	0		
80–82	3	0	3	0	100	0		
Total	668	227	310	358	46.41	53.59	1:1.15	3.45

Of the 733 fish, 633 were sexed by external sex characters (n=406) or histological examination of the gonads (Table 4) (n=227). The remaining 102 fish were too small (< 30 mm) to sex with external characters and were not sexed by histological examination or macroscopic examination of gonads due to time constraints; 52.5% of those individuals that could be reliably sexed were females and 47.5% were males.

Overall, there were slightly more females than males giving a sex ratio of 1:1.15 however they did not significantly vary from an expected 1:1 ratio (χ^2= 3.45 $p<0.05$ where Chi squared critical value, p=0.05, d.f. 1 is 3.841). Sex ratio varied between size classes, with significantly ($p<0.05$) more males at lengths >50 mm (TL) and significantly more females at lengths <50 mm (TL) (Table 2).

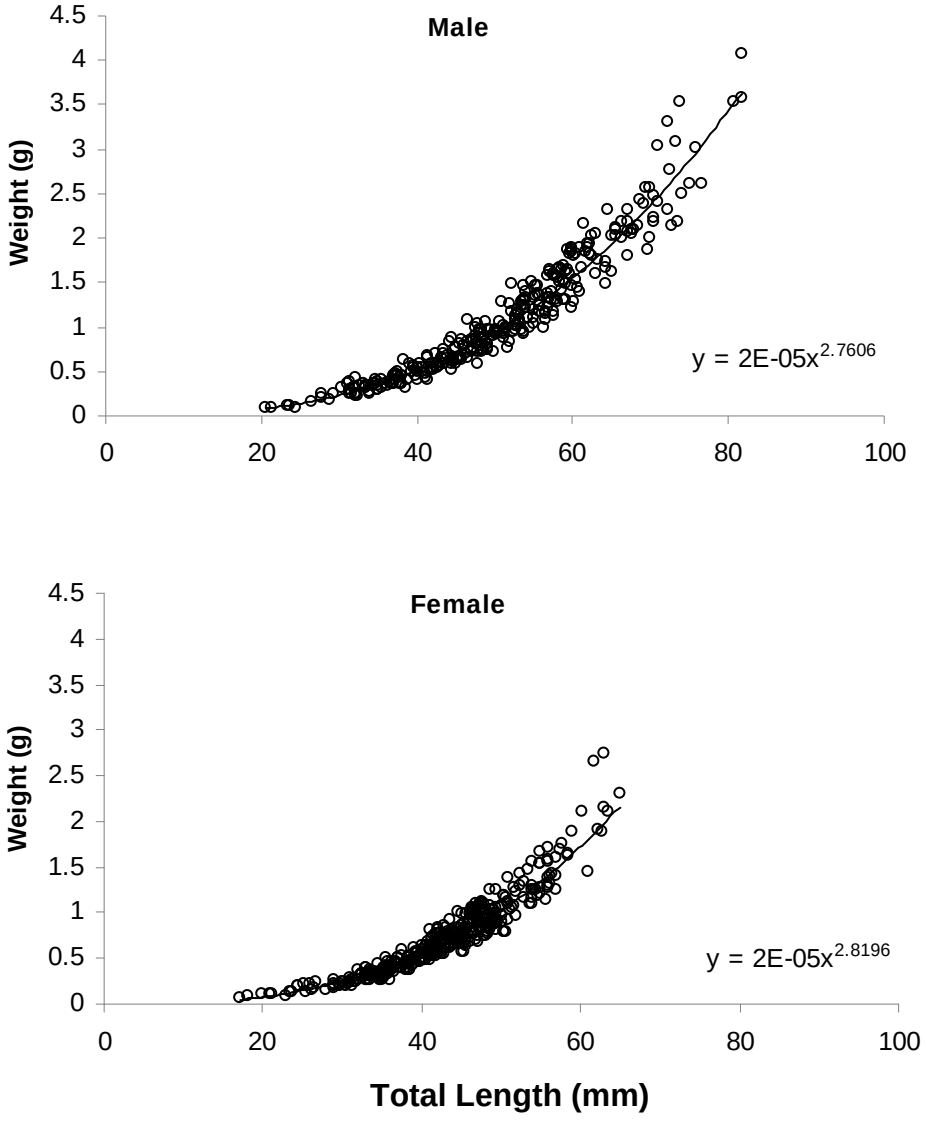

Figure 19. Allometric relationship between length and weight of *O. anolius*.

Nests

Thirty-two nests were found between 25 November 2008 and 31 March 2009; 31 were found inside an oyster shell and one was found in a pholad hole with an oyster 'cap' (Figure 20b). Eggs in different stages of development were laid in a single layer on the upper, and lower, valves of oyster shells. In the case of the pholad hole, eggs were found evenly distributed on the outer and inner surface. Of the 32 nests identified, all contained a single male fish only. Eggs from 17 nests were counted, and plotted against the total length of each parent male. The mean number off eggs per nest was 1464 (SE 209), range 252–2611. The mean length of the accompanying male was 55.52 mm (SE 2.39), range 39.7–73.5 mm TL. General linear regression (Figure 21) revealed that the number of eggs significantly increased with total length ($F_{(1,16)}$=9.15; p=0.009; R^2=0.379); this correlation was increased by log transforming the data ($F_{(1,16)}$=13.9; p=0.002; R^2=0.480).

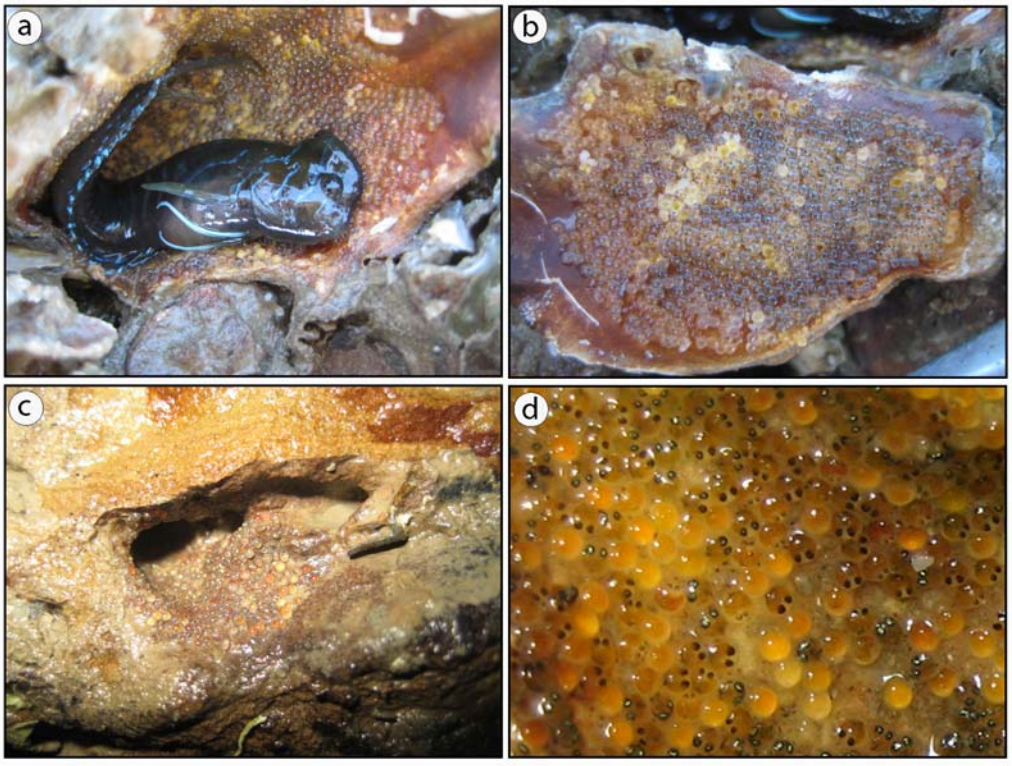

Figure 20. Nests of *O. anolius*. (a) Lower valve of oyster with male in the nest, (b) upper valve of same oyster as (a) with eggs, (c) eggs in a pholad hole with oyster cap removed, (d) eggs in different stages of development.

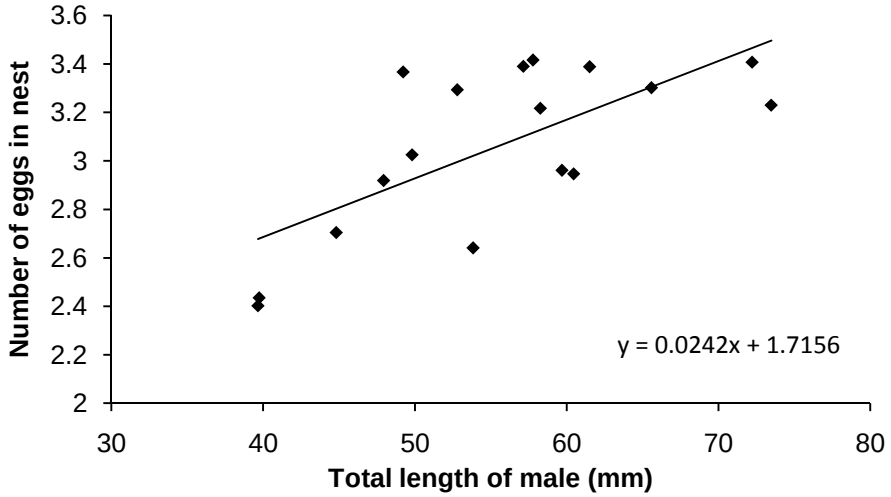

Figure 21. Number of eggs found in nests log transformed in relation to size of male found with nest.

Ovaries

The ovaries of *O. anolius* consisted of a pair of "sausage" shaped structures that varied in colour and size throughout the year. Immature fish had small milky-white ova which became bright orange and occupied much of the body cavity in mature fish. The development of oocytes is described below. All stages of development could be observed at the same time within a single ovary indicating that *O. anolius* is an asynchronous spawner.

1. **Primary growth stage** (Figure 22a). Consists of two distinct phases: the chromatin nucleolar phase and the perinucleolar phase. **Chromatin nucleolar phase**. (>30 μm). Oocyte appears dark. The thin cytoplasm is highly basophilic with a large centrally located nucleus that is difficult to distinguish. No nucleoli are visible. **Perinucleolar phase** (Figure 22a, b). (35–115 μm). Early in this stage the cytoplasm appears dark with the nucleus clearly visible in the middle of the cell with nucleoli. As the oocye continues to grow (70–115 μm) the cytoplasm starts to appear lighter and many more nucleoli can be seen in the nucleus; often situated around the periphery of the nucleus.

2. **Cortical alveoli stage** (Figure 22c). (115–280 μm). The start of this stage occurs when small cortical alveoli start to appear in the cytoplasm looking like empty vacuoles. These continue to increase in number until they fill the entire cytoplasm forming block like structures. The nucleus is in the middle of the cell with

prominent nucleoli spread all over it. During this stage the adherent disc becomes visible.

3. **Vitellogenic stage** (Figure 22d) (300–450 μm). The start of this stage is signalled with the appearance of yolk granules associated with the vacuoles. These granules continue to increase until the entire cytoplasm is filled.

4. **Ripe stage** (Figure 22f) (450–800 μm). The cytoplasm becomes filled with large yolk platelets and the nucleus is not visible. This stage is difficult to observe because of the distortion of the cells during processing and because the platelets fall out of the cell.

Testes and testicular gland

The male gonads of the *O. anolius* consist of two very distinct structures: the testicular gland and testis which surrounds it (Figure 24a). The size of the testes varies throughout the year, increasing in size during the spawning season.

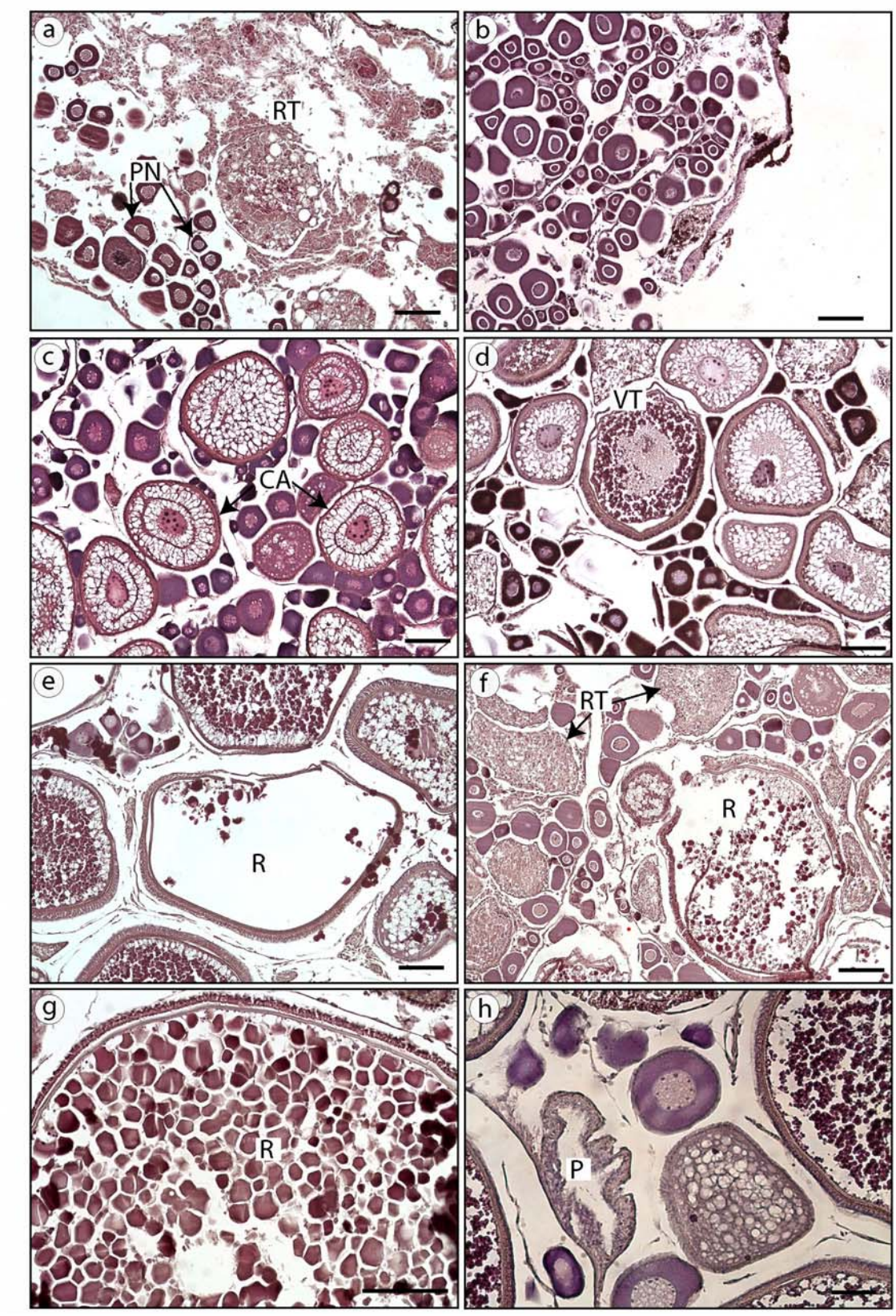

Figure 22. Ovaries of *O. anolius*. (a) April – with brown bodies (B) and perinucleolar oocytes (PN); (b) March, with only perinucleolar oocytes ; (c) September, cortical alveoli oocytes (CA); (d) October, with vitellogenic oocytes (VT) and adherent disc (AD); (e) January, with ripe oocytes (R); (f); March, with brown bodies; (g) Ripe oocyte; (h) December, with postovulatory follicle (P). (Scale: 100 µm)

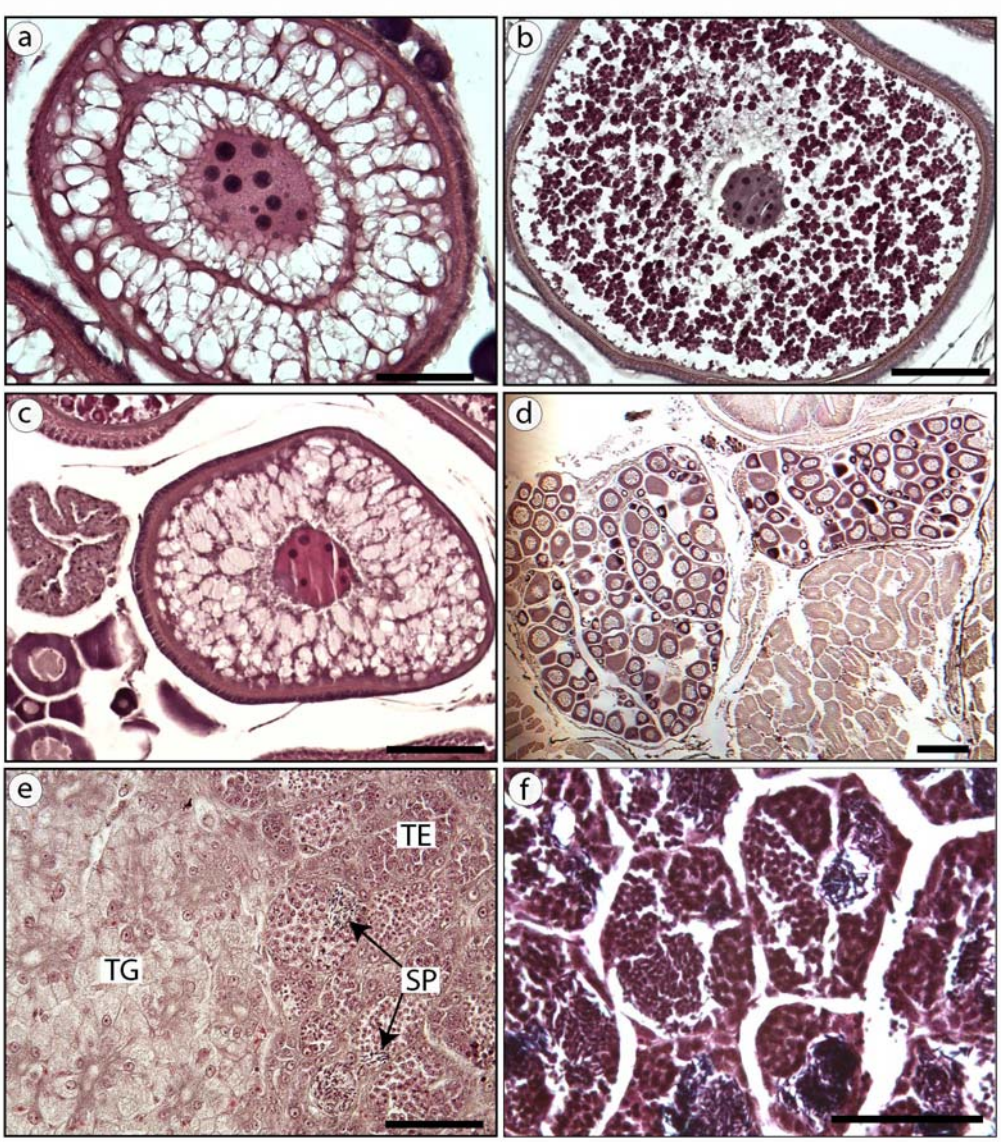

Figure 23. Histology of *O. anolius* gonads. (a) cortical alveoli oocyte (scale: 50 µm); (b) vitellogenic oocyte (scale: 100 µm); (c) cortical alveoli oocyte and postovulatory follicle (scale: 50 µm); (d) section of fish trunk with two ovaries (scale: 100 µm); (e) spermatids present in male 27.8 mm TL (scale: 50 µm); (f) spermatids and spermatocytes in seminiferous tubules (scale: 50 µm).

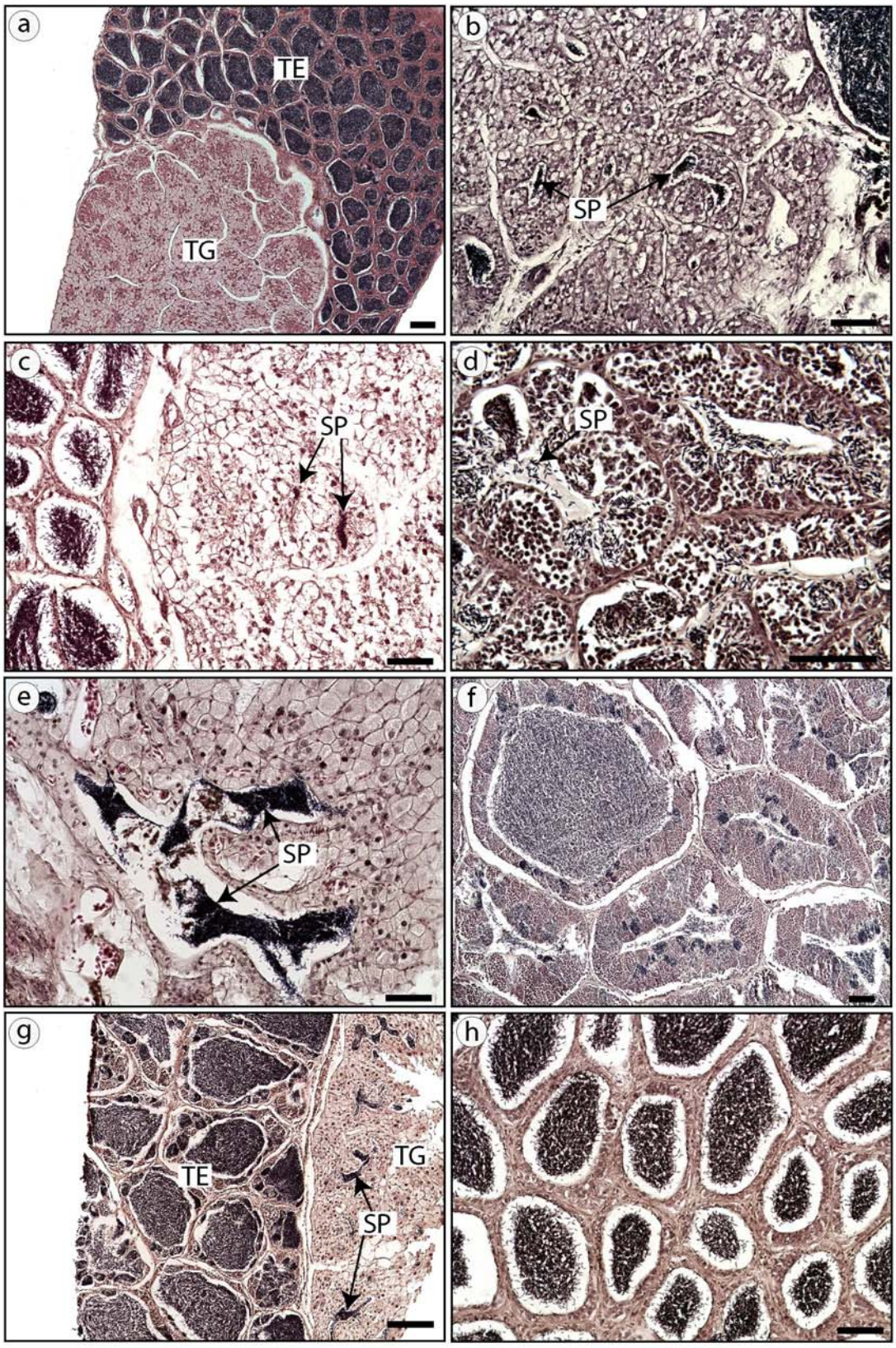

Figure 24. Histology of male *O. anolius* gonads. (a) April – testis (TE) and testicular gland (TG); (b) May – spermatids (SP) in testicular gland; (c) July – testicular gland; (d) October – spermatocytes and spermatids in seminiferous tubules; (e) November – abundant spermatids in testicular gland; (f) March – testes with low numbers of spermatids in seminiferous tubules; (g) March – testis and testicular gland with numerous spermatids in seminiferous tubules, also present in testicular gland; (h) July – seminiferous tubules full of spermatids. (Scale: 50 µm)

Spawning season

The occurrence of nests from the end of November to the end of March gives an indication of the spawning season, with the percentage of males found accompanying nests peaking in January (Figure 25).

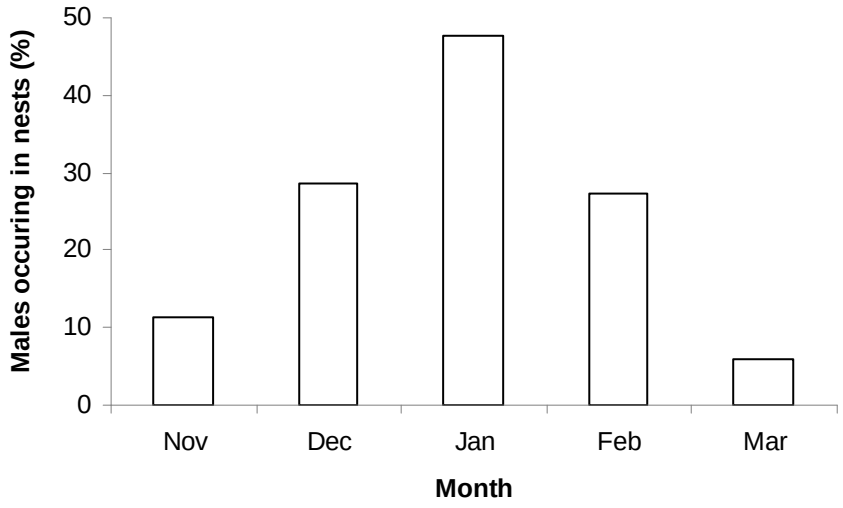

Figure 25. Temporal distribution of male *O. anolius* found with nests.

There is a significant seasonal difference in GSI values for both males and females (Table 5). Tukey's HSD groupings on female GSI values indicate that April to September cluster together, as do the higher GSI values from October to February. March GSI values were intermediate and clustered with both of these groups (Figure 26). This indicates that the spawning season occurs from October through to March. Male GSI values followed a similar trend, but the Tukeys HSD groupings do not show as clear seasonal clusters as in the use of female GSI (Figure 26).

Table 5. ANOVA of monthly GSI values for *O. anolius*.

	SS	df	MS	f	P
Females					
Intercept	10.6715	1	10.6715	110.0328	0.0000
Month	19.6821	9	2.1869	22.5490	0.0000
Error	15.5175	160	0.0969		
Males					
Intercept	3.0199	1	3.0199	30.8176	0.0000
Month	10.3381	9	1.1486	11.7219	0.0000
Error	13.6212	139	0.0979		

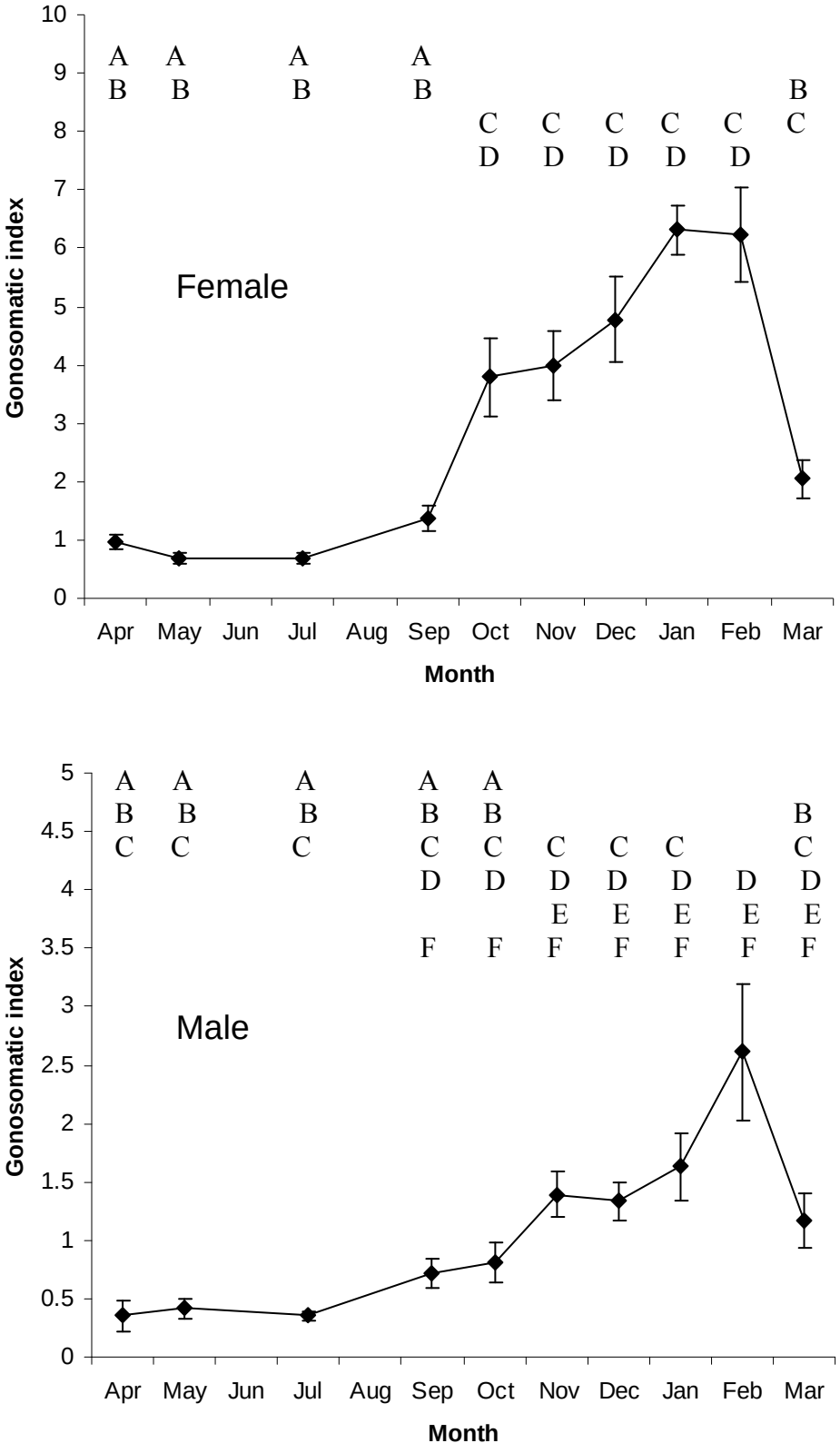

Figure 26. GSI values for *O. anolius* from April 2008 to March 2009. Capital letters indicate Tukeys HSD groupings where months with the same letter do not differ significantly (P<0.05). Error bars = SE.

The presence of ripe oocytes in ovaries lends further evidence that the spawning season runs from Ocober to March (Figure 27). On October 17, 2008, 31% of ovaries contained ripe oocytes, however these represented only a small proportion of oocytes with the ovary dominated by oocytes in the cortical alveoli stages and some in the vitellogenic stage (Figure 22d). By December nearly all ovaries have oocytes in the ripe stage, and vitellogenic and ripe cells oocytes are prevalent within the ovary and this continues through to February (Figure 22e). During this period post-ovulatory follicles (POF) occur (Figure 22h) and are evidence of recent spawning. In March, 64% of ovaries have ripe oocytes, however within these ovaries the number of ripe oocytes present are less than occur in the preceding months; and atretic oocytes and browns bodies start to dominate the ovaries (Figure 22f). Atretic oocytes are oocytes that have failed to be released or mature and brown bodies represent a more advanced stage of atresia. Their prevalence in the ovaries indicates the spawning season coming to an end. In April no ripe oocytes are present in any of the ovaries and brown bodies dominate much of the ovary (Figure 22a) indicating that the spawning season has finished and the ovaries are now starting to recover. In May through to July cortical alveoli are present in many ovaries, however the ovaries mainly contain cells in the primary growth phase (Figure 22b). In September this changes with cortical alveoli dominating much of the ovary (Figure 22c).

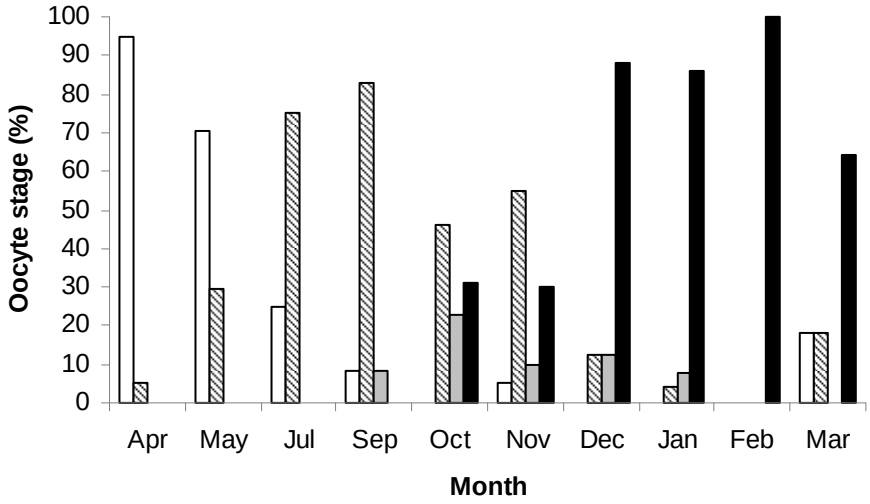

Figure 27. Monthly variation in maturity stages of female *O. anolius*. Black bars=Ripe Stage, white bars =Primary Growth Stage , grey bars = Vitelogenic Stage, cross-hatch bars = Cortical Alveoli Stage.

In male teleosts, stages of development are not clearly distinguished. In *O. anolius* the size of the testes was reduced post spawning and increased in size again once spawning commenced. However the gonads exhibited all stages of development throughout the year without a clear pattern. In maturing testes, spermatocytes and spermatids were observed (figure 24d) and these maturing testes occurred in greater numbers in October, November, December; although they occurred throughout the year. During the spawning season large numbers of spermatids travelled from the seminiferous tubules into the ducts of the testicular gland (Figures 24e, g). However spermatids were observed in the testicular gland in May and July also (Figures 24b, c). Throughout the year the seminiferous tubules were observed to be completely full with spermatids (Figures 24g, h), however some gonads had empty semiferous tubules (Figure 24h) towards the end of the spawning season, indicating that they were spent.

Liver

There was a significant seasonal difference in hepatosomatic index for both females and males (Table 6), however a Tukey's post-hoc test revealed no clear trend over the course of the year (Figure 28).

Table 6. ANOVA of monthly HSI values for *O. anolius*.

	SS	df	MS	f	P
Females					
Intercept	37.0425	1	37.0425	1961.233	0.0000
Month	0.5612	9	0.0623	3.302	0.0010
Error	3.0597	162	0.0188		
Males					
Intercept	11.3410	1	11.3412	370.7124	0.0000
Month	0.9461	9	0.1051	3.4362	0.0008
Error	4.1912	137	0.0306		

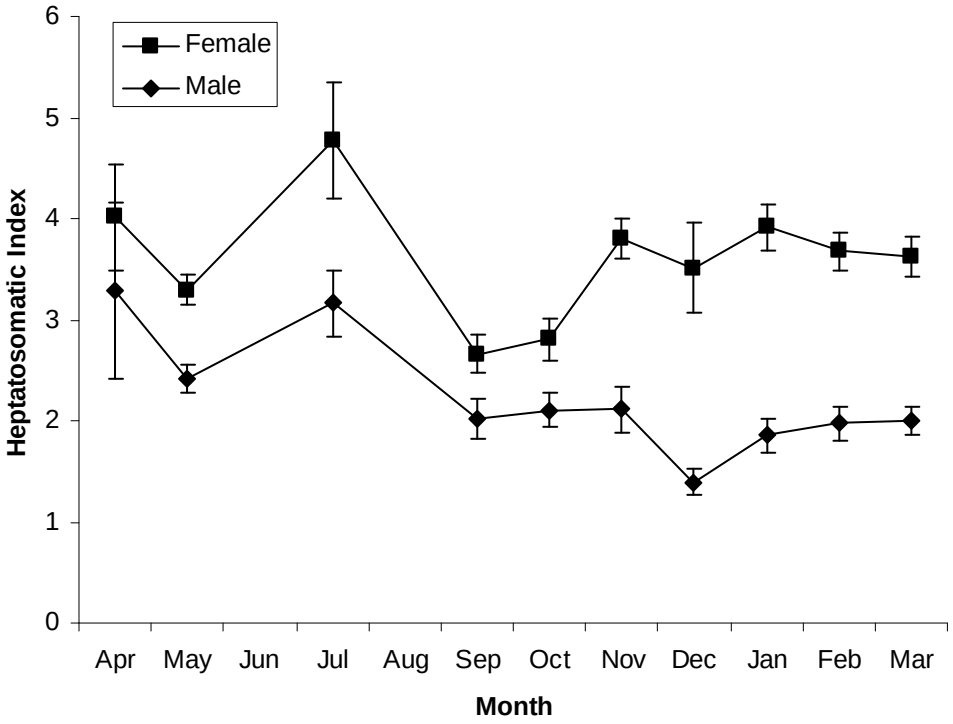

Figure 28. Hepatosomatic Index of *O. anolius* from April 2008 to March 2009. Error bars = SE.

Batch Fecundity

The size frequency distribution of oocytes measured from two individual fish (Figures 29a, b) confirmed the efficacy of using hydrated oocytes to determine the batch fecundity of these fish. On both fish examined in this way, there is a clear "batch" of oocytes greater than 800 mm, corresponding to the minimum diameter of hydrated oocytes. The mean batch fecundity was 142 ± 17.4SE and ranged from 58 to 302 hydrated oocytes. The general liner regression (Figure 30) revealed that batch fecundity significantly increased with total length ($F_{(1,14)} = 48.94$; p=0.000; $R^2 = 0.79$).

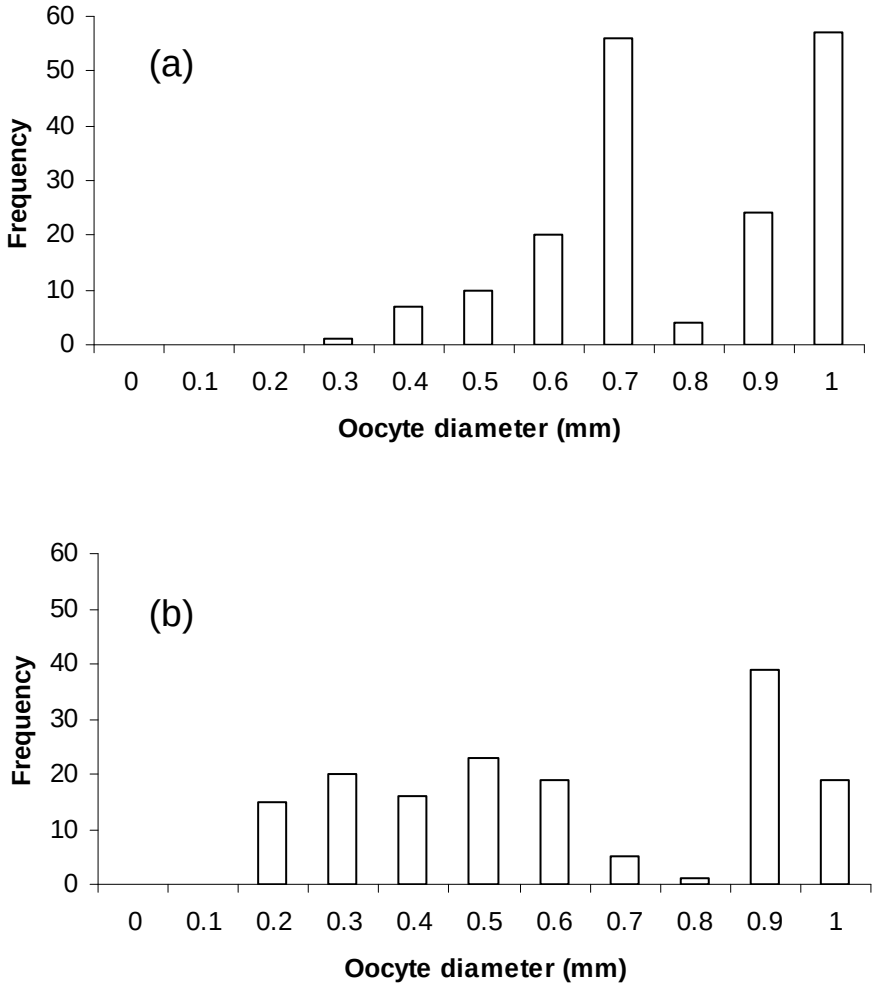

Figure 29. Size classes of oocytes from two individual *O. anolius*.

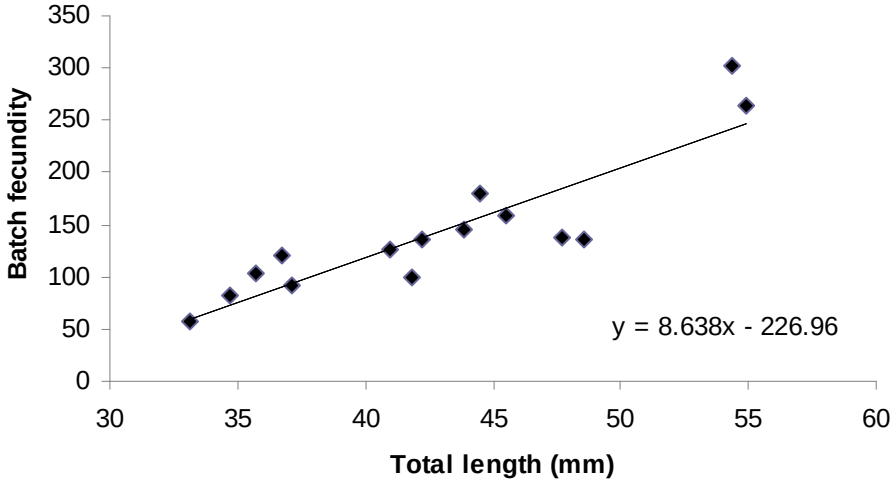

Figure 30. Number of hydrated oocytes in relation to fish length in *O. anolius*.

Length at maturity

The smallest mature female was 28.8 mm and all females over that length were mature. The smallest mature male was 23.5 mm TL and all males above 27.8 mm were mature. The first male with a distinguishable head crest appeared at 27.8 mm and all males over 30 mm had head crests. In no males over 30 mm was the prominent head crest absent and no females had prominent head crests, though large females could have weakly developed head crests. From December to March only four males and only three females collected were smaller than 30mm TL. Due to insufficient data in this size class a logistic regression curve was unable to be fitted to estimate length at 50% maturity (L_{50}) and 95% maturity (L_{95}).

Discussion

This study is the first to provide a detailed account of the reproductive life history of *O. anolius*. Although various aspects of reproduction and early development have been described for other species in the Omobranchini Tribe (Thomson & Bennett 1953, Neira *et al*. 1998, Sunobe 1998, Kawaguchi *et al*. 1999, Rao 1970, Dotsu & Oota 1973, Ismail & Clayton 1990, Kubo & Sasaki 1999), this study represents the first histological examination of gonads from this Tribe.

While little is known about *O. anolius*, there has been considerable study on the Blenniidae family. The testes of blennioids and gobies are unique among teleosts in possessing a testicular gland (Patzner & Seiwald 1987). In blennies this gland stores lipids and spermatids (Patzner & Seiwald 1987, Lahnsteiner *et al*. 1990, Lahnsteiner & Patzner 1990a). Unlike most teleosts, spermiogensis in these fish is not confined to the testes, as spermatids are released into the testicular gland where they further differentiate and receive nutrition (Lahnsteiner *et al*. 1990). The final development into spermatozoa occurs in the spermatic duct, which also acts as storage for mature spermatozoa (Lahnsteiner & Patzner 1990b). It has been suggested that since the final differentiation and storage occurs outside of the testes, this may shorten the time taken for spermiogensis which could be important in blennies spawning over successive days (Santos 1995). The testicular gland also adds sialomucins to the seminal fluid which are believed to increase its viscosity; this may be important in helping eggs adhere to the substratum (Lahnsteiner *et al*. 1990, Lahnsteiner & Patzner 1990a). At the end of the

spawning season the testicular gland cells have high phagocytotic activity (Lahnsteiner *et al*. 1990).

The proportion of testes to testicular gland varies among blennies with those species that have small testes releasing spermatids into the testicular gland at an earlier stage of maturation (Lahnsteiner & Patzner 1990c). Two types of spermiogensis occur in blennies: In Type I spermiogensis elongated spermatozoa are produced. This occurs in species with proportionally large testes. In Type II spermiogensis spherical spermatozoa are produced. This occurs in species with proportionally small testes (Lahnsteiner & Patzner 1990c). In this study, the volume of the testes in the *O. anolius* was not measured, but the spermatids were elongated so *O. anolius* appears to conform to type I spermiogensis. In *O. anolius* spermatids were present in the testicular gland before and after the female spawning period which may help to ensure that all ripe eggs get fertilised (Patzner & Seiwald 1987). Well outside of the spawning period lower numbers of spermatids were present in the testicular gland, which has also been observed in other blennies (Patzner & Seiwald 1987, Santos 1995, Carrassón & Bau 2003). The reason that spermatids occur in the testicular gland and are found in high numbers in the testes outside of the spawning season is unclear. The presence of spermatids immediately post spawning could be explained by the phagocytotic function of the gland but this does not explain spermatids found in the gland in July or the high numbers of spermatids in the testes outside of the spawning season. This warrants further study.

The sequence and general development of oocytes in *O. anolius* is similar to that of other teleost fish (Wallace & Selman 1981). In the final ripe stage coalescence of the yolk granules occurs in many marine teleosts and this is particularly so in those fish that spawn pelagic eggs (Wallace & Selman 1981). In *O. anolius* this coalescence was not observed, with the final ripe stage having large yolk platelets similar to other blennies and fish species that lay dermersal eggs (Patzner 1983, Santos 1995, Carrassón & Bau 2003). This can make distinguishing between the vitellogenic and ripe stage difficult (Carrassón & Bau 2003). In the fixing and staining technique used in this study the large yolk platelets proved difficult to fix and would often smear across the sections making the identification between these stages more difficult. As found in other species the final development of the ripe stage was not able to be successfully sectioned using standard techniques due to the shrinkage and distortion of these cells (West 1990).

Classical histological signs of sex change are ovaries that contain spermatogenic tissue or testes that contain ovarian tissue (Sadovy & Shapiro 1987). These were not present in any sections of *O. anolius* gonads sampled so it appears that *O. anolius* does not change sex. This is the same as all other blennies examined thus far (Patzner *et* al.2009).

This study reports the first record of an *O. anolius* nest found outside of an oyster shell, demonstrating that they are not restricted to using oysters for nests. The mean number of eggs per nest (1464) found in this study was higher than the 468 eggs that hatched in Thomson & Bennett's (1953) study. The maximum number of eggs reported in other *Omobranchus* species is 277 for *O. Japonicas* (Rao 1970), 291 for *O. bhattacharyae* (Rao 1970) and approximately 900 eggs for *O. loxozonus* (Dotsu & Oota 1973). Male blennies often spawn with multiple females and can even spawn with more than one female at the same time (Shibata & Kohda 2007). Therefore the number of eggs in one nest does not represent the fecundity of an individual female. The size of the male correlated with the numbers of eggs found in the nest which may mean that larger males have the ability to attract larger, more fecund females, or attract a larger number of females into the nest. As most nests collected appeared to be completely covered in eggs, an alternate explanation for this correlation might be that larger males simply have larger nests. Unfortunately nest size was not measured in this study so further study is required to confirm this.

Batch fecundity in *O. anolius* was correlated with fish length, with larger females more fecund. Without knowing the individuals' spawning period and spawning frequency, absolute fecundity (how many eggs are spawned in a given season) is very difficult to determine for batch spawners (Murua *et al*. 2003). Absolute fecundity is generally low in fish which show parental care, however this low fecundity is generally remedied by higher survivorship (Wootton 1999).

Introduced species may grow larger in their introduced range compared to their native range (Grosholz & Ruiz 1996). The oyster blennies maximum reported total length in Australia usually varies from 70 mm to 80 mm (Ogilby 1911, McCulloch 1917, Marshall 1964, Grant 1987, Kuiter 1993, Smith-Vaniz 2008) though it has been reported as high as 92 mm (Gomon *et al*. 1994, cited in Francis *et al.* 2004 & Neira *et al*. 1998). The largest specimen of 82 mm (TL) collected in this study was slightly larger than the usual size range in Australia however not outside of the largest size reported therefore there is no evidence that the *O. anolius* attains a larger size in New

Zealand. Male oyster blennies were significantly larger than females, a trend in 18 of 20 *Omobranchus* species (Springer & Gomon 1975). This is typical of blennies in general and has been attributed to their courtship and territorial behaviour (Oliveira *et al*. 2001).

All temperate marine teleosts have a regular and consistent spawning season which can be correlated with day length, temperature or plankton biomass (Bye 1990). As in New South Wales, the *O. anolius* females in New Zealand spawn batches of eggs over the summer months. In the northern part of its range in Australia the spawning season may be different because seasonal changes in temperature and day length have much smaller amplitudes at higher latitudes (Wootton 1999). Spawning over the summer months has also been observed in other *Omobranchus* species, for example *O. loxozonus* (Dotsu & Oota 1973) and *O. punctatus* (Ismail & Clayton 1990). Teleost species that spawn in the summer often lay multiple batches of eggs as the eggs develop faster in warmer temperatures (Wootton 1999). Interestingly most intertidal species in New Zealand breed from late winter to late spring (Paulin & Roberts 1992). Paulin and Roberts (1992) suggest that this may ensure that juveniles and pelagic larvae are in the water column when plankton production peaks in spring and summer. The production of multiple batches of eggs reaching maturity at different times over an extended spawning season may offer some advantages. Releasing eggs at different times avoids the risk to losing progeny to a one-off negative environmental event and it also may have implications for dispersal with different winds and currents on different days. This may be particularly true for *O. anolius*, in which eggs were observed to hatch daily over 17 days (Thomson & Bennett 1953)

The hepatosomatic index (HSI) measures liver weight in relation to body weight which can vary according to differences in sex, season, age, reproduction, feeding and stress (Brusle & Anadon 1996). The HSI is generally higher in female fish (Brusle & Anadon 1996) which was also the case in *O. anolius*. In some blennies it has been shown that as the gonosomatic index (GSI) increases, HSI drops because energy reserves of the liver are made available to the growth and differentiation of the gonads (Podroschko *et al*. 1985, Santos 1995) but this variation does not occur in all blennies (Carrassón & Bau 2003) and was not observed in *O. anolius*.

Springer & Gomon (1975) commented that for Omobranchiini the length at which the head crest first becomes apparent is variable and may be attributable as much to a state

of sexual maturity as to the size attained. In this study, it was not possible to fit a logistic regression curve to estimate length at 50% maturity (L_{50}) and 95% maturity (L_{95}) because there were insufficient smaller specimens collected during the spawning season. Outside of those months, particularly in April and May, many very small specimens were encountered, which may indicate a settlement event. The lack of fish in the smaller size ranges later in the year may indicate that this cohort became sexually mature within the first year. This is likely as they would only have to grow around 10 mm over that time. However, without ageing the fish using their otoliths this cannot be confirmed. Early maturity can be advantageous as it increases the chances of survival to a reproductive state and shortens the generation time. There are also disadvantages as the fish may not be as effective parents (i.e. guarding) and there may be a reduction in fecundity (Hutchings 2002).

The results from this study provide the first concrete evidence that introduced *O. anolius* are breeding successfully in New Zealand. The life history of *O. anolius* may have provided a number of advantages to help it establish in New Zealand. First, parental care has been shown in introduced freshwater fishes to increase the chances of establishment as it ensures a higher degree of survivorship which is important to very small founding populations (Marchetti *et al*. 2004). Second, by releasing multiple batches over an extended period it reduces the chance of a one-off event wiping out the progeny, important to a fledgling population (Peterson *et al*. 2004). Third, it seems likely that *O. anolius* matures within its first year, a critical factor in increasing the likelihood of establishing an introduced population (Sakai *et al*. 2001).

Chapter 4: General Discussion

The results of this study have demonstrated that *Omobranchus anolius* has established in New Zealand waters and within five years it has become both widespread and abundant in the region it was first discovered. While they mainly occur in dead shells of the invasive oyster *Crassostrea gigas* in the intertidal zone, they are also found in known vectors for dispersal of NIMS i.e. hull fouling and marine farms. This research has also provided the first detailed information on the life history of this species, outlining some of the life history characteristics that have aided its establishment in New Zealand.

Most marine fish species that are introduced to a new environment fail to establish viable populations. Even those that do manage to establish will not expand their range and become abundant (Baltz 1991). In 2003 the Australian oyster blenny, O. *anolius* was discovered in Auckland, and a survey found just 24 specimens, most at a single location (Francis *et al*. 2003). Little was known about this species and it had no prior history of invasion, making it difficult to know whether it would successfully establish and spread, and if it did, what impact it might have.

To test for adventism in New Zealand marine species, Cranfield *et al*. (1998) adopted the criteria of Chapman & Carlton (1994) (see below), where a species is classified as an adventive species if it meets at least three of the listed criteria. In New Zealand this system has been employed to test for adventism in the bridled goby, *Arenigobius bifrenatus* (Willis *et al*. 1999), and the Asian goby *Arenigobius pflaumii* (Francis *et al*. 2003).

Criteria for assessment of "adventive" status (*sensu* Chapman & Carlton 1994).

Has the species suddenly appeared locally where it had not been found before?

Has the species spread subsequently?

Is the species distribution associated with human mechanisms of dispersal?

Is the species associated with, or dependent on, other introduced species?

Is this species prevalent in, or restricted to, new or artificial environments?

Is the species distribution restricted compared to natives?

The worldwide distribution of the species is tested by a further three criteria:

Does the species have a disjunctive worldwide distribution?

Are dispersal mechanisms of the species inadequate to reach New Zealand, and is passive dispersal in ocean currents unlikely to bridge ocean gaps to reach New Zealand?

Is the species isolated from the genetically or morphologically most-similar species elsewhere in the world?

This study shows that *O. anolius* fulfils at least eight of the nine criteria:

Criteria 1) *O. anolius* was not known in New Zealand before 2003 (Francis *et al*. 2004)

Criteria 2) Increase in geographical spread

Criteria 3) *O. anolius* is presumed to have arrived in New Zealand via shipping (Francis *et al*. 2004) and it has now been found on hull fouling and in marine farms

Criteria 4) *O. anolius* is not only associated with the invasive Pacific oyster *Crassostrea gigas*, but seems to prefer it as a habitat

Criteria 5) *O. anolius* has been found on artificial structures and closely associated with *C. gigas* which create new biogenic habitats

Criteria 6) *O. anolius* was not found further north of Whangateau Harbour or further east that the western side of the Coromandel Peninsula though similar native species were

Criteria 7) *O. anolius* is only known from Australia and New Zealand

Criteria 8) *O. anolius* movement appears to be limited and larvae are released at a large size therefore they are unlikely to have arrived here through passive dispersal.

There is insufficient information to establish the final criterion.

Whether a species will successfully invade a novel environment is based on the characteristics of the invading species but also on the health and diversity of the receiving environment (Landis 2003). *Omobranchus anolius* has a number of characteristics which may have aided its establishment.

Blennies have a history of invasion success and this success has been attributed to their habit of seeking small holes for refuge and nesting sites which may allow them to take advantage of sea chests and crevices within vessels (Wonham *et al*. 2000). This behaviour can also mean they find immediate habitat within the spaces created by artificial structures inside and around ports (Wonham *et al*. 2000). Within the tribe Omobranchiini there have been successful invaders (e.g. *O. punctatus* (Baltz 1991, Gerhardinger *et al*. 2006, Golani 2004); *O. ferox* (Englund & Baumgartner 2000) and *O. rotundiceps obliquus* (Baumgartner 2002).

Intertidal fish are eurythermal and euryhaline and can tolerate considerable water loss (Gibson 1993), attributes shared by *O*. *anolius*. These attributes have been recognised in other invaders (Kolar & Lodge 2002, Marchetti *et al*. 2004) as harsh conditions are often encountered en route to the novel environment and during establishment.

Parental care in introduced freshwater fishes increases the chances of establishment by ensuring a higher degree of survivorship, which is important to very small founding populations (Marchetti *et al*. 2004, Peterson *et al.* 2004).

Releasing multiple batches of eggs over the spawning season reduces the chance of a one-off event wiping out the progeny (Peterson *et al*. 2004).

Maturing in one year may also add to success of an invading species, as it is able to breed rapidly after introduction (Sakai *et al*. 2001).

Ecosystems containing healthy and diverse assemblages may be more able to repel invaders (Elton 1958). Within the marine environment, harbours and estuaries have been identified as sites most common to invasion (Baltz 1991, Ruiz *et al*.1997, Paavola *et al*. 2005). These have been identified as susceptible habitat as they often have lower species diversity and are impacted by anthropogenic influences such as pollution, sedimentation and the erection of artificial structures (Landis 2003, Paavola *et al*. 2005, Piola & Johnston 2008). Auckland is a highly modified habitat that has been affected by reclamation, sedimentation, pollution and the introduction of at least 39 NIMS (Dromgoole & Foster 1983, Hayward *et al*.1997, Lohrer *et al*. 2008).

Where there is available habitat, invading species spread far more rapidly (Olden *et al*. 2006) and introduced species are more likely to be successful where there have been prior invasions or anthropogenic habitat modifications. This makes the introduction of

O. anolius of particular concern, as the invasive oyster *C. gigas* continues to spread in New Zealand waters, increasing the available preferred habitat for this fish.

Prior invasion success has been attributed as a characteristic that increases the likelihood of a species successfully invading (Kolar & Lodge 2001, 2002; Marchetti *et al*. 2004; Wotton & Hewitt 2004). As *O. anolius* is now a proven invader it seems likely that it will spread further within New Zealand waters and may also spread to other countries. Finding *O. anolius* on oyster farms is of particular concern as farmers routinely move both oysters and farming equipment between farms in different harbours. Certainly there is much available habitat for *O. anolius* further afield than the Hauraki Gulf.

Future research on this species should focus on a) determining the impact it is having on local species in terms of competitive interactions for food and habitat, b) the amount of transportation due to aquaculture activities and on hull fouling, c) monitoring the spread of this species, and d) further elucidation of the reproductive behaviour and dispersal mechanisms.

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Appendices

Appendix I – Protocols

Tissue processing, sectioning and staining procedures were modified from Bancroft & Stevens (2008) and from the Histological Techniques 775511 Laboratory Manual 2008, Bachelor of Medical Laboratory Science degree, Auckland University of Technology.

Tissue processing

Fixed gonads were dehydrated, cleared and infiltrated in a tissue processor (Shandon Elliot DM3) to the following schedule.

Dehydration	50% ethanol	1hour
75% ethanol	1hour	
75% ethanol	1hour	
95% ethanol	1hour	
95% ethanol	1hour	
100% ethanol	1hour	
100% ethanol	1hour	
100% ethanol	1hour	
Clearing	Xylol	1.5hour
Xylol	1.5hour	
Infiltration	Paraffin Wax	1.5hour
Paraffin Wax	1.5hour	

Embedding – Using an embedding centre (Thermolyne Histo-Center II-N) a thin layer of paraffin wax was dispensed into a mold and the gonad orientated, the mold was then transfered to the cold plate to solidify wax to hold the gonad in position. A cassette was then attached to the mold which was then filled with wax and transferred to the cold plate. Once the wax was solid, the mold was removed and the paraffin block with cassette was stored until sectioning.

Paraffin section cutting

The cassette with the paraffin block was clamped to microtome (Leica RM2235) and using the coarse feed mechanism ‘rough cut’ until the gonad was reached. Sections were then cut at 5–7 µm using smooth slow strokes with each individual section cut sticking edge to edge forming a ribbon. Once several sections had been cut the ribbon was floated in a 50ºC water bath and the folds removed by gentle teasing with tweezers. Sections were then floated onto a slide and placed into a 100ºC oven for 30 minutes to dry in preparation for staining.

Staining Procedure for Haematoxylin and Eosin (Progressive)

Slides were placed in racks and using the schedule below they were transferred between the solutions to deparaffinise, rehydrate, stain and counter stain the sections.

Xylol	5 minutes (Dewaxing)
Xylol	5 minutes (Dewaxing)
100% (Absolute) ethanol	1 minutes (Hydration)
95% ethanol	1 minute
95% ethanol	1 minute
70% ethanol	1 minute

Wash in running tap water

Haematoxylin (Harris's)	8 minutes
Running tap water	Rinse approx 20 seconds

Blue in Scotts Sub Tap Water(observe sections) 1 minute

Wash well in tap water	2 minutes
Eosin	5 minutes

Rinse in tap water

Slides were then dehydrated by taking them back through the alcohols in reverse order. Starting with the 95% for approx 8 dips in absolute; (so 8 dips in the 2 changes of 95% then 8 dips in the absolute).

Place slides in a 50/50 mix of absolute ethanol and xylol 20 seconds

Xylol	1 minute
Xylol	1 minute

Slides were then air dried and mounted using DPX and glass cover slips.

Appendix II – Marine Farm Questionnaires

20 December 2008

XXXXXXXX
(Oyster Farm)

(Postal Address)
XXXXXXX
XXXXXXX
Auckland, NZ

Dear XXXX,

I am a postgraduate student from Auckland University of Technology, and for my Master of Applied Science Degree I am studying the introduced Australian oyster blenny (*Omobranchus anolius*). As part of my thesis I am trying to establish the current distribution of this fish in New Zealand, as well as investigating its diet and reproductive biology. As the name suggests, this fish has an association with oysters and can often be found within the valves of dead oysters. In no way is this fish thought to harm the oysters, it simply uses them as a place of refuge.

I have found this fish in wild oyster populations around the Auckland region and I believe that this fish is present in some oyster farms; and it would be of benefit to my research to know which ones. I am writing to all oyster farmers in the Auckland region to ask them to fill in a short questionnaire to establish whether this fish may be present in their farm. If the results are positive then I shall send questionnaires to oyster farmers in other regions. Included with the questionnaire is an information sheet to aid in the identification of this fish species.

I intend to publish the results of this questionnaire, along with my other results in a peer-reviewed journal and in my Master's thesis. In these publications, sites where this fish is present or absent will be displayed on a map, and the results will appear in a summarised form; however the names of individuals, individual marine farms or organisations will not be mentioned. A copy of the report generated will be made available on request to those who participate in this research.

Your participation is entirely voluntary, however I would really appreciate it if you would take a moment of your time to check with your employees to see if they have noticed this fish and fill in the attached questionnaire. If you choose not to that is fine, and if you choose to and then change your mind any time before February 1st, you may remove yourself, and any information you provide without any adverse consequences. If you choose to assist me in this study could you please return the questionnaire within two weeks using the self addressed envelope supplied. To fully protect your confidentiality could you also please fill in the enclosed consent form and return it with the questionnaire. A follow up phone call may be required to confirm some details and if you are comfortable with this could you please tick the appropriate box on the consent form.

If you have any questions or would like further information please feel free to contact myself (details below) or my supervisor Dr Lindsey White, (Ph: 921-9999, Fax: 921-9743, Email: lindsey.white@aut.ac.nz). Should you have any concerns regarding the conduct of the research, please direct these to the Executive Secretary, AUTEC, Madeline Banda, *madeline.banda@aut.ac.nz* , 921 9999 ext 8044.

I look forward to hearing from you.

With kindest regards

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Approved by the Auckland University of Technology Ethics Committee on 2 December 2008, AUTEC Reference number 08/241

Distribution of the Oyster blenny Questionnaire

It would be greatly appreciated if you would use the attached identification sheet to check whether you or your employees have noticed the oyster blenny while processing your oysters. Once you have done this could you please fill out this questionnaire to the best of your ability and return it in the self addressed envelope provided.

Correspondent's Information

Name: _____

Organization: _____

Farm location (be as specific as possible):

_____

_____

_____

Office Address:

_____

Telephone No. _____

Email address: _____

Distribution Survey

1. During the processing of oysters, have you ever noticed fish amongst the oyster shells?

   ☐ **Yes**

   ☐ **No**

2. Do believe they are the oyster blennies as described and depicted on the information sheet?

   ☐ **Yes** they are this fish

☐ **No** they are another fish

Comments:_____

_____

If you answered yes to question 1 or 2, when did you first notice these fish?

☐ 1 year ago

☐ 2 years ago

☐ 3 – 5 years ago

☐ Longer than 5 years ago

Comments:_____

_____

Please feel free to make any other comments or observations regarding this research.

_____

_____

Participant's signature:
.....
.....

Participant's name:
.....
.....

Date:

Approved by the Auckland University of Technology Ethics Committee on 2 December 2008 AUTEC Reference number 08/241.

Oyster blenny Identification Sheet

The oyster blenny is a small (3cm – 7cm) greenish brown fish, with males differentiated from the females by the presence of a fleshy head crest (see fig 1 & 2). A unique feature of this fish is that it is closely associated with oysters and is often found hidden inside the valves of dead oyster shells (see fig 5 & 6).

If fish are being regularly found during the processing of the oysters they are most likely oyster blennies, however on occasion rock fish (fig 3) and triplefins (fig 4) can be found associated with oysters also. In general appearance oyster blennies can be distinguished from these two other fish in that they are a slender fish while the other two are more solid, but the definitive characteristic that distinguishes oyster blennies from these other two fish is the presence of a fleshy head crest in the males.

Figure 1: Male oyster blenny with head crest (3 – 7cm).

Figure 2: Female oyster blenny without head crest (3 – 7cm).

Figure 3: Rockfish with a solid body and large mouth, no head crest (3 – 15cm)

Figure 5: Upper valve of dead oyster removed to reveal male oyster blenny. Note the head crest folded over.

Figure 6: Upper valve of dead oyster removed to reveal a female oyster blenny hidden inside

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Consent Form

Project title: Distribution and ecology of the introduced Australian oyster blenny, *Omobranchus anolius* within the Hauraki Gulf.

Project Supervisor: Dr Lindsey White

Researcher: Jeremy Barker

- O I have read and understood the information provided about this research project in the accompanying letter dated 20 October 2008.
- O I have been given the opportunity to ask questions and to have them answered.
- O I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- O If I withdraw, I understand that all relevant information will be destroyed.
- O I agree to take part in this research.
- O I would you be happy to receive a follow up phone call if one is required to clarify any details (please tick one): YesO NoO.
- O I wish to receive a copy of the report from the research (please tick one): YesO NoO

Participant's signature:
.....

Participant's name:
.....

Participant's Contact Details (if appropriate):

.....

.....

Date:

***Approved by the Auckland University of Technology Ethics Committee on 2 December 2008 AUTEC Reference number* 08/241**

Note: The Participant should retain a copy of this form.

Appendix III – Fish collected in the study

X and Y = Map co-ordinates; HC=Headcrest where 0 is absent and 1 is present; TL=total length (mm); SL=standard length (mm); Wt=Weight (g).

Date	Location	X	Y	HC	TL (mm)	SL	Wt
7/04/08	Tahuna torea	174.8856	-36.8732	0	40.0	34.5	0.591
				0	26.5	22.5	0.168
				0	36.0	31.0	0.425
				0	45.0	37.9	0.859
				0	46.4	39.9	1.055
				0	46.5	40.0	0.964
				0	16.1	13.8	0.043
				0	32.4	28.3	0.326
				0	32.5	28.0	0.320
				0	23.8	20.6	0.123
				0	33.5	28.5	0.315
				0	27.0	23.2	0.185
				1	29.2	24.8	0.249
				1	34.7	29.8	0.360
				1	31.8	26.7	0.323
				1	34.8	30.3	0.407
				1	46.6	40.4	0.827
				1	53.8	45.1	1.460
				1	31.3	27.0	0.282
9/04/08	Tahuna Torea	174.8856	-36.8732	0	21.4	18.5	0.093
				0	38.0	33.1	0.454
				0	43.3	37.0	0.741
				0	35.2	29.9	0.388
				0	21.1	17.1	0.105
				0	18.3	15.7	0.063
				1	39.0	33.7	0.585
				1	26.2	22.8	0.175
				1	32.7	28.3	0.334
				1	48.0	41.0	0.975
				1	27.8	23.6	0.205
18/04/08	Tahuna Torea	174.8856	-36.8732	0	23.5	19.7	0.123
				0	17.1	14.8	0.072
				0	18.0	15.8	0.087
				0	23.4	19.4	0.135
				0	24.5	20.4	0.191
				0	24.5	21.1	0.192
				0	25.2	22.2	0.229
				0	25.9	21.6	0.215
				0	26.8	22.3	0.243
				0	18.3	15.3	0.072
				0	22.4	19.0	0.155
				0	23.3	19.4	0.161
				0	23.3	20.3	0.187

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				0	23.5	20.0	0.144
				0	26.3	22.0	0.228
				0	27.0	23.0	0.254
				0	27.8	23.8	0.276
				0	28.7	24.5	0.283
				0	29.0	24.4	0.220
				0	32.0	28.0	0.378
				0	33.0	29.7	0.393
				0	33.5	29.1	0.371
				0	34.5	29.5	0.445
				0	35.6	30.2	0.495
				0	37.5	30.9	0.588
				0	41.1	35.2	0.818
				0	42.0	35.7	0.827
				0	42.6	36.7	0.753
				0	42.8	36.5	0.867
				0	43.3	37.4	0.793
				0	43.5	38.0	0.919
				0	44.7	39.3	1.019
				0	47.5	40.6	1.101
				0	54.0	46.4	1.561
				0	25.6	22.4	0.236
				0	29.6	25.0	0.287
				0	41.6	36.5	0.737
				0	45.2	38.0	0.993
				0	47.0	39.0	1.063
				0	48.5	41.8	1.244
				1	27.8	23.6	0.248
				1	29.3	24.7	0.276
				1	30.1	25.6	0.327
				1	30.4	25.7	0.325
				1	31.3	26.5	0.378
				1	32.2	27.0	0.435
				1	46.5	38.0	1.084
				1	47.8	41.0	1.043
				1	48.8	42.3	1.053
				1	58.0		1.542
				1	60.0	48.8	1.891
				1	62.5	51.0	2.035
				1	25.2	21.1	0.157
				1	31.2	26.5	0.357
				1	38.4	32.6	0.639
				1	44.6	37.8	0.879
				1	52.1	43.8	1.476
22/04/08	Harbour Bridge	174.7518	-36.8173	0	20.0		0.101
				0	21.0		0.114
				0	33.0		0.328
				0	38.5		0.527
				0	49.0		1.038

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				0	55.0		1.538
				1	30.0		0.286
				1	37.5		0.486
				1	42.5		0.695
				1	43.5		0.733
				1	53.5		1.282
				1	57.0		1.615
				1	57.5		1.570
				1	53.5		1.235
5/05/08	Hobson Bay	174.8022	-36.8557	0	40.0	34.3	0.630
				0	35.2	31.4	0.315
				1	69.7	56.8	1.863
				1	73.9	58.8	2.487
				1	64.4	53.0	1.478
7/05/08	Okahu Bay	174.8139	-36.8516	0	29.0		0.260
				0	31.0		0.237
				0	44.0		0.773
				0	44.5		0.715
				0	29.0		0.200
				0	29.0		0.214
				0	18.0		0.055
				0	18.5		0.070
				0	28.0		0.172
				0	28.5		0.211
				0	32.5		0.330
				0	33.0		0.306
				0	41.5		0.664
				0	43.0		0.658
				0	45.0		0.770
				1	37.0		0.371
				1	51.0		1.281
				1	55.5		1.379
				1	29.0		0.226
				1	33.5		0.313
				1	37.0	31.9	0.445
				1	39.5		0.531
				1	57.0		1.637
8/05/08	Okahu Bay	174.8139	-36.8516	0	20.5		0.099
				0	24.5		0.153
				0	31.0		0.252
				0	36.0		0.402
				0	33.0		0.297
				1	36.0		0.363
				1	46.0		0.798
25/05/08	Little Shoal Bay	174.7397	-36.8171	0	34.0	29.0	0.340
				1	28.0	24.0	0.207
				0	44.0	37.0	0.763
				0	53.0	45.0	1.330
				0	55.0	46.5	1.662

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				1	55.2	46.7	1.462
				1	60.0	50.5	1.840
				1	59.0	45.0	1.476
				0	47.0	40.0	0.878
				0	51.0	43.0	1.118
				0	40.0	35.0	0.494
				0	46.5	39.0	0.793
				1	62.0	51.0	1.924
				1	33.5	28.5	0.339
				1	35.0	29.5	0.385
				1	35.0	29.5	0.340
				1	45.0	39.0	0.767
				1	65.0	53.0	2.030
				1	65.5	52.0	2.033
				1	55.0	46.0	1.323
				1	31.5	26.5	0.237
30/05/08	Tahuna Torea	174.8856	-36.8732	0	47.0	40.0	0.920
				0	50.5	43.5	1.180
				0	52.0	44.0	1.223
				0	42.5	36.0	0.688
				0	52.5	45.0	1.288
				0	57.0	47.5	1.594
				0	19.9	17.0	0.073
				0	33.0	28.5	0.324
				0	39.5	34.0	0.579
				0	43.0	36.0	0.701
				0	48.0	41.0	0.998
				0	48.5	41.0	1.023
				0	44.0	37.5	0.759
				1	52.0	44.0	1.255
				1	58.0	48.5	1.292
				1	60.0	48.0	1.873
				1	61.0	50.5	1.898
				1	62.0	50.5	1.891
				1	41.5	36.5	0.675
				1	54.0	44.0	1.302
				1	65.5	59.0	2.102
				1	48.0	40.0	0.912
				1	54.0	44.5	1.334
15/06/08	Island Bay	174.6886	-36.8100	0	19.8	16.7	0.059
				0	48.9	42.6	0.911
				0	45.1	38.9	0.709
				0	26.4	23.1	0.133
				0	38.9	32.9	0.417
				0	40.5	35.1	0.478
				0	56.2	47.6	1.322
				1	50.2	42.9	0.972
				1	52.7	43.7	1.020
01/07/08	Hilders Park	174.6789	-36.7896	0	62.8	53.5	1.877

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				1	63.3	53.1	1.763
21/07/08	Tahuna Torea	174.8856	-36.8732	0	29.8	26.2	0.218
				0	30.1	26.0	0.255
				0	34.0	29.9	0.355
				0	39.4	34.0	0.513
				0	41.3	36.9	0.719
				0	45.5	39.7	0.805
				0	29.0	25.1	0.182
				0	48.0	40.5	0.742
				0	22.4	19.2	0.088
				0	28.1	23.5	0.177
				0	31.4	27.3	0.246
				0	25.0	21.4	0.114
				0	26.0	22.9	0.169
				0	26.3	22.5	0.173
				0	43.1	36.7	0.605
				1	40.1	35.3	0.537
				1	45.7	38.6	0.658
				1	51.2	44.3	0.885
				1	51.2	43.0	0.920
				1	55.1	47.3	1.193
				1	55.8	47.9	1.234
				1	62.5	51.6	1.806
				1	67.2	57.1	2.073
				1	29.9	24.2	0.216
				1	34.5	29.8	0.352
				1	31.3	26.9	0.242
5/09/08	Rangitoto Island	174.8636	-36.8068	0	61.8	53.3	2.647
9/09/08	Harbour View	174.6656	-36.8367	0	30.0	25.0	0.186
				0	31.0	27.5	0.279
				0	32.0	27.5	0.267
				0	48.0	41.0	0.830
				0	54.0	46.0	1.300
				0	33.5	28.5	0.273
				0	41.0	35.0	0.494
				0	49.0	41.0	0.980
				0	61.0	50.0	1.447
				0			1.134
				0	47.0	40.6	0.740
				1	41.5	35.5	0.504
				1	81.6	65.2	4.063
				1	35.0	29.5	0.303
				1	36.5	31.0	0.385
				1	42.0	36.0	0.577
				1	42.0	35.0	0.514
				1	49.0	41.5	0.761
11/09/08	Little Shoal Bay	174.7397	-36.8171	0	24.4	20.3	0.089
				0	45.4	38.6	0.580
				0	46.8	40.2	0.703

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				0	48.3	41.0	0.780
				0	50.0	42.0	0.902
				0	50.8	43.3	0.925
				0	54.6	48.1	1.180
				0	22.9	19.4	0.097
				0	26.4	22.6	0.124
				0	35.2	30.8	0.288
				0	45.5	39.5	0.628
11/09/08	Okahu Bay	174.8139	-36.8516	0	29.2	24.6	0.174
				0	30.5	26.1	0.206
				0	35.0	29.9	0.280
				0	35.6	31.0	0.362
				0	36.0	30.2	0.265
				0	39.7	34.0	0.465
				0	40.4	34.6	0.486
				0	41.2	35.4	0.519
				0	43.6	37.5	0.620
				0	47.3	40.6	0.802
				0	48.4	41.8	0.963
				0	50.5	43.2	0.785
11/09/08	Pollen Island	174.6786	-36.8672	0	19.6	16.9	0.05
				0	33.3	29.2	0.32
				0	42.5	36.3	0.58
				0	54.1	47.5	1.25
11/09/08	Point England	174.8731	-36.8840	0	35.8	30.4	0.325
				0	41.9	35.5	0.521
				0	45.0	37.5	0.560
				0	53.6	45.2	1.089
				0	29.7	25.2	0.147
				0	57.0	48.4	1.259
				0	51.8	43.4	0.972
11/09/08	Little Shoal Bay	174.7397	-36.8171	1	37.0	32.3	0.353
				1	53.6	44.6	0.957
				1	53.8	45.3	0.936
				1	60.1	49.9	1.292
				1	65.2	54.7	1.630
				1	70.5	58.1	2.192
11/09/08	Okahu Bay	174.8139	-36.8516	1	36.3	31.2	0.329
				1	38.0	31.9	0.363
				1	40.7	35.1	0.530
				1	45.0	38.1	0.583
				1	45.7	38.4	0.640
11/09/08	Pollen Island	174.6786	-36.8672	1	35.4	30.8	0.32
				1	51.3	43.8	1.02
				1	53.6	44.8	1.23
				1	56.7	46.4	1.09
				1	68.7	55.1	2.42
				1	69.9	58.3	2.00
11/09/08	Pollen Island	174.6876	-36.8715	1	52.3	45.0	0.95

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				1	72.2	60.7	2.32
11/09/08	Point England	174.8731	-36.8840	1	36.9	31.6	0.363
				1	69.5	57.3	2.563
				1	55.1	47.0	1.092
				1	41.5	33.5	0.411
23/09/08	Halfmoon Bay	174.8955	-36.8810	0	35.0	30.0	0.302
				0	37.5	32.0	0.385
				0	38.0	32.5	0.382
				0	49.0	41.5	0.878
				0	56.0	47.5	1.295
				1	38.0	33.0	0.357
				1	42.0	35.5	0.547
				1	59.0	49.0	1.297
				1	67.0	56.0	1.789
				1	44.5	37.5	0.577
				1	48.5	41.5	0.745
23/09/08	Harbour View	174.8487	-36.8769	0	30.0	25.0	0.186
01/10/08	Panmure Basin	174.8525	-36.9021	0	42.2	36.8	0.595
				0	50.7	43.8	1.167
17/10/08	Dunkirk Reserve	-36.8958	174.8684	0	42.2	36.1	0.766
				0	46.5	39.6	0.974
				0	33.9	29.0	0.318
				0	50.8	43.8	1.388
				1	55.3	46.2	1.351
17/10/08	Panmure Wharf	174.8679	-36.9030	0	38.3	33.7	0.450
				0	39.1	33.2	0.516
				0	49.4	42.9	0.893
				0	45.4	39.6	0.771
				1	36.8	31.7	0.356
				1	49.4	43.0	0.974
				1	59.2	50.4	1.612
				1	37.8	32.9	0.422
				1	43.2	36.9	0.643
17/10/08	Waiotaki Bay	174.5254	-36.5270	0	45.5	40.0	0.978
				0	42.0	36.0	0.613
				0	40.4	34.5	0.498
				0	42.7	36.3	0.614
				0	48.3	40.9	0.759
				0	23.8	20.4	0.120
				0	36.9	31.5	0.504
				1	35.4	30.5	0.404
				1	37.9	32.5	0.443
				1	41.3	35.5	0.655
				1	53.0	45.6	1.190
				1	55.6	47.2	1.471
				1	61.0	49.9	1.401
				1	58.9	50.1	1.682
14/11/08	Tahuna Torea	174.8856	-36.8732	0	34.7	29.2	0.269
				0	44.4	37.8	0.675

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				0	47.9	42.4	0.881
				0	45.5	39.3	0.791
				0	35.8	30.8	0.328
				0	44.2	38.2	0.659
				0	44.8	38.5	0.652
				0	45.3	39.0	0.734
				0	33.7	29.9	0.337
				0	33.3	29.3	0.261
				0	45.4	38.3	0.664
				0	48.8	41.7	0.935
				0	36.0	31.2	0.391
				0	38.7	33.5	0.493
				0	37.2	32.0	0.406
				0	25.4	22.2	0.122
				0	28.1	24.0	0.156
				0	29.7	26.0	0.187
				0	30.1	25.7	0.183
				0	30.1	25.8	0.231
				0	31.2	27.0	0.201
				0	35.1	30.9	0.259
				0	25.1	21.6	0.119
				0	27.8	23.7	0.154
				0	29.5	25.1	0.189
				0	29.7	25.6	0.190
				1	45.4	39.3	0.654
				1	35.0	30.5	0.299
				1	35.1	29.7	0.289
				1	40.0	34.5	0.531
				1	39.2	33.4	0.500
				1	43.5	37.3	0.574
				1	44.7	38.1	0.658
				1	70.6	58.5	2.474
				1	28.8	24.8	0.177
				1	32.3	27.9	0.236
				1	34.1	29.7	0.275
25/11/08	Bayswater Marina	174.7706	-36.8187	0	30.6	25.3	0.223
				0	35.6	30.4	0.328
				0	28.3	24.0	0.168
				0	43.9	37.2	0.629
				1	49.8	42.9	0.713
25/11/08	Little Shoal Bay	174.7397	-36.8171	0	30.1	25.1	0.170
				0	42.7	37.2	0.560
				0	46.2	39.6	0.686
				0	48.7	42.7	0.808
				0	54.4	46.1	1.250
				0	54.9	46.9	1.216
				1	32.2	27.6	0.233
				1	33.8	29.3	0.248
				1	37.8	32.1	0.351

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				1	38.6	33.7	0.324
				1	38.9	33.1	0.402
				1	41.2	35.3	0.478
				1	49.1	41.5	0.808
				1	51.7	43.4	0.758
				1	52.0	43.9	0.828
				1	54.5	46.1	0.996
				1	55.0	46.2	1.032
				1	67.9	57.4	2.089
				1	72.8	59.1	2.131
29/11/08	Kawakawa Bay	175.1503	-36.9448	0	29.5	25.5	0.184
				0	31.8	28.1	0.240
				1	41.1	34.7	0.428
				1	46.4	40.0	0.656
				1	59.4	51.5	1.636
				1	40.1	34.0	0.402
				1	75.0	61.8	2.610
30/11/08	Eric Armshaw Park	174.6940	-36.8685	0	34.2	29.0	0.314
				0	36.1	31.0	0.370
				0	37.1	31.8	0.433
				0	46.6	40.3	0.757
				0	51.5	44.6	1.082
				1	32.2	27.6	0.255
				1	56.8	48.2	1.210
				1	64.2	54.1	1.672
				1	68.4	57.4	2.132
30/11/08	Raymond Reserve	174.6988	-36.8593	0	29.9	26.4	0.238
				0	28.8	25.4	0.219
				0	24.7	21.2	0.126
				0	35.5	30.3	0.417
				0	43.7	37.9	0.754
				0	44.0	37.7	0.734
				0	44.8	38.1	0.773
				0	50.2	42.9	1.071
				0	50.4	43.0	1.187
				1	32.1	27.9	0.258
				1	38.2	32.6	0.443
				1	42.8	40.0	0.615
				1	45.0	38.6	0.758
				1	46.2	39.0	0.696
				1	47.2	39.9	0.763
				1	53.2	43.9	1.083
				1	59.6	49.9	1.601
				1	59.7	50.3	1.820
				1	61.5	50.1	2.168
13/12/08	Waiwera Bridge	174.7056	-36.5408	0	63.6	54.8	2.106
18/12/08	The Boulevard	174.8773	-36.9009	0	23.5	20.4	0.112
				0	34.1	29.1	0.355
				0	36.2	30.7	0.465

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				0	36.3	31.2	0.412
				0	38.4	33.6	0.495
				0	42.8	37.7	0.739
				0	55.8	47.9	1.381
				0	40.4	34.5	0.632
				0	30.0	26.4	0.231
				0	28.2	23.9	0.177
				0	32.2	27.6	0.301
				1	60.5	50.4	1.523
				1	48.4	41.4	0.877
				1	51.3	42.5	0.969
				1	53.9	44.7	1.204
				1	55.8	45.6	1.168
				1	57.4	47.9	1.316
				1	73.5	58.7	2.187
				1	39.7	33.2	0.440
				1	45.6	39.7	0.815
				1	46.9	39.5	0.849
				1	47.0	40.5	0.717
				1	47.1	40.3	0.745
				1	47.6	41.4	0.812
				1	53.8	43.9	0.913
15/01/09	Little Shoal Bay	174.7397	-36.8171	0	34.1	28.9	0.358
				0	36.7	31.3	0.466
				0	41.0	35.1	0.572
				0	42.1	36.0	0.597
				0	43.3	36.8	0.767
				0	44.5	38.3	0.712
				0	45.2	38.8	0.682
				0	47.2	40.4	0.903
				0	50.1	43.1	0.965
				0	36.2	31.4	0.364
				0	38.5	33.3	0.380
				1	40.0	34.2	0.487
				1	42.7	36.2	0.538
				1	43.5	37.3	0.594
				1	50.0	43.2	0.917
				1	57.1	47.7	1.330
				1	57.8	47.9	1.302
				1	37.6	32.6	0.449
				1	42.9	36.3	0.567
				1	44.8	38.0	0.653
				1	47.9	41.1	0.720
				1	48.4	41.0	0.799
				1	49.8	42.7	0.892
				1	52.8	44.8	0.967
				1	53.1	43.8	1.061
26/01/09	Edgewater Drive	174.8717	-36.9244	0	56.4	47.7	1.437
				0	41.2	35.0	0.585

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				0	48.3	42.0	0.970
26/01/09	Princes Street	174.8658	-36.9342	0	52.3	44.7	1.417
				1	65.6	52.4	2.111
26/01/09	Edgewater Drive	174.8717	-36.9244	1	58.3	48.7	1.675
				1	49.2	41.3	0.743
26/01/09	Tahuna Torea	174.8856	-36.8732	0	33.0	28.5	0.403
				0	33.2	28.5	0.313
				0	35.7	30.3	0.455
				0	41.1	35.0	0.646
				0	41.8	35.7	0.765
				0	43.9	37.1	0.807
				0	42.2	36.3	0.759
				0	26.3	22.8	0.163
				0	42.1	37.2	0.803
				0	49.3	43.5	1.243
				0	38.9	33.3	0.615
				1	45.8	37.7	0.845
				1	34.2	28.6	0.347
				1	39.7	33.8	0.571
				1	40.3	34.0	0.574
				1	53.7	45.4	1.303
7/02/09	Tarau	175.5243	-37.1174	0	49.3	42.3	0.873
				1	48.8	41.8	0.843
				1	56.4	47.6	0.992
8/02/09	Colville Bay	175.4658	-36.6252	1	56.5	47.4	1.145
				1	72.2	60.3	3.315
8/02/09	Coromandel	175.4887	-36.7630	0	48.9	41.5	0.866
				0	56.0	47.6	1.282
				1	43.4	36.9	0.688
				1	44.4	38.1	0.635
				1	49.2	41.4	0.962
				1	56.5	47.5	1.176
				1	57.5	48.5	1.166
				1	58.7	48.2	1.412
				1	60.6	51.0	1.432
4/03/09	Farm Cove	174.8783	-36.8955	0	42.6	36.9	0.553
				0	53.8	45.9	1.162
				0	40.1	34.1	0.500
				0	47.7	41.2	0.901
				0	36.5	31.8	0.353
				0	40.9	35.8	0.601
				0	41.9	35.7	0.556
				0	42.0	36.0	0.578
				0	44.9	38.8	0.716
				0	47.0	40.4	0.939
				0	48.4	41.3	0.872
				0	48.6	42.2	0.933
				0	48.7	41.6	0.837
				0	57.1	49.5	1.411

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				1	56.7	47.8	1.368
				1	51.3	43.3	0.947
				1	64.4	52.2	1.727
				1	67.6	55.4	2.040
				1	76.7	61.5	2.618
				1	50.5	43.7	0.915
				1	52.8	44.3	1.016
				1	43.0	38.0	0.558
				1	45.2	38.6	0.644
				1	48.4	41.7	0.752
				1	49.7	41.5	0.901
10/03/09	Bayswater	174.7679	-36.8180	0	64.9	56.2	2.302
				0	51.5	43.8	1.061
				0	53.5	46.5	1.472
				1	54.3	45.6	1.101
				1	57.9	47.5	1.564
10/03/09	Stanly Point	174.7813	-36.8271	0	44.5	38.5	0.683
				0	45.5	39.1	0.709
				0	45.8	39.4	0.778
				0	39.1	33.8	0.437
				1	45.4	38.4	0.628
				1	57.5	48.6	1.133
				1	58.8	49.1	1.304
				1	59.0	49.8	1.298
				1	55.8	47.9	1.243
11/03/09	Brookfield Point	174.9511	-36.9127	0	42.3	35.8	0.322
				0	50.4	43.5	0.792
				0	51.0	43.8	1.035
				1	59.9	50.5	1.463
11/03/09	Puriri Avenue	174.9849	-36.8862	0	25.0	21.5	0.126
				0	26.5	23.0	0.146
				0	33.3	29.2	0.300
				0	34.7	30.0	0.341
				0	34.8	29.8	0.359
				0	35.1	30.1	0.327
				0	35.3	30.6	0.339
				0	36.9	31.6	0.431
				0	37.7	32.5	0.431
				0	38.0	33.3	0.391
				0	38.3	33.7	0.380
				0	38.5	33.7	0.434
				0	39.9	34.5	0.487
				0	41.3	36.0	0.550
				0	41.3	35.6	0.562
				0	43.7	37.2	0.645
				0	49.4	42.8	0.809
				0	49.5	42.7	0.979
				0	53.0	45.4	1.174
				0	54.0	46.6	1.255

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				1	44.0	38.2	0.665
				1	44.4	37.9	0.523
				1	47.5	42.0	0.980
				1	47.8	40.1	0.800
				1	47.9	39.5	0.592
				1	48.3	41.0	0.727
				1	53.9	46.3	1.386
				1	57.6	48.2	1.602
11/03/09	Te Puru Park	175.0146	-36.8790	0	33.0	28.2	0.268
				0	20.0	17.4	0.068
				1	52.8	44.7	1.131
12/03/09	Riverlea Road	174.6170	-36.7741	0	57.8	49.7	1.746
				0	58.6	51.1	1.626
				0	62.3	54.0	1.920
				0	63.0	55.7	2.155
				0	63.1	53.8	2.735
				1	80.7	66.2	3.533
				1	71.1	57.2	2.403
12/03/09	Scotts Reserve	174.6711	-36.8049	0	47.0	40.5	0.678
				0	47.8	41.0	0.736
				0	50.5	42.9	0.792
				0	36.0	31.2	0.351
				1	44.7	37.8	0.610
				1	51.4	43.1	0.990
				1	58.8	48.8	1.503
				1	59.9	49.3	1.210
				1	70.4	57.4	2.237
12/03/09	Waimaire	174.6387	-36.7740	0	54.9	47.6	1.264
				0	59.0	51.4	1.885
				0	60.1	51.4	2.098
				0	53.8	46.5	1.097
				0	55.8	48.2	1.135
				0	56.2	48.2	1.399
				1	75.8	62.4	3.007
13/03/09	Point Erin	174.7395	-36.8378	0	34.9	30.3	0.349
				0	43.7	36.8	0.651
				0	42.5	35.9	0.600
				0	44.1	38.3	0.721
				0	47.1	40.7	1.055
				0	47.4	40.4	0.890
				0	49.5	42.4	0.903
				0	50.1	44.1	1.078
				1	67.7	56.8	2.096
13/03/09	Westhaven Marina	174.7458	-36.8401	1	67.0	57.1	2.190
17/03/09	Omaha Causeway	174.7657	-36.3409	1	58.1	49.5	1.643
				1	61.7	50.7	1.841
25/03/09	Little Shoal Bay	174.7397	-36.8171	0	37.1	31.9	0.515
				0	37.5	31.5	0.527
				0	40.1	34.1	0.538

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				0	43.0	37.3	0.831
				0	46.0	38.3	0.978
				0	46.8	39.6	0.999
				0	47.9	40.8	1.103
				0	46.9	40.0	1.102
				0	51.6	43.7	1.268
				0	46.4	39.3	1.006
				0	54.9	46.7	1.534
				0	29.1	24.7	0.234
				0	39.2	34.3	0.573
				0	46.2	39.7	0.930
				1	44.2	37.3	0.837
				1	52.1	43.4	1.160
				1	54.7	47.1	1.501
				1	56.8	46.7	1.573
				1	59.5	48.8	1.861
				1	60.3	49.3	1.803
				1	37.2	31.9	0.441
				1	47.1	39.6	0.825
				1	48.3	41.3	0.961
				1	48.5	40.3	0.875
				1	71.1	58.5	3.039
				1	73.9	60.0	3.527
28/03/09	Judges Bay	174.7895	-36.8506	0	35.8	30.2	0.365
				0	40.2	33.7	0.548
				0	44.7	37.8	0.826
				0	44.7	37.2	0.713
				0	47.8	40.5	1.040
				1	33.1	28.2	0.364
				1	42.6	35.1	0.581
				1	48.4	40.5	0.963
				1	52.8	43.5	1.083
				1	62.1	49.7	1.813
				1	62.2	51.7	1.944
				1	63.1	53.3	2.039
				1	66.4	54.2	2.189
				1	67.2	52.6	2.317
				1	72.4	58.4	2.773
				1	73.3	59.8	3.077
28/03/09	Parnel Baths	174.7939	-36.8519	0	40.8	34.8	0.679
				0	45.2	38.6	0.853
				0	47.5	40.9	1.101
				0	47.6	40.6	1.048
				0	47.6	39.9	1.115
				0	48.0	40.8	0.997
				0	58.4	50.0	1.648
				1	53.0	44.3	1.147
				1	54.3	45.9	1.096
				1	57.3	47.0	1.389

Date	Location	X	Y	HC	TL (mm)	SL	Wt
				1	58.3	48.9	1.503
				1	70.0	55.8	2.556
29/03/09	Glendowie	174.8831	-36.8616	0	50.9	43.7	1.093
				0	27.7	23.0	0.178
				1	56.9	47.4	1.330
29/03/09	Karaka Bay	174.8775	-36.8501	0	46.4	39.8	0.772
29/03/09	Victoria Avenue	174.7999	-36.8619	0	37.2	31.6	0.391
31/03/09	Fallstaff Reserve	174.8945	-36.8846	0	47.0	40.5	0.976
				0	57.4	49.4	1.692
				0	26.4	22.8	0.150
				0	40.2	34.2	0.579
				0	42.7	36.6	0.740
				0	42.8	37.2	0.689
				0	42.9	36.5	0.706
				0	43.5	37.0	0.724
				0	45.3	38.4	0.871
				0	45.6	39.2	0.828
				0	46.4	39.8	1.029
				0	47.3	40.8	1.081
				0	55.8	47.8	1.551
				0	55.9	48.1	1.582
				1	66.4	53.6	1.996
				1	37.4	32.7	0.472
				1	50.6	41.4	1.061
				1	64.6	52.9	2.312
				1	69.2	57.3	2.381
				1	81.6	64.7	3.568
				1	47.4	40.3	0.850
31/03/09	Hathaway	174.8990	-36.8613	0	39.2	33.9	0.548
				0	41.2	34.9	0.617
				0	41.3	35.4	0.731
				0	48.6	41.9	1.082
				0	49.3	42.4	1.050
				0	49.8	42.5	1.061
				0	56.0	48.4	1.704
				0	43.6	36.5	0.777
				0	44.0	37.3	0.781
				0	33.6	28.0	0.350
				0	41.6	34.9	0.645
				0	47.7	40.4	1.022
				1	42.3	35.0	0.528
				1	50.1	41.0	0.940
				1	58.6	48.3	1.654
				1	61.1	49.7	1.668
				1	62.9	50.9	1.590
				1	33.5	27.6	0.319
				1	47.5	39.8	0.899
				1	60.3	49.0	1.818

Date	Location	X	Y	HC	TL (mm)	SL	Wt
31/03/09	Buckland	174.9049	-36.8779	0	25.4	21.9	0.158
				0	31.1	27.0	0.258
				0	45.7	39.3	0.885
				0	46.7	40.2	0.914
				0	33.3	29.2	0.321
				1	55.9	55.6	1.354