

Biology and ecology of the non-indigenous goby
***Acentrogobius pflaumi* (Bleeker 1853) in the Swan-**
Canning Estuary

Submitted by
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This thesis is presented for the Degree of Honours in Marine Science

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B.Sc

Murdoch University

Declaration

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Abstract

Non-indigenous species can have significant deleterious impacts on the ecosystems in which they become established. Following the recent establishment of the Striped Sandgoby *Acentrogobius pflaumi* in the Swan-Canning Estuary, south-western Australia, a study was initiated to determine its spatial and temporal distribution and biological characteristics. Although *A. pflaumi* was not recorded in the coarse sandy sediment present in the nearshore, shallow waters of the estuary, substantial numbers were recorded on soft muddy sediments in the deeper waters, where it comprised 55% of the total number of gobies. While *A. pflaumi* dominated the gobiid fauna in Lower Melville Water (~98%), its contributions declined progressively upstream, indicating a preference for waters with a salinity close to that of full strength sea water. Size and age compositions determined that the oldest individual was 3.9 years old and 89 mm in total length, but that the population is dominated by 1+ individuals. Population mortality and turn-over rates are therefore likely to be very high. Both males and females attained > 87% of their asymptotic lengths (L_{∞}) of 74.9 and 69.3 mm, respectively, during the first year of life, which is characteristic of smaller, shorter-lived species of fish. The results from gonadosomatic indices and the histological examination of gonads suggest that *A. pflaumi* is able to spawn throughout most of the year, with a peak from November to February. The presence of mature, spawning and depleted gonads in *A. pflaumi* suggests that this species spawns within the Swan-Canning Estuary. *Acentrogobius pflaumi* can be thus considered an estuarine & marine species like *Favonigobius lateralis*. As *A. pflaumi* attains high densities over a relatively large part of the estuary and can breed within the system, it is likely to be a permanent resident and further work is needed to determine its impact on the native gobiid fauna.

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Chapter 1: General Introduction

In this Thesis, I have investigated the biology of the non-indigenous goby species, *Acentrogobius pflaumii*, in the Swan-Canning Estuary in south-western Australia. This chapter firstly describes the nature of estuaries, followed by a short overview on the fish fauna of these ecosystems, with particular focus on the significance of gobies. The impacts of non-indigenous or invasive species are then briefly highlighted before introducing *A. pflaumii* and the aims of this study. Note that further information on the ecology of non-indigenous species in estuaries is provided in a literature review in Chapter 2.

1.1. Estuaries

The term estuary applies to a range of water bodies, which exhibit a range of geomorphological and hydrological characteristics (McLusky and Elliott, 2007). Early attempts to define an estuary (*e.g.* Lyell, 1833; Dionne, 1963; Pritchard, 1967) focused mainly on large systems located in macrotidal regions of North America and Europe, which were typically funnel-shaped, maintained a permanent connection to the ocean and contained ‘seawater that is measurably diluted by freshwater’ (Pritchard, 1967). Such definitions, however, did not encompass the types of estuaries found in microtidal regions of the world, *e.g.* south-western Australia and southern Africa, where the connection between the estuary and the ocean can be temporarily disconnected via the formation of a sand bar and in which salinities can become greater than full strength seawater and sometimes markedly so (Potter *et al.*, 2015a; Tweedley *et al.*, *in press*) In order to provide a more holistic and all-encompassing definition of an estuary, Potter *et al.* (2010) proposed that an estuary is a “*partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural sea water and varies temporally and along its length, it can become hypersaline*

in regions when evaporative water loss is high and freshwater and tidal inputs are negligible”.

As estuaries are located at the interface between marine and freshwater ecosystems, they are unique aquatic environments influenced by processes that characterise the marine and freshwater systems interacting within their boundaries (Gillanders and Kingsford, 2002; McLusky and Elliott, 2007). Thus, these system may experience simultaneous influences from both marine and freshwater processes, such as salinity variation due to tidal influence and freshwater runoff (Gillanders and Kingsford, 2002; Kennish, 2002), or increased nutrient availability and cycling due to nutrient rich water being discharged from the catchments via rivers and allochthonous inputs from marine waters (Sheaves *et al.*, 2014). As a result of the interactions between freshwater and marine inputs, estuaries are regarded as dynamic environments where the physico-chemical conditions change rapidly (Kennish, 2002; Sheaves *et al.*, 2014).

Despite posing considerable physiological and physical challenges to the organisms that inhabit them, the productive nature of estuaries provides an environment conducive to the rapid growth of those species that possess the necessary physical, behavioural and osmoregulatory characteristics to cope with the varied stressors present in these systems (Elliott and Quintino, 2007). Indeed, estuaries are considered amongst the most productive and ecologically important ecosystems (Jackson *et al.*, 2001; Kennish, 2002; Sheaves *et al.*, 2014) and are globally recognised nursery areas for many recreationally and economically important fishery species (Robinson 2001; Sheaves *et al.* 2014; Potter *et al.* 2015b). In addition, these systems provide some less obvious ecosystem services, such as nutrient cycling and water purification (Costanza *et al.*, 1997).

1.2. Unique environment of south-western Australian estuaries

It has become increasingly apparent over the last few decades that the geomorphological, hydrological, sedimentological and biotic characteristics of temperate microtidal estuaries (*i.e.* tidal range < 2 m), such as those in south-western

Australia and southern Africa, differ markedly from the coastal plain estuaries and rias found in temperate macrotidal regions of the world (*i.e.* tidal range > 2 m), such as north-western Europe (Potter and Hyndes, 1999; Elliott and Whitfield, 2011; Tweedley *et al.*, *in press*). From an environmental perspective, microtidal estuaries typically have narrow entrance channels, unlike the wide funnel shaped coastal plain estuaries. This narrow entrance channel, combined with the low tidal range, restricts exchange with the ocean, limits flushing and thus only a small proportion of the water body in the estuary is exchanged during each tidal cycle. This low exchange rate means that the residence time for water in microtidal estuaries, such as the Swan-Canning, can be up to 235 days in summer (Kalnejais *et al.*, 1999), compared to less than a week in 12 macrotidal estuaries in northern Spain (Borja *et al.*, 2006). This high residence time is thought to be a key factor in the natural susceptibility of south-western Australian estuaries to degradation (Tweedley *et al.*, 2014b, 2015a).

As a result of the limited water exchange with the ocean, the salinity at any single point in a microtidal estuary undergoes minimal changes during a tidal cycle, unlike in macrotidal systems, where rapid and substantial changes in salinity occur during each tidal cycle. The salinity in microtidal estuaries changes rapidly, however, when large volumes of freshwater enter these systems during and following periods of heavy rainfall. South-western Australia experiences a Mediterranean climate, where on average, ~70% of rainfall occurs between May and September, *i.e.* from late autumn to early spring (Hodgkin and Hesp, 1998) and thus some of these systems, which receive lower volumes of freshwater discharge either seasonally or throughout the year, can be cut off from the ocean by the formation of a sand bar at their mouths. The highly seasonal rainfall and limited exchange with the ocean can also result in parts (or all) of the estuary becoming hypersaline and in some cases, such as Culham Inlet, on the south coast of Western Australia, salinity can reach ~300 (Chuwen *et al.*, 2009) and can result in the death of fish (Hoeksema *et al.*, 2006a).

1.3. Estuarine fish faunas

Estuaries form an essential part of the life cycle for a diverse array of fish and invertebrate species due to the provision of habitat, refuge, food and migratory routes for diadromous species (Nagelkerken *et al.*, 2013; Potter *et al.*, 2015a, 2015b). Species utilise estuaries, in both a facultative and obligatory sense, and can be classified according to the ways in which they use these systems (Potter *et al.*, 2015b).

The ichthyofaunas of macrotidal estuaries are dominated, both in terms of number of species and individuals, by species which spawn in the marine environment, *i.e.* marine stragglers and marine estuarine-opportunists (*e.g.* Thiel and Potter, 2001). This reflects the presence of pronounced tidal water movements, which provide a mechanism for the passive and selective transport of larvae and juveniles of various species from their spawning grounds in marine waters to their nursery areas within the estuary (Forward and Tankersley, 2001). As a result, the species composition undergoes pronounced cyclical changes each year due to time-staggered immigration and emigration of juveniles of marine species and of diadromous species (Potter *et al.*, 1997; Maes *et al.*, 2005). Moreover, macrotidal estuaries, due to their turbidity and flushing capacity, provide a hostile environment for the spawning and survival of eggs and larvae, thus resulting in such systems containing relatively few estuarine-resident species (Potter and Hyndes, 1999).

In contrast to the dynamic hydrological nature of macrotidal estuaries, the physico-chemical conditions present in microtidal estuaries throughout summer are relatively stable and benign, due to low inputs of freshwater discharge that occur at that time of year (Potter and Hyndes, 1999; Potter *et al.*, 2015a). This stable environment, in which salinity approaches that of full strength seawater, water temperature is relatively high and flushing is very limited, promote the survival and retention of eggs within the estuary and thus provide a stable and conducive environment for the growth of the resultant larvae and juveniles (Tweedley *et al.*, *in press*). As a result, the fish faunas of microtidal estuaries are dominated, in terms of the numbers of individuals, by species

capable of spawning within the estuary (Gill and Potter, 1993; Chuwen *et al.*, 2009; Potter *et al.*, 2015a).

Unlike in macrotidal estuaries, where pronounced changes in fish faunal composition occur throughout the year due to the to time-staggered immigration and emigration of juveniles of marine species and of diadromous species, the species composition of the ichthyofauna in both nearshore and offshore waters of microtidal estuaries in south-western Australia is determined by season, and, to a much greater extent, region within the estuary (Loneragan *et al.*, 1987, 1989). It has been hypothesised that the greater influence of region reflects the marked differences between the morphological and thus hydrological characteristics of the different regions of particularly the larger and permanently-open estuaries, such as the Swan-Canning Estuary. Thus, abundant species belonging to the Atherinidae and Gobiidae families are largely partitioned between the entrance channel, *i.e.* lower estuary, basins, *i.e.* middle estuary, and saline reaches of the tributary rivers, *i.e.* upper estuary (Fig.1.1; Prince and Potter, 1983; Gill and Potter, 1993). The species present with each family also partition dietary resources among themselves and, together with the spatial separation, has resulted in them dominating the near shore fish faunas (Gill and Potter, 1993; Humphries and Potter, 1993; Potter and Hyndes, 1999). Such is the dominance of these species, in the estuaries on the south coast of Western Australia, that they can represent as much as 99% of the total ichthyofauna in some estuaries (Hoeksema *et al.*, 2009; Tweedley, 2011).

1.4. Non-indigenous species

Non-indigenous species (NIS), or introduced species, are those species translocated to environments outside of their natural distribution, typically as a result of anthropogenic activities, such as shipping, aquaculture and the construction of canals connecting various previously disparate waterbodies (Williams and Grosholz, 2008; Ojaveer *et al.*, 2014). If a NIS is shown to have a deleterious impact on the ecology of

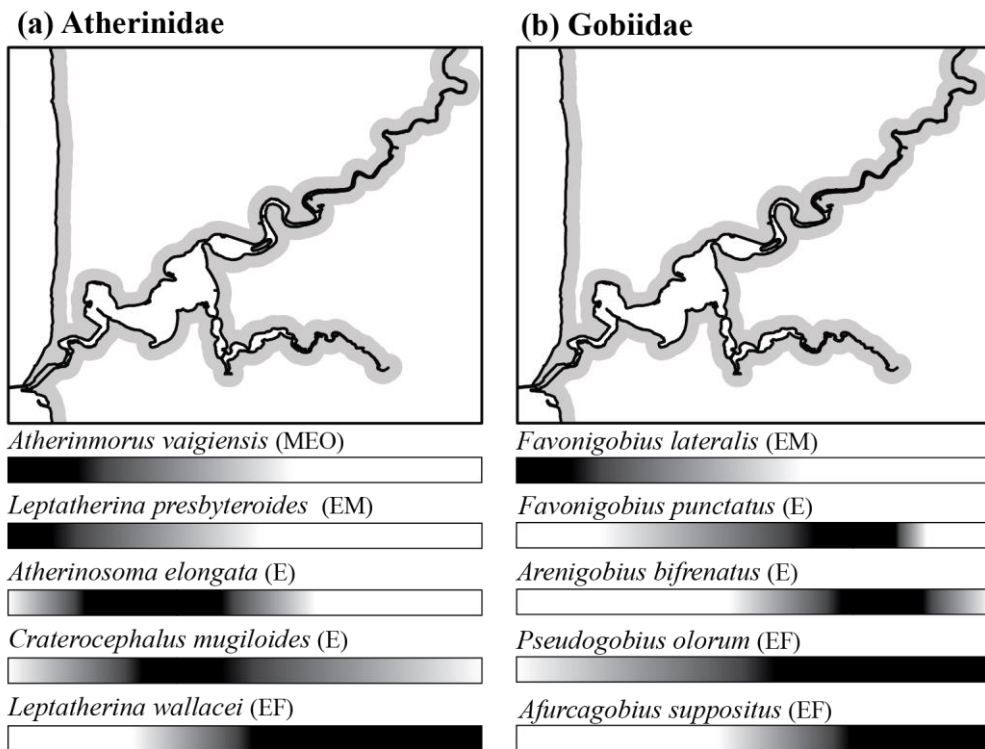


Figure 1.1. Distribution of five atherinid species and five goby species in the Swan-Canning Estuary. The gradation from black to white for each species reflects their relative abundance, ranging from maximum to zero densities, respectively. Life cycle guilds for each species are allocated on the basis of Potter *et al.* (2015a), namely solely estuarine (E), estuarine and marine (EM), estuarine and freshwater (EF) and marine estuarine-opportunist (MEO). Modified from Tweedley *et al.* (*in press*).

the receiving environment, it is then regarded as an invasive species. Therefore, while all NIS species are introduced, not all are invasive. Unfortunately, however, many NIS have been found to negatively impact ecosystems outside of their native ranges and thus invasive species have been identified as one of the most pervasive threats to marine ecosystem health worldwide (*e.g.* Vitousek *et al.* 1997; Mcneely and Schutyser 2003; Molnar *et al.* 2008; Williams and Grosholz 2008).

Estuaries have long been focal points for human colonisation. They initially provided an abundant food source both within their waters and via the development of agriculture on the surrounding land (Wilson, 1988). These characteristics, and the provision of a suitable environment for docking ships, led to the establishment of

permanent settlements around these systems (Wilson, 1988; 2002). This, in turn, resulted in the creation of various industries revolving around estuaries and the associated global importation and exportation of trade products (McLusky and Elliott, 2004). These factors help explain why so many cities have been built around estuaries. Indeed, seven of ten of the world's largest megacities are located on or close to estuaries, including Tokyo, Guangzhou, Jakarta, New York, Seoul and Shanghai (Potter *et al.*, 2015a). As coastal populations continue to expand and utilisation of marine ecosystems increase, so too do the impacts associated with anthropogenic activities (Jackson *et al.*, 2001; Small and Nicholls, 2003).

Estuaries are particularly susceptible to introductions of NIS due to their nature as a centre for anthropogenic activities that also conjointly function as major pathways of introduction (Williams and Grosholz, 2008), such as shipping activities (*e.g.* Carlton *et al.*, 1995; Davidson *et al.*, 2009), aquaculture (*e.g.* Naylor *et al.*, 2001; GISP, 2004) and canals (*e.g.* Holeck *et al.*, 2004; Por, 2012). Moreover, the rate of invasions is increasing exponentially worldwide as marine activity increases (*e.g.* Tamburri *et al.*, 2002; Hewitt *et al.*, 2004; Worm *et al.*, 2006). Indeed, the prevalence of NIS in estuaries and coastal waters is illustrated by the San Francisco Bay and delta (estuary), which is arguably the most invaded of all aquatic systems containing 234 introduced species and at least 125 species of unknown origin (Cohen and Carlton, 1998).

1.5. Gobiids as non-indigenous species

The vectors (*i.e.* methods of transport) of NIS introduction are generally non-selective in terms of the types of taxa transported to new locations and studies have yet to elucidate the key factors determining the success of invasion by NIS, with the exception of climate/habitat match (Williams and Grosholz, 2008). It is therefore striking to note that globally, members of the Gobiidae have been successfully introduced into non-natal systems more frequently than any other fish family (Wonham

et al., 2000). Examples of invasions by non-indigenous goby species include: *Acanthogobius flavimanus* in New South Wales (Australia) and California (USA) (Middleton, 1982; Workman and Merz, 2007), *Proterorhinus marmoratus* in the North American Great Lakes (Jude *et al.*, 1992), *Arenigobius bifrenatus* in New Zealand (Willis *et al.*, 1999) and *Neogobius melanostomus* in the North American Great lakes, the River Scheldt (Belgium), the Hron River (Slovakia), Gulf of Gdańsk (Baltic sea) and the River Rhine (Germany) (Jude *et al.*, 1992; Sapota, 2004; Stráňai and Andreji, 2004; Borcherdig *et al.*, 2011; Verreycken *et al.*, 2011). Furthermore, in a study of ballast water fish dispersal, Wonham *et al.* (2000) found that of the 24 species that managed to successfully establish in a new environment post-transport, 6 were gobies, the highest proportion of species belonging to any family.

The prevalence of goby introductions, which are predominantly associated with ballast discharge, is due, in part, to their crevicolous nature; as they seek out small holes or cracks in order to hide and lay eggs and it is this cryptic nature that gives them the ability to avoid detection and escape predation (Carlton *et al.*, 1995; Wonham *et al.*, 2000). This strategy also potentially predisposes them to surviving in port environments in which they are discharged as other alternative sources of prey may be more ‘visible’ to potential predators. Furthermore, gobies have an advanced lateral line system with superficial neuromasts, which is thought to aid them in navigating and finding food at night when the risk of being predated on themselves is reduced (Kasumyan, 2003; Janssen, 2004). Indeed, some species of goby, such as the Round Goby *Neogobius melanostomus*, prefer to feed at night and can do so in complete darkness (Charlebois *et al.*, 1997b). When combined with an opportunistic diet (*e.g.* Darcy 1981; Kostrzewa and Grabowski 2003), such an adaptation would, no doubt, increase the chance of being able to feed in the ballast hold of ships, thereby increasing their chances of survival.

1.6. Gobiid biology

Gobiid gobies, referred to as gobies hereafter, form part of the Gobioidae sub-order within the Perciformes and represent the most speciose marine fish family in the

world, comprising ~1,950 species (Helfman *et al.*, 2009). In general, gobies are predominantly small, *i.e.* < 10 cm in total length, typically have short lifespans (< 1 year) and are characterized by rapid growth and early sexual maturity (Helfman *et al.*, 2009). Indeed the shortest documented lifespan of any vertebrate species is that of the Coral Reef Pygmy Goby *Eviotta silligata*, at a maximum of ~59 days (Depczynski and Bellwood, 2006; Randall and Delbeek, 2009). In contrast, some species display a propensity for longevity that belies their relatively minute stature, such as the Yellow Clown Goby *Gobiodon okinawae*, which has been recorded to surpass at least 13 years of age despite reaching a total maximum length of just 38 mm (Randall and Delbeek, 2009).

Gobies tend to live on mud or sand or in association with burrowing/burying invertebrates such as shrimps (Helfman *et al.*, 2009) and occur throughout a diverse range of marine and freshwater habitats (Patzner *et al.*, 2011). In fact, some gobies are able to osmoregulate over a wide range of salinities, for example, the Bluespot Goby *Pseudogobius olorum*, a species able to reproduce in freshwater (Potter *et al.*, 2015b), was found in hypersaline conditions in an estuary where the salinity was 76 (Hoeksema *et al.*, 2006b).

Gobies also exhibit a variety of reproductive strategies to enhance the survival of their offspring. This typically involves producing demersal or benthic eggs, which, in rivers and estuaries, prevent their eggs being swept downstream and out to sea (Miller, 1984; Hoese, 1994; Potter and Hyndes, 1999). In contrast, many freshwater goby species on tropical islands, particularly the members of the Sicydiinae, have developed an amphidromous lifecycle, where they are swept out to sea as larvae, but later return to rivers where they eventually become mature (Keith, 2003; Tweedley *et al.*, 2013). Such a life history strategy enables these species to be dispersed across the marine divide and into the rivers of recently emerged high islands, thus enhancing the range of the species (McDowall, 2010).

Parental investment is also common in many members of the Gobiidae family (Patzner *et al.*, 2011) and is an intrinsic component of courtship rituals and reproductive

success for some species, such as in the sand goby *Pomatoschistus minutus* (Lindström *et al.*, 2006). Indeed the males of the River Goby *Glossogobius callidus* (Wasserman *et al.*, 2015) and Freshwater Goby *Tridentiger brevispinis* fan and aggressively guard nests (Mashiko, 1998), while yet others such as the Black Goby, *Gobius niger*, build elaborate nests in addition to guarding and fanning eggs (Vaas *et al.*, 1975). Gobies show a diversity of reproductive lifestyles, with some species being gonochoristic (*e.g.* Leopard Goby *Elactinus saucrum*; Robertson and Justines, 1982) and others displaying hermaphroditism (*e.g.* Blue-banded Goby *Lythrypnus dalli*; Mary 1993).

1.8. Striped Sandgoby

In its native range, the Striped Sandgoby, *Acentrogobius pflaumi*, is found in estuarine environments and coastal embayments in China, Korea, Japan, Taiwan and the Philippines (Masuda *et al.*, 1984; Shao *et al.*, 1993; Kanou *et al.*, 2004; Nan *et al.*, 2009). This goby predominantly inhabits soft substrates, sand and sea grass (Horinouchi and Sano, 2000; Kanou *et al.*, 2004; Horinouchi, 2008) and has been recorded cohabiting with burrowing alpheid shrimps in Japan (Yanagisawa, 1978). Studies in coastal waters off South Korea have demonstrated that *A. pflaumi* feeds predominantly on detritus and benthic macroinvertebrates, such as polychaetes and amphipods, with an ontogenic shift observed towards larger food items (Horinouchi and Sano, 2000; Kanou *et al.*, 2004; Horinouchi, 2008).

Like most members of the Gobiidae, *A. pflaumi* is a relatively small fish, which, in the north-west Pacific, averages 80 mm (TL) (Baeck *et al.*, 2004) but has been reported to reach lengths of up to 109 mm (Wang *et al.*, 2015). Individuals reach sexual maturity at 40 mm and proceed to spawn, during their first year of life, in late spring to early summer (Baeck *et al.*, 2004). The eggs produced by these gobies are benthic and adhesive and thus attached to solid substrate, such as the underside of bivalve shells (Masuda *et al.*, 1975). After hatching, the larvae undergo a ~30 day pelagic larval phase and then settle at a size of ~5-8 mm (Lockett and Gomon, 1999; Kanou *et al.*, 2004).

Unfortunately, following translocation, likely via ballast water discharge, *A. pflaumii* established populations in Waitemata and Whanapoua harbour in New Zealand in 2001 (Francis *et al.*, 2003) and in Moreton Bay (Queensland) and Port Phillip Bay (Victoria) in Australia (Lockett and Gomon, 2001; Gomon *et al.*, 2008). The species was first recorded in Cockburn Sound, a marine embayment near Perth, Western Australia, in 2004 (Mead-Hunter, 2005) and was recorded by SCUBA divers in the Swan-Canning Estuary ~20 km to the north later that same year (Maddern and Morrison, 2009). Limited investigations of the biology and ecology of this species in Australia and New Zealand indicate that the species may grow up to 70-80 mm TL and has a preference for open soft silty substrates (Francis *et al.*, 2003; Maddern and Morrison, 2009).

1.9. Aims

Although the fish fauna of the Swan-Canning Estuary has been surveyed extensively since the mid-1970s (Chubb *et al.*, 1979; Loneragan *et al.*, 1989; Gill and Potter, 1993; Potter and Hyndes, 1999; Hoeksema *et al.*, 2006a; Valesini *et al.*, 2009, 2014; Hallett and Tweedley, 2014, 2015), *A. pflaumii* was not recorded during any of those studies, several of which have been undertaken post 2004. However, in addition to *ad hoc* sightings by SCUBA divers (Maddern and Morrison, 2009), substantial numbers of *A. pflaumii* have been recorded during a study to determine the spatial and temporal distribution of the Western School Prawn (*Metapenaeus dalli*) in the Swan-Canning Estuary (Tweedley *et al.*, 2014a). Given that gobies represent a significant proportion of the total number of fish inhabiting estuaries in south-western Australia and the fact that *A. pflaumii*, once established in Port Phillip Bay, was shown to proliferate rapidly (Lockett and Gomon, 2001), there is a clear need to investigate the extent of the colonisation of the Swan-Canning Estuary by this non-indigenous species.

In light of the above, the overall aim of this study was to use the comprehensive ‘monthly’ sampling regime already in place to monitor *M. dalli*, which started in October 2013 and runs until March 2016, to collect information and specimens of

A. pflaumi from which the general biology and ecology of the non-native population of this species can be determined.

More specifically, the aims of my Honours project were to:

1. Undertake a literature review of the ecology of invasive species in estuarine and coastal waters, focusing on the vectors of transmission, impacts of the invasions and the management strategies employed to mitigate their effect and reduce the likelihood of future introductions (Chapter 2).
2. Determine the abundance and distribution of *A. pflaumi* in the shallow, nearshore and deeper, offshore waters of the Swan-Canning Estuary and detail if this changes throughout the year (Chapter 3).
3. Compare the spatial and temporal patterns of abundance and distribution of *A. pflaumi* to that of native goby species (Chapter 3).
4. Determine whether the otoliths of *A. pflaumi* possess periodically formed growth increments and if the numbers of such zones can be used to age this species (Chapter 4).
5. Determine the growth rates for female and male *A. pflaumi* (Chapter 4).
6. Compare the biological/ecological traits of *A. pflaumi* to other goby species globally, with reference to native gobies when possible (Chapter 4).
7. Determine whether *A. pflaumi* is spawning in the Swan-Canning Estuary and, if so, the timing and duration of reproduction and the lengths and ages at maturity for females and males (Chapter 4).
8. Use the information generated above to provide some future directions for research on this non-indigenous species in estuaries in south-western Australia (Chapter 5).

Chapter 2: Non-indigenous species in estuaries and coastal marine waters-vectors of transmission, biological and ecological implications and management strategies

2.1. Abstract

Estuaries are highly productive coastal ecosystems that provide a variety of valuable goods and key ecosystem services, such as the provision of nursery habitats and water purification. However, the ability of estuaries to continue to provide quality goods and services, upon which we depend, is undermined by the existence of threats such as global warming, eutrophication and non-indigenous species (NIS). As significant threats to estuarine health, NIS are typically accompanied by adverse effects upon the systems they inhabit and are increasingly common occurrences in coastal environments as their rate of dispersal and subsequent establishment increases due to their association with anthropogenic vectors of dispersal, such as shipping, the aquarium industry and canals/seaways. Shipping, as the most prominent pathway of dispersal, is the focus of increasing scrutiny as management efforts towards NIS repeatedly fail. Future success in the prevention of NIS relies upon the development of effective risk assessment strategies, both species and region specific, in order to accurately identify threats and allocate resources and management efforts to prevent them.

2.2. Introduction

Non-indigenous species (NIS) are organisms that have been translocated to regions outside of their natural range, typically through direct or indirect association with anthropogenic activities (Williams and Grosholz, 2008; Ojaveer *et al.*, 2014). The rate at which NIS are successfully colonising estuaries and coastal ecosystems throughout the world is increasing markedly (Cohen and Carlton, 1998; Ruiz *et al.*, 1999; Tamburri *et al.*, 2002; Hewitt *et al.*, 2004; Gollasch, 2006; Worm *et al.*, 2006). Furthermore, the range of impacts of NIS on coastal ecosystems, both positive and negative, are also accumulating (Ruiz *et al.*, 1999), which in turn places additional stress on the estuarine and marine environments they inhabit.

Estuaries were initially attractive to humans as they provided an abundant food source both within their waters and from the development of agriculture on the surrounding fertile land (Wilson, 1988). These characteristics, and the convenience of estuaries as sheltered docking points for ships, for the transport of goods, led to the establishment of permanent settlements around these systems (Wilson, 1988, 2002). Thus, estuaries are regarded as hubs of maritime activity and resource use (Lotze *et al.*, 2006), and, in fact, seven of ten of the world's largest megacities (*e.g.* Shanghai, Tokyo, New York City) are located on or close to estuaries (Potter *et al.*, 2015a). Given this, it is not surprising that estuaries are regarded as the most degraded of all aquatic ecosystems (Jackson *et al.*, 2001; Kennish, 2002).

While awareness of some anthropogenic pressures exerted on estuaries are well established, *e.g.* eutrophication, climate change and urbanisation, the recent and ongoing increase in the number of NIS and their subsequent impacts on coastal ecosystems is now being regarded as one of the most pervasive threats to estuarine health (*e.g.* Vitousek *et al.* 1997a,b; Thresher 1999; Kappel 2005; Gollasch 2006; Venter *et al.* 2006; Molnar *et al.* 2008). The impact of these threats to estuaries ultimately manifests in the degradation of ecological processes and the reduction in the ability of estuaries to provide key ecosystem goods and services, such as water purification and the provision of nursery habitats (Barbier *et al.*, 2011).

Estuaries are defined as “A *partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural sea water and varies temporally and along its length, it can become hypersaline in regions when evaporative water loss is high and freshwater and tidal inputs are negligible*” (Potter *et al.*, 2010, 2015a). These complex systems provide a variety of benefits to society, such as provision of refuge, nursery and transitional water regions for valuable finfish and crustacean estuarine dependant fisheries, in addition to water purification and erosion control (*i.e.* sediment stabilisation and soil retention via vegetation root structure) services (*e.g.* Costanza *et al.* 1997; Beck *et al.* 2001; Barbier *et al.* 2011; Sheaves *et al.* 2014; Potter *et al.* 2015a). The global decline in the health of estuarine and coastal ecosystems, which is partly due to NIS, is known to have affected at least three services, namely: (1) the number of viable fisheries, (2) the extent and quality of nursery habitats and (3) filtering and detoxification services provided by suspension feeders, submerged vegetation and wetlands (Worm *et al.*, 2006).

In light of the above, this review will describe the increasing frequency of NIS establishing themselves in estuarine and coastal ecosystems worldwide, with particular emphasis on the impacts associated with the occurrence of these species through the use of documented cases of NIS establishment. Mechanisms of NIS dispersal are also reviewed, highlighting trends in their occurrence, both past and present, and their respective relevance to contemporary society. In addition, the current management strategies of estuarine NIS will be reviewed, identifying gaps in their effective management and making suggestions for management improvement based on case studies of successful management. It is hoped synthesising global knowledge about NIS and their impacts will lead to greater understanding and, therefore, greater success in preventing the establishment of NIS and managing and mitigating the impacts of established NIS.

2.3. Pathways and vectors of dispersal

2.3.1. Types of non-indigenous species

Non-indigenous species, also referred to as alien and/or introduced species, are species that occur in regions outside of their ‘natural distribution’ through the intervention of anthropogenic avenues of dispersal or favourable circumstances that overcome otherwise limiting factors (Williams and Grosholz, 2008; Ojaveer *et al.*, 2014). While NIS pose a suite of unpredictable socio-economic, ecological and health impacts, it is the subset of NIS, known as invasive species, which are of particular cause for concern (Ruiz *et al.*, 1997). These species are NIS that have spread, are spreading or have the capability to spread elsewhere and whose occurrence is accompanied by adverse effects on biological diversity, ecosystem function, socio-economic values and/or human health (Occhipinti-Ambrogi and Savini, 2003; Williams and Grosholz, 2008; Ojaveer *et al.*, 2014). Indeed, Thresher (1999) considered NIS to be “ecological pollutants” rather than a problem like the invasive subset. Accurately identifying NIS can be difficult given the knowledge of the system at hand, which results in the use of the term cryptic species, *i.e.* those species for whom no reliable proof exists for the allocation as either native or introduced (Carlton, 1996b). It should be noted that this application of the term cryptic species differs markedly to that employed by an evolutionary biologist, in which cryptic species allude to those organisms that are morphologically identical but are separate species.

2.3.2. Pathways of transmission

Occurrences of NIS are becoming increasingly frequent as the global rate of introduction and subsequent establishment increases (*e.g.* Fig. 2.1; Cohen and Carlton 1998; Ruiz *et al.* 1999, 2000; Tamburri *et al.* 2002; Hewitt *et al.* 2004; Worm *et al.* 2006). The establishment of NIS in such large numbers, particularly post 1960s (Fig. 2.1), stems from increases in the frequency and variety of dispersal mechanisms provided by anthropogenic activities, in addition to increased documentation and sampling (Hewitt *et al.*, 2004). The proliferation of vectors provided multiple routes that

inadvertently circumvented the biogeographical barriers typically restrictive of natural marine invasion events (Ruiz *et al.*, 1997; Ricciardi, 2007).

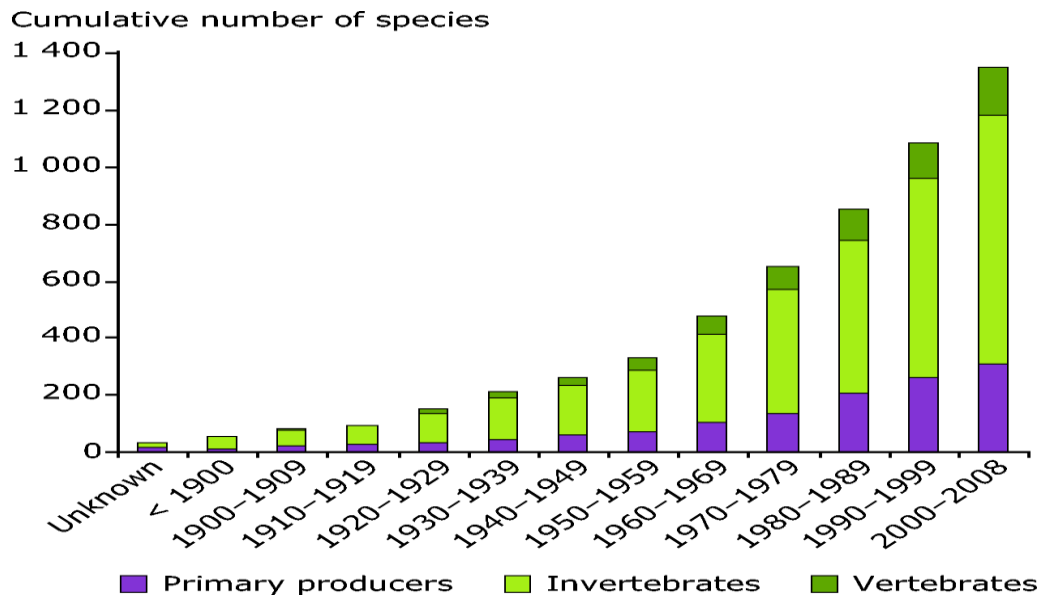


Fig. 2.1. Non-indigenous species in European marine/estuarine waters. Taken from European Environment Agency (EEA, 2009).

The pathways and vectors of transmission have varied throughout history, reflecting the activities and technology relevant to the time period (Hewitt *et al.*, 2004) and the dominance of any given vector representing a progression in marine activities and global connectivity (Ruiz *et al.*, 1997; Gollasch, 2006). Globalisation has ensured that the diversity of vectors is dynamic, complex and ever increasing (Bax *et al.*, 2003; Perrings *et al.*, 2005). For example, historic shipping practices provided bio-fouling and ballast vectors, while current shipping activities present multiple opportunities for dispersal through ballast, hull fouling and recesses in vessel hulls, *i.e.* sea chests (*e.g.* Table 2.1; Williams *et al.* 1988; Ruiz *et al.* 1997; Bax *et al.* 2003; Davidson *et al.* 2009; Frey *et al.* 2014). Five major pathways of NIS transmission have been recognised within the marine environment (Table 2.1), of which the first three: shipping; aquaculture and fisheries; and waterways, are considered the most frequent modes of primary dispersal (Ojaveer *et al.*, 2014). In addition, there are also various minor vectors available for species transfer, such as bait bucket introductions,

scuba/snorkelling equipment contamination, escape from ponds and dams, drifting debris, tourism, dredge spoil and deliberate release for cultural/religious reasons (*e.g.* Walters 1988; Carlton and Geller 1993; Bax *et al.* 2003; GISP 2004; Lintermans 2004).

The current global exponential increase in invasive species is predominantly due to a previously predicted increase in shipping activities in the 21st century (*e.g.* Valiela 1995; Cohen and Carlton 1998; Kennish 2002). Prior to this, the majority of traceable NIS introductions can be allocated to events occurring within two distinct timeframes: (i) the establishment of modern world trade in the early 19th century and (ii) the industrialisation era, commencing during the end of the 19th century (Hewitt *et al.*, 2004). Both of these time periods were characterised by rapid and significant advances in marine technology and subsequently new vectors for transmission and new regions for colonisation became available.

Table 2.1. Summary of the predominant pathways of NIS dispersal within the marine environment and representative NIS attributed to transport via their vectors.

Pathway	Vector
Shipping activities (commercial, recreational and military)	<p>Hull fouling/encrusting: e.g. The polychaete <i>Sabella spallanzanii</i> in Australia (Currie <i>et al.</i>, 2000; GISP, 2004)</p> <p>Ballast discharge: e.g. The introduction of the goby <i>Tridentiger trigonocephalus</i> (Lockett and Gomon, 2001) and the starfish <i>Asterias amurensis</i> (GISP, 2004) into Australia. Also, the goby <i>Neogobius melanostomus</i> into the North American Great Lakes during 1990 (Jude <i>et al.</i>, 1992) and <i>Acanthogobius flavimanus</i> into San Francisco (Brittan <i>et al.</i>, 1970)</p> <p>Sea chests: e.g. The introduction of mussel <i>Mytilus galloprovincialis</i> into the Antarctic during 2006 (Lee and Chown, 2007).</p>
Aquaculture and Fisheries/Fishery products	<p>Escape from aquaculture facilities: e.g. Atlantic salmonid regularly escape from breeding pens (Naylor <i>et al.</i>, 2005)</p> <p>Transfer on commercial fishing gear: e.g. Transfer of eggs on nets (Lintermans, 2004) or the spread of green algae <i>Caulerpa taxifolia</i> on otter trawls and trammel nets in the Ligurian Sea since 1984 (Relini <i>et al.</i>, 2000).</p> <p>Accidental transfer in aquaculture stock: e.g. The brown alga <i>Sargassum muticum</i> in the English Channel (Engelen <i>et al.</i>, n.d.; Tweedley <i>et al.</i>, 2008), the crab <i>Callinectes sapidus</i> to the sea of Galilee (Snovsky and Galil, 1990).</p> <p>Live Seafood and bait fishing: e.g. The crab <i>Eriocheir seninsis</i> is partly dispersed through live seafood in Singapore (Low <i>et al.</i>, 2013)</p> <p>Intentional introduction for fishery: e.g. The oyster <i>Crassostrea gigas</i> into at least 45 ecoregions worldwide (Molnar <i>et al.</i>, 2008)</p>
Canals, seaways and internal waterways	<p>Water flow change as a result of canal construction or alteration: e.g. Suez Canal construction resulted in ~1000 species establishing in the eastern Mediterranean (GISP, 2004; Katsanevakis <i>et al.</i>, 2014; Occhipinti-Ambrogi and Savini, 2003) and the Welland canal opening resulted in the sea lamprey <i>Petromyzon marinus</i> invading the Great Lakes (GISP, 2004).</p> <p>Existing waterways: e.g. The mussel <i>Dreissena polymorpha</i> invading North America via the Great Lakes (GISP, 2004) and the goldfish <i>Carassius auratus</i> moving from rivers into estuaries (Beatty <i>et al.</i>, 2014; Tweedley <i>et al.</i>, 2012).</p>

Table 2.1. cont. Summary of the predominant pathways of NIS dispersal within the marine environment and representative NIS attributed to transport via their vectors.

Pathway	Vector
Pet trade and Aquarium industry	<i>Escape from facilities/release into wild:</i> e.g. The green algae <i>Caulerpa taxifolia</i> escape from aquarist tanks into the Mediterranean region (Jousson <i>et al.</i> , 1998).
Biological control	<i>Intentional:</i> e.g. The sea urchin <i>Evechinus chloroticus</i> to control an invasive kelp species in Fiorland, New Zealand (Atalah <i>et al.</i> , 2013). <i>Natural:</i> e.g. The ctenophore <i>Beroe ovate</i> establishment in the Black sea (Shiganova <i>et al.</i> , 2003), whose predatory habits curbed the influence of the previously established invasive ctenophore <i>Mnemiopsis leidyi</i> (Shiganova, 1998)

2.3.3. Shipping activities as a pathway of transmission

The commencement of substantial amounts of world trade, by ship, in the early 19th century resulted in what is considered the beginning of significant numbers of NIS being dispersed, with most species fouling and encrusting the hulls of vessels (*e.g.* Ruiz *et al.* 1997; Bax *et al.* 2003; Hewitt *et al.* 2004; Davidson *et al.* 2009). Recorded establishments of NIS escalated during this period, however, shortly after this escalation began it plateaued as most donor species and applicable vectors had been exploited (Fig. 2.1). Technological advances in the 1960s prompted the increased adoption of anti-fouling paints (*e.g.* Tributyltin), metal hulls, shorter residency time in ports and faster travelling speeds, all of which reduced the possibility of colonisation using this vector (Ruiz *et al.*, 1997). GISP, 2004; Hewitt *et al.*, 2004; Gollasch, 2006)

At the same time, however, greater volumes of ballast water were being transported as a result of the expanding global fleet and trade demands, which increased the abundance, density and survival of the species entrained and transferred in ballast waters (Carlton, 1996a). Thus began the second invasion period, which was characterised by ballast water discharge becoming the dominant vector of dispersal (*e.g.* Ruiz *et al.* 1997; Thresher 1999; Hewitt *et al.* 2004). Increases in global connectivity and the extent of marine activities led to the access of new NIS donor and recipient regions and hence species. For example, the construction of the Suez Canal in 1869 resulted in increased Australian military shipping activity via Mediterranean regions (Hewitt *et al.*, 2004), as well as providing a new pathway of natural range expansion which, in conjunction with other vectors, resulted in the establishment of ~1000 new tropical indo-pacific species into the Mediterranean (Por, 2012; Katsanevakis *et al.*, 2014).

As estuaries are focal points of anthropogenic activity and resource use (Lotze *et al.*, 2006) it is not surprising that they are consistently the areas most afflicted by NIS (Ruiz *et al.*, 1997, 1999). The historical nature of NIS establishment, corresponding to changes in anthropogenic activities associated with two generic time epochs of colonisation and globalisation, is perfectly illustrated by Port Phillip Bay (PPB) in

Victoria, Australia and San Francisco Bay (SFB) in California, USA. The first of these systems is arguably the most invaded estuarine system in the southern hemisphere, with the documentation of 160 introduced and 61 cryptogenic species (Hewitt *et al.*, 2004). The majority of introductions to PPB occurred either during the early 1800s, coinciding with the colonisation of the area and establishment of PPB as a trading port or during the 1960s, strongly correlating with global development trends, the industrialisation of marine trade and the introduction of the use of ballast water (Hewitt *et al.*, 2004; Hewitt and Campbell, 2010).

San Francisco Bay is regarded as the most invaded of all aquatic ecosystems, not just estuaries, harbouring at least 234 introduced and 125 cryptogenic species (Cohen and Carlton, 1998). Virtually all habitats in San Francisco bay and delta are dominated by invasive species (Bax *et al.*, 2003) and, according to Molnar *et al.* (2008), high proportions of those are considered high impact species (Fig. 2.3). Over 50% of the invasions recorded since 1851 in this system occurred after 1960, with, on average, one new NIS being established every ~100 days, compared to the rate of one new species every year prior to 1960 (Cohen and Carlton, 1998). The ongoing and elevated rate of NIS establishment in SFB is thought to be supported by the invasion meltdown theory proposed by Simberloff and Von Holle (1999), which states that, contrary to the traditionally accepted biotic resistance paradigm, accumulated positive interactions amongst introduced species may act synergistically to facilitate further invasions.

Currently, although bio-fouling is still considered by some to be the predominant vector of NIS dispersal (*e.g.* Lintermans 2004; Ferreira *et al.* 2006), given the aforementioned technological advances resulting in the reduced impact of bio-fouling, many now consider ballast discharge to be, either now or in the near future, the single greatest vector of species dispersal worldwide (*e.g.* Williams *et al.* 1988; Carlton and Geller 1993; Cohen *et al.* 1995; Ruiz *et al.* 1997, 2000; Wonham *et al.* 2000; Hewitt *et al.* 2004). Underpinning this change in the influence of these vectors is the acceptance that bio-fouling's impacts are potentially historically-biased as it is the oldest known vector and, as such, may have been over-emphasised in comparison to

modern vectors such as aquaculture and ballast discharge (Hewitt and Campbell, 2010). Indeed, Wonham *et al.* (2000) suggest that the significance of ballast discharge as a vector for NIS has been underestimated to date.

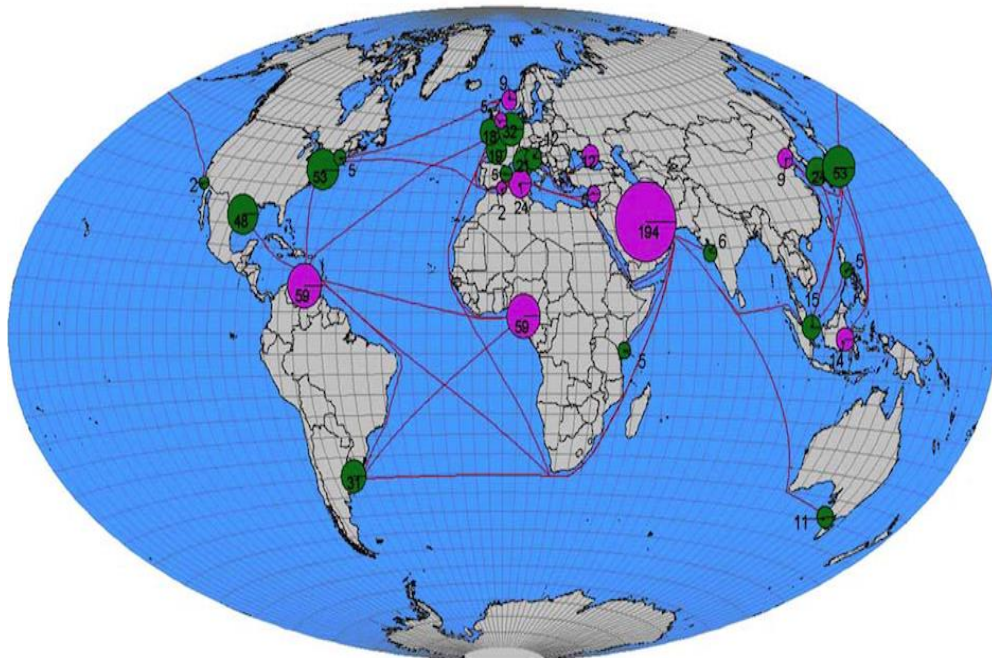
Although the establishment of only ~20% of Australia's introduced species can be attributed directly to ballast discharge (Thresher, 1999; Hewitt *et al.*, 2004), 8 of the 18 successfully established fish species reported in the decade post 1990 were attributed to ballast discharge (Ruiz *et al.*, 2000). Furthermore, at any given point in time, as many as 10,000 species are being transported in ballast tanks alone (Carlton 1999). The repetitive underestimation of introductions resulting from ballast water discharge stems from the inherent difficulty of accurately identifying the vector responsible, especially for species which could have been translocated by multiple vectors (Gollasch, 2006). In addition to this, the role of cryptogenic species in underreporting and underestimating NIS presence and impact is well accepted (Carlton, 1996b).

The ambiguity inherently involved in tracing species capable of being transported by multiple vectors is linked to one of the strengths of ballast transport. Thus, unlike other vectors, such as bio-fouling which favours substrate dependant taxa, ballast water discharge is an indiscriminate method of transport, both in terms of phylum or ecological role of the NIS, which effectively ensures that ballast water has the ability to facilitate the simultaneous establishment of a far wider range of taxa than other vectors (Carlton and Geller, 1993). The success of ballast water as a vector and its dominance as a mechanism for the establishment of NIS, is clarified when it is understood that the most prominent vectors are those that maximise a) frequency and geographic range of vector activity and b) abundance, diversity and survivorship of associated organisms (Davidson *et al.*, 2009).

The proliferation in vector frequency associated with shipping is illustrated by the increases in annual world seaborne trade from 5,984 in 2000 to 9,548 million metric tonnes of goods in 2013 (UNCTAD, 2014; UNCTADstat, 2015). In addition to this is the introduction of over 6,000 new trade vessels since 2011 and an increase in global fleet DWT (dead weight tonnage in thousands), for seagoing propelled merchant ships

with a gross tonnage of ≥ 100 metric tonnes, from 793,771 metric tonnes in 2000 to 1,749,222 metric tonnes in 2015 (UNCTADstat, 2015). Endresen *et al.* (2004) provided a snapshot of crude oil tanker shipping activities during 1996 and in doing so identified the estuaries and ports most afflicted by ballast discharge, particularly those in north-western Europe, and these areas generally contain larger number of NIS (Fig. 2.2; Fig. 2.3). The exception being Gulf State waters, which contain low numbers of NIS despite high levels of shipping due to high levels of stress present, *e.g.* high salinities in the Red Sea, severe eutrophication and tidal inundation combined with a lack of sediment deposition (Mendelssohn and Kuhn, 2003; Mitsch *et al.*, 2001).

Fig. 2.2. Areas experiencing ballast water discharge (via importing) and uptake (via exporting) as a result of interregional transport by large crude oil carriers during the year 1996. Taken from Endreson et al. (2004). ● Ballast water discharge and ● ballast water loading volumes (million tonnes). Purple lines denote major oil tanker routes.



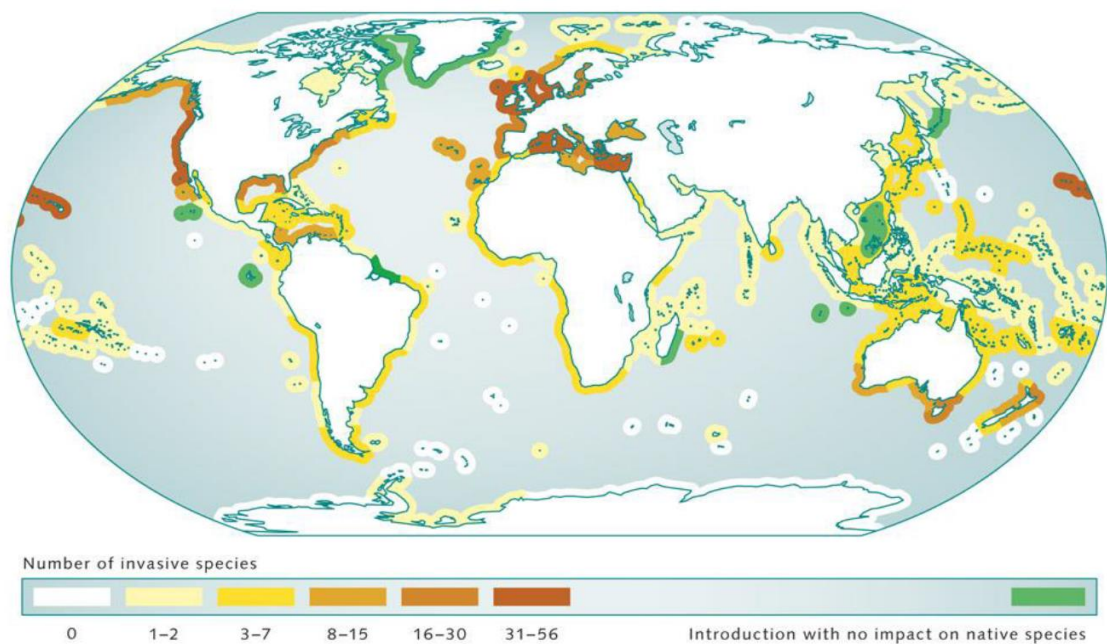


Fig. 2.3. Number of known detrimental NIS existing per ecoregion globally, regions of red denote areas with greater number of species with high ecological impact based upon assessment criteria devised by Molnar et al. (2008). Blue regions denote areas with invasive species considered to be harmless in all relevant categories, *i.e.* health, economics and ecology. Taken from Molnar et al. (2008).

2.3.4. Waterways, aquaculture and aquariums as pathways of transmission

Canals and seaways/waterways have been implicated in the dispersal of multiple species within the marine environment (Table 2.1; GISP 2004; Katsanevakis *et al.* 2014). The existence of such waterbodies provide corridors of dispersal that can act as rapid ‘stepping stones’ in the colonisation of environments (GISP, 2004), particularly in the presence of other vectors, like shipping, which act synergistically. A particularly good example is provided by the construction of the Suez Canal in 1869, which resulted in the ‘Lessepian migration’ (GISP, 2004; Por, 2012). This migration refers to the unidirectional movement of Red Sea species into the Mediterranean Sea following the opening of the canal via vectors such as direct access, shipping and aquaculture (Fig. 2.4.A, B, C; Por 2012; Katsanevakis *et al.* 2014). These vectors have resulted in 986 NIS becoming introduced to the region, with 420 of the 799 species that could confidently be assigned to a vector being directly attributed to the canal (Fig. 2.4.A;

Katsanevakis *et al.* 2014). Such species were typically highly mobile and predominantly fish and some invertebrates (Fig. 2.5).

The role of aquaculture in the dispersal of NIS, intentional and unintentional, is well documented (*e.g.* Naylor *et al.* 2001, 2005; Stickney 2001; Katsanevakis *et al.* 2014). Aquaculture presents various pathways of introductions, the most common of which are contamination of stocking organisms, intentional introduction for establishment of a fishery and escape from aquaculture facilities (Table 2.1). Such introductions are becoming increasingly common as demand for sources of marine protein rises, which has resulted in a recent doubling in global aquaculture activity (Naylor *et al.*, 2001). In continuation of the Suez Canal/ Mediterranean example, 64 species, predominantly macrophytes, were introduced in this region through aquaculture (Fig. 2.4.C; Katsanevakis *et al.* 2014).

Contrary to canals and waterways, the spread of NIS through the aquarium industry is a contemporary issue. The dispersal of primarily ornamental or exotic species through this vector is due to typically lax regulations concerning industry practices and the responsibility of owners (Strecker *et al.*, 2011). In Florida, for example, >100 introduced fish species have established as a result of the aquarium trade via escape from breeding facilities and pet owners (Mcneely and Schutyser, 2003), with the Lionfish *Pterois volitans* being a particularly well researched example (*e.g.* Whitfield *et al.* 2002; Ruiz-Carus *et al.* 2006). A classic non-fish example from the same region is provided by the Burmese python *Molurus bivittatus*, which due to its large size (7-8 m and 90 kg) is thought to have been released into the Everglades by overwhelmed pet owners (*e.g.* Snow *et al.* 2007; Rodda *et al.* 2009).

2.3.5. Spatial variation in the pathways of transmission

It should be noted, however, that the influence of all pathways and vectors vary regionally, due to variation in the locally dominant marine activities and their related pathways of invasion (Ruiz *et al.*, 1997; Gollasch, 2006; Katsanevakis *et al.*, 2014).

Such variation is evident in the well-studied NIS fauna of the Mediterranean Sea (Fig. 4.A, B, C). For example, the Thau and Venice lagoons are focal regions for aquaculture and, unsurprisingly, they host the largest numbers of NIS species attributed to transport via aquacultural activities (Fig. 2.4.C; Fig. 2.5).

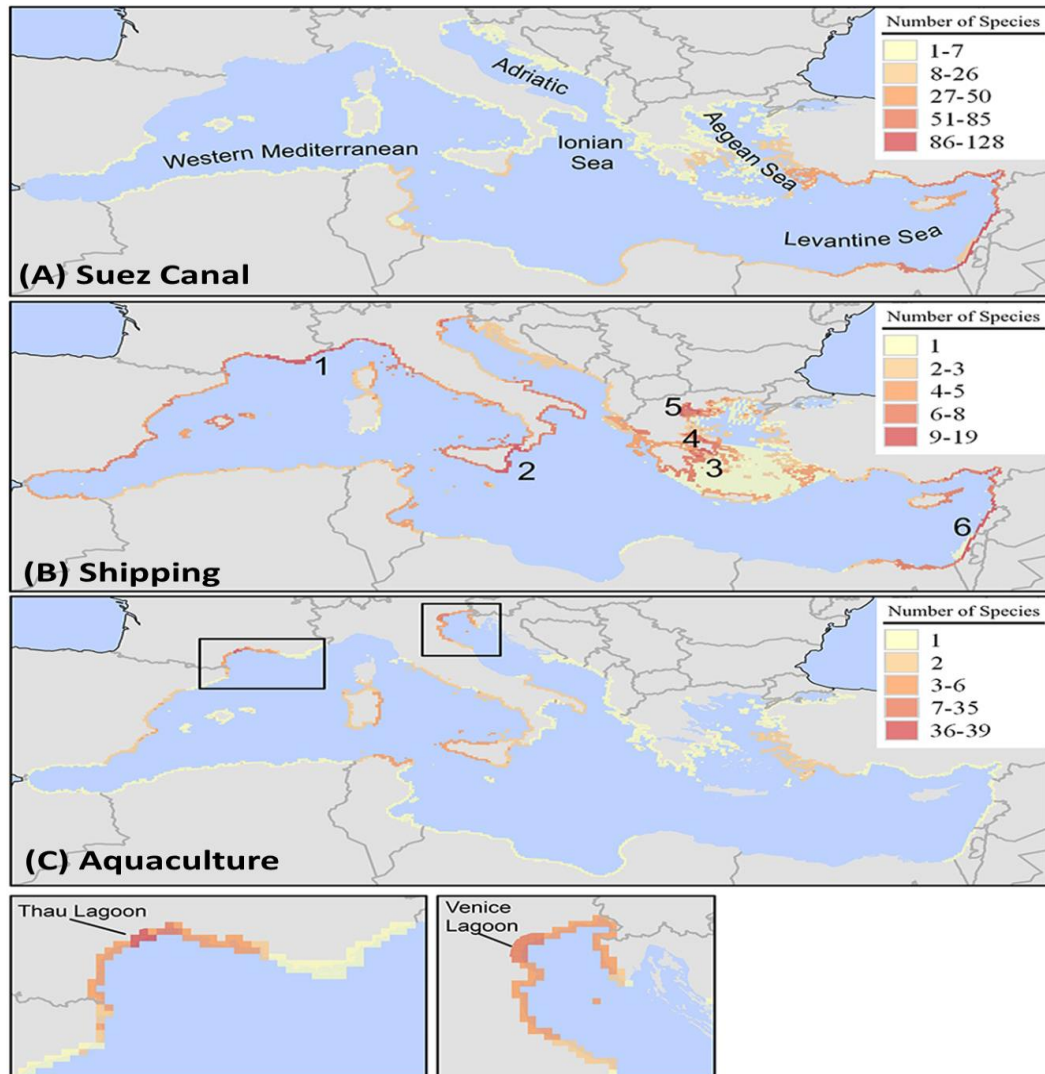
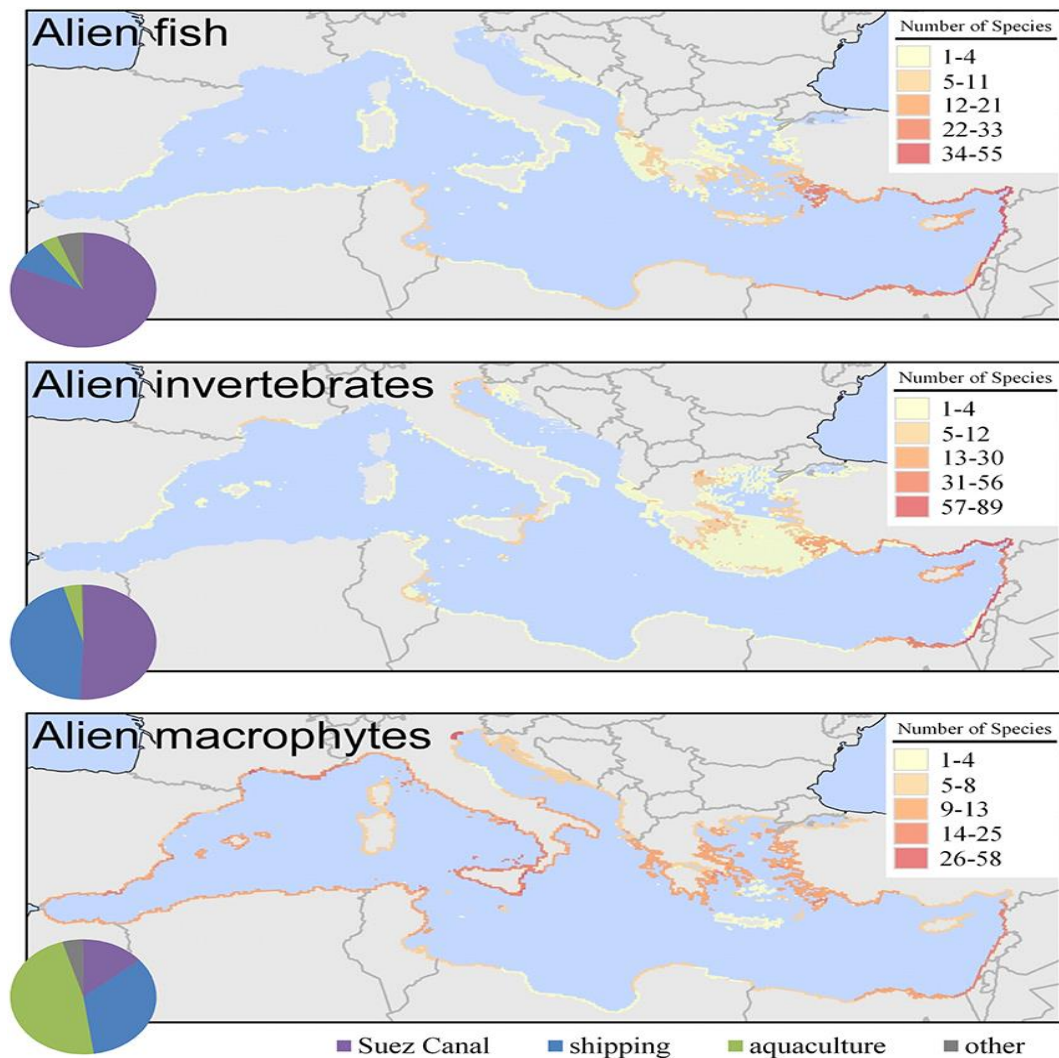


Fig. 2.4. Abundance of introduced species in the Mediterranean Sea (in 10 km by 10 km grid cells) due to: A) the Suez Canal, B) shipping and C) aquaculture. The numbers in (B) 1-6 indicate areas of high impact. Taken from Katsanevakis *et al.* (2014).

Interestingly, 41 of the 64 species dispersed through aquaculture were macrophytes (Katsanevakis *et al.*, 2014). This suggests taxa specific vector affiliation, a view that is shared by Bax *et al.* (2003). Further evidence of this lies in similar associations between groups of taxa and particular pathways, as fish were generally

associated with direct access through the Suez Canal (Fig. 2.5) and invertebrates with shipping and the canal (Fig. 2.5). Moreover, in the case of alien fish species, the number of introductions decreases from a peak in the Eastern Mediterranean near the entrance the canal and decrease progressively westward (Fig. 2.4.A; Fig. 2.5).

Fig. 2.5. Abundance of introduced alien fish, invertebrate and macrophyte species in the Mediterranean Sea (10 km by 10 km grid cells). Pie charts show the respective vectors attributed to the dispersal of those taxa. Taken from Katsanevakis *et al.* (2014).



2.4. Impacts of NIS species

2.4.1. Community health and intrinsic value

While the impacts of most NIS upon human health are mostly unknown, two major risks have been identified, (i) increase in toxic algae/dinoflagellate blooms, (Anderson, 1989; Hallegraeff, 1993) and (ii) spread of cholera virus *Vibrio cholerae* (Ruiz *et al.*, 1997). Among the various algal taxa of particular concern is the increasing occurrence of toxic algae blooms, *i.e.* ‘red tides’, which can have flow on effects due to biomagnification of their toxin in fisheries and ecosystems (*e.g.* Anderson 1989; Boesch *et al.* 1997; Van Dolah 2000). This, in turn, can lead to prominent health issues, such as shellfish poisoning (Van Dolah, 2000). In addition to this, algal blooms also affect the value of the system by degrading ecological processes and aesthetic appeal, *e.g.* noxious smell of algae decomposition (Howard-Williams, 1979; Potter *et al.*, 2015a). The spread of the cholera virus into multiple water systems, predominantly through ballast discharge, resulted in the deaths of thousands in South and Central America (Ruiz *et al.*, 1997). Parasites may also travel with introduced species, such an example is provided by the Chinese mitten crab *Eriocheir sinensis*, which is a widely successful NIS and has been attributed to the spread of human liver flukes (Bax *et al.*, 2003).

2.4.2. Socio-Economic impacts

Ecosystem community structure and function alteration due to NIS can result in changes to trophic composition and interactions between organisms (Ruiz *et al.*, 1997; Vitousek *et al.*, 1997a). Due to human exploitation of ecosystems and the services they provide, economic side effects are typically associated with the introductions (Ruiz *et al.* 1997; Williams and Grosholz 2008). Although the economic impacts of NIS are understudied, usually qualitative and hence hard to evaluate accurately (Williams and Grosholz, 2008), there are several examples of economically significant invasive species establishment throughout history. One such example of this is the establishment of the Zebra mussel *Dreissena polymorpha* in North America. This bivalve is an efficient filter feeder and as it reached densities as high as 10,000 individuals m⁻²;

removed large amounts of phytoplankton, which lowered food supplies for native species of zooplankton, mussels and fish (GISP, 2004). This has resulted in ~140 native species, predominantly other bivalves, being threatened with immediate extinction (Ricciardi *et al.*, 1998). Furthermore, this NIS causes damage to water treatment and electricity generation plants by clogging and smothering of infrastructure such as cooling pipes. In total, the cost of *D. polymorpha* and its congener the Quagga mussel *Dreissena bugensis* in North America is estimated > US \$1 billion y^{-1} in damages and management costs (Pimentel, 2005). Another prominent example of an economically devastating invasion is that of the ctenophore *Mnemiopsis leidyi* in the Black Sea during the 1980's. *Mnemiopsis leidyi* caused the widescale collapse of fisheries in the area (Shiganova, 1998), at a conservative cost estimate of US \$16.7 million y^{-1} (Knowler, 2005).

Pathogens may also be spread. For example, the White spot syndrome virus was introduced to the Americas from Thailand via imported frozen bait shrimp (Bax *et al.*, 2003; Sánchez-Paz, 2010). This virus is capable of decimating large populations of crustaceans like those in prawn aquaculture facilities/farms in several days (Sánchez-Paz, 2010) and has caused millions of dollars of damage to prawn farm stock throughout its native Asiatic region (Kautsky *et al.*, 2000).

2.4.3. Biology/ecology of the system

Invasive species can affect local ecosystems by altering primary productivity levels, decomposition rates, hydrological regimes, geomorphology, bioturbation, nutrient cycling and habitat change (Ruiz *et al.*, 1997; Vitousek *et al.*, 1997a) and can influence fauna by introducing parasites/disease, hybridization with native species, altering food webs and predator/prey relationship and creating competition (Ruiz *et al.*, 1999; Hayes and Sliwa, 2003). A contemporary example of a NIS having deleterious biological impacts in Australian estuaries and coastal waters is that of the Pacific Sea Star *Asterias amurensis*. This species was first detected in Port Phillip Bay in 1995, with its population expanding to ~150 million individuals within a decade (Parry and

Cohen, 2001). The highly active predatory habits of *A. amurensis* threatens native benthic fauna, particularly bivalves (Ross *et al.*, 2002), and thus it is considered a significant threat to local mariculture and wild shellfish fisheries. This conclusion is substantiated by the fact that this species was shown to be responsible for the wide-scale depletion, and subsequent rarity, of large bivalves in Tasmania (Ross *et al.*, 2002).

2.5. Contemporary NIS management approaches and suggested amendments

It goes without saying that prevention is the most cost-effective tool in mitigating NIS, however, once such a species has become successfully established, management options are limited to three principles, (i) eradication (ii) containment/quarantine (iii) mitigation and control (Carlton *et al.*, 1995; Thresher, 1999; Bax *et al.*, 2003; GISP, 2004; Williams and Grosholz, 2008). Eradication and containment approaches typically involve (i) mechanical/physical control, comprising the removal by hand or mechanical harvesting *i.e.* trawling or dredging; (ii) chemical control, such as toxic baits, application of an organic or inorganic herbicide, larvicide or other pesticide; (iii) biological controls, *e.g.* target specific pathogen, parasite, predator, biopesticide, genetic manipulation, reproduction manipulation or habitat modification, *i.e.* change salinity and (iv) Integrated Pest Management (IPM), which utilises a mixture of the above three approaches (Thresher, 1999; GISP, 2004). It should be noted that mitigation and control approaches are employed when there is an inability to control the organisms and thus these are worst-case scenario approaches (GISP, 2004).

The success of the above tactics is highly-dependant on early identification, species knowledge and the availability of resources to act quickly (Wotton *et al.*, 2004; Williams and Grosholz, 2008). The importance of acting quickly with appropriate information is highlighted by the successful eradication of the ‘killer algae’ *Caulerpa taxifolia* in California (GISP, 2004), the zebra mussel *Dreissena polymorpha* in Australia (GISP, 2004) and the kelp *Undaria pinnatifida* in New Zealand (Wotton *et al.*, 2004). These are among the very few cases of invasive species that have successfully been identified, managed and eradicated.

The historical lack of success in managing invasive species is mainly due to a lack of centralized leadership or standardised policies, which has resulted in an overlapping mosaic of independent approaches that are ineffective and contradictory (Williams and Grosholz, 2008). In the near global absence of any similar regulated or even deregulated management policies concerning vectors, particularly shipping related ones, there is a necessity for a transition from expensive and ineffective containment procedures to prioritising prevention based measures (Mcneely and Schutyser, 2003; Mcneely *et al.*, 2003; Simberloff, 2005). The expensive, often unsuccessful and long-term nature of many eradication and mitigation approaches has resulted in the overall consensus that prevention is the most effective management strategy (*e.g.* Thresher 1999; Bax *et al.* 2003; Mcneely and Schutyser 2003; Mcneely *et al.* 2003; GISP 2004; Ojaveer *et al.* 2014).

Significant gaps exist concerning baseline data on estuarine biotic community health and composition for developing and even first world countries, which substantially increases the difficulty in achieving successful and applicable management actions (Kennish, 2002). The shifting baseline syndrome, which describes the gradual accommodation of invalid reference points based on contemporary conditions, *i.e.* the progressive evolution of what is considered normal (Pauly, 1995), is particularly applicable to modern NIS management. The relevance of progressive reference points to invasive species management is clarified when considered in conjunction with the current lack of knowledge concerning ecosystem structure, function and species. Without previous information concerning the individual systems at hand, we have no way of knowing if what is occurring is in fact normal. Therefore, it becomes even harder to identify invasive species in some cases, much less what their impacts upon the community function and structure are, hence the common occurrence of cryptogenic species in regions such as San Francisco Bay.

Understanding the crucial role that information concerning species and ecosystems plays in the successful application of management actions (Williams and Grosholz, 2008) has influenced the construction of several recommendations (Table 2.2)

regarding the requirements of NIS management to increase prevention and management efficiency. In addition to these suggestions concerning improvements in management efficiency, there is a focus upon developing ballast water management strategies in particular. Although multi-vector management is advocated by many workers (*e.g.* Thresher 1999; Ojaveer *et al.* 2014), the nature of ballast discharge as the principal contemporary dispersal pathway of marine NIS (*e.g.* Ruiz *et al.* 1997; Hewitt *et al.* 2004) requires emphasis being placed upon developing and applying management techniques that are specific to ballast discharge.

Table 2.2. Recommendations and benefits for the successful assessment and management of non-indigenous species. Compiled from Bax *et al.* (2003), Williams and Grosholz (2008) and Ojaveer *et al.* (2014).

Recommendation	Benefit
Availability of taxonomic expertise	Accurate identification of organisms occurring in the environment, particularly useful for con-familial species
Application of molecular tools	Aids knowledge gathering concerning pathways of invasion, (useful in determining stepping stone <i>vs</i> direct invasion nature) site of origin, identification/discrimination of morphologically similar species
Common surveillance and monitoring guidelines	Reduce underreporting occurrences associated with modern restrictions in sampling methodology and feasibility
Early detection and monitoring	Emphasise monitoring efforts concerning activities of the highest threat in order to increase chances of early detection and hence higher success in eradicating the NIS before it establishes
Standardisation of data and information systems	Currently problems arise with comparability of the data, due to differing policies/formats increased availability of data due to universal guidelines would solve this problem and increase management useability
Investigation and assessment of propagule pressure	Help deduce future invasions or occurring establishments and what actions to take in order to combat them
Careful choice of indicators	Universally applicable indicators of ecosystem health that translate well into other areas would result in more effective comparison
Multi-vector management	Vector based prevention and management is best and as there are multiple vectors they all need to be addressed simultaneously
Cooperation with stakeholders	Increased communication between all ecosystem users will increase the operability and implementation of future actions.
Adoption of the single authority approach	Most effective management is prevention. Fragmented responsibilities, powers, legislations restrain this from taking place effectively

2.6. Case study: management of ballast discharge

Current ballast water management methods revolve around open ocean exchange with a stipulation that 95% of ballast water must be exchanged (Bax *et al.*, 2003; Tamelander *et al.*, 2010), however, further requirements are predicted pending the ratification of the 2004 International Convention for the Control and Management of Ships Ballast Water and Sediments (BMW) (IMO, 2004). Presently, water treatment methods are not consistently employed due to lack of technology or concerns over safety and ecological and/or economic concerns (Tamburri *et al.*, 2002; Bax *et al.*, 2003). In light of this, it is evident that prevention based management must be economical and efficient as well as effective, particularly when considering that there is currently no legally binding international enforcement and thus a lack of responsibility for industry stakeholders. Proposals for treatment methods of ballast water are not lacking, however, and hold promise for future endeavours. Proposed treatment methods include: (i) intensive filtration, (ii) thermal treatment, (iii) UV irradiation/ filtration, (iv) mid/open ocean exchange (currently employed), (v) chemical treatments (*e.g.* biocides) and (vi) oxygen deprivation (Carlton *et al.*, 1995; Tamburri *et al.*, 2002; Bax *et al.*, 2003; Endresen *et al.*, 2004; GISP, 2004). Amongst these proposed treatment methods, oxygen deprivation holds the most promise as it is cheap, safe and has been shown to cause >75% mortality in 72 hours. Furthermore, it also combats rust and thus increases the economic life of the ship (Tamburri *et al.*, 2002).

In addition to the various treatment methods currently proposed, there is a growing movement, supported by the BMW convention, promoting operational changes to currently active ballast discharge processes. The changes include: (i) designation of ballast discharge zones, (ii) establishment of mandatory reporting systems, (iii) adoption of port state control and (iv) risk based assessment systems (Bax *et al.*, 2003; Hewitt and Campbell, 2007; Liu *et al.*, 2014). Designated discharge zones aim to provide regions where, due to prevailing site conditions, *e.g.* currents and geography, discharge of ballast water is unlikely to disperse organism into areas where they can establish a

viable population (Liu *et al.*, 2014). These zones are increasingly likely to utilise areas designated as marine dumping zones. The addition of port control is essentially a last line of defence and would enable a port authority to deny ships entry if they fail to meet appropriate standards regarding ballast water treatment and discharge (Liu *et al.*, 2014).

The provision of mandatory reporting systems, such as ballast record books, would maintain traceability and transparency in maritime shipping activities, which, in turn, provides the information necessary to make effective and appropriate decisions regarding treatment plans for individual ships. The risk of NIS will vary amongst ships depending upon frequency of trips, time in transit, ship characteristics and design, volume of ballast water and flag state, *i.e.* the state authority the vessel is registered and hence answerable to (Liu *et al.*, 2014). Different treatment approaches should be utilised in order to minimise delays and increase efficiency, whilst still preventing NIS transmission. Here, the adoption of a risk based assessment system holds particular merit, as it will counter the inability to apply standardised protocols to every facet of marine activities and inherent flaws in such a “one size fits all” approach (Endresen *et al.*, 2004). Currently, several risk assessment systems are already employed, such as the EU Marine Strategy Framework Directive and Australia's National System for the Prevention and Management of Marine Pest Incursions (Department of Agriculture 2014; Olenin *et al.*, 2014). The adoption of the latter system has already proved valuable (*e.g.* Piola and McDonald, 2012; Dias *et al.*, 2015). For an in-depth review of current risk assessment approaches and risk scoring systems, see Leung *et al.* (2012). A Generalised approach to risk assessment can be seen below (Fig. 2.6).

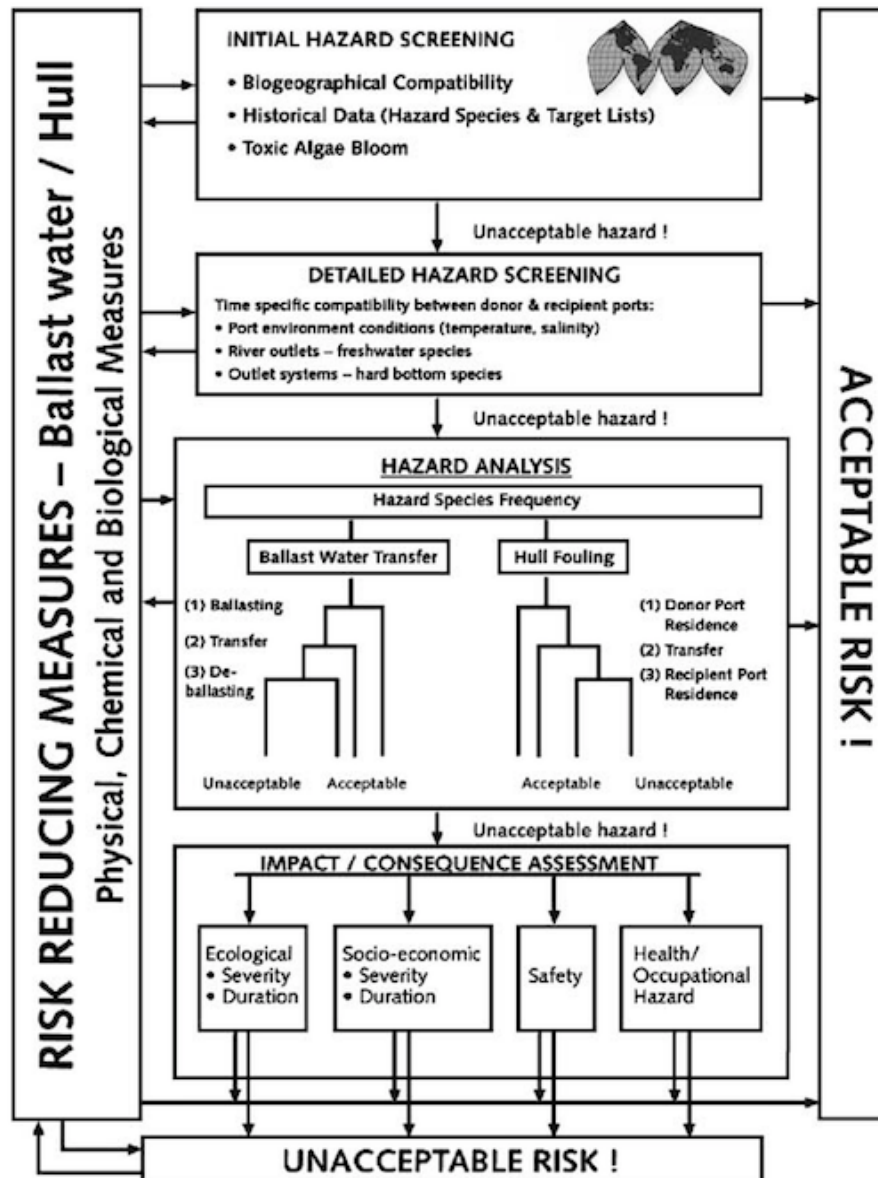


Fig. 2.6. EMBLA risk assessment strategy by Behrens et al. (2002) and modified by Endresen et al. (2004).

Risk assessments, such as that in Figure 2.6, can help to determine the safest and most applicable treatment methods for the activity given the circumstances. Considering this, most risk assessment systems, while they vary in terms of structure and focus depending on the region and activities involved, must take into account port characteristics, the potentially invasive species present at each port and their spatial and temporal distribution (Endresen *et al.*, 2004). In order to be economically feasible on a broad scale, Barry *et al.* (2008) advocate the use of species-specific risk assessments

concerning a select number of high risk invasive species whilst utilising environmental similarity matching criteria. This approach functions on the premise that not all NIS are bad, nor is it possible to effectively manage all of them and therefore we should instead focus resources on preventing establishment of species known to be problematic (Barry *et al.*, 2008). This strategy requires a method of determining the “environmental distance”, *i.e.* the effective distance between sites based on prevailing environmental parameter similarity, of the sites in question, which currently has not been established, in addition to a wealth of information, concerning species and ecosystems, which is presently lacking (Kennish, 2002). Despite these barriers, Hayes and Sliwa (2003) provide an excellent working example of species-specific risk assessment of potential future high-risk invasive species in Australia based on their ability to be transported to this region and the subsequent likelihood of establishment. This risk assessment strategy demonstrates the value of standardised data and reporting systems concerning NIS biology and ecology, as the availability of information made the creation of this risk assessment strategy possible.

The overall consensus is that it is almost impossible to determine which species will invade and where and how, and thus more species and site specific regulations and research are required for any conclusive predictive framework to be formed (Hayes and Barry, 2008). This is, of course, in addition to the orientation of management to all vectors and applying a holistic approach that accounts for all scenarios and avenues of invasion. Indeed, in order to stem the increasingly negative impacts on estuarine and coastal ecosystems and the services they provide, the adoption of a synergistic model of management that promotes interconnectivity of the marine landscape is required (Barbier *et al.*, 2011). Modern approaches to management are most likely to adopt a legislative and sociological tact, which will rely upon a nationally enforced and unified approach complemented by computer based decision support systems (Thresher, 1999). This approach will support shipping agents, port managers and shipmasters in assessing risk associations with practices undertaken and then allow them to apply the appropriate treatment (Thresher, 1999). It is important to note that many countries currently do not

possess the resources necessary to monitor and control the flow of invasive species (Perrings *et al.*, 2005) and this is where the value of a universally integrated management framework becomes apparent. Common guidelines concerning marine activities and their management options, such as those proposed by the BMW convention (IMO, 2004), encourage cooperation between relevant regions, which increases management success of less able regions and subsequently reduces the likelihood of stepping stone invasion for further regions down the track. Increasing stakeholder involvement in prevention of NIS based on the 'polluter pays' principle can only create positive momentum towards achieving the recommended internationally homogenous framework and is a crucial step in future movements concerning reducing the externalisation of costs associated with NIS, which is suggested as a factor behind the current lack of management (Perrings *et al.*, 2005).

2.7. Biological characteristics of successful NIS

To date, very few universal biological characteristics have been identified in successful NIS across multiple taxonomic groups (Hayes and Barry, 2008). Indeed, a study conducted by Wonham *et al.* (2000) found little correlation between the biology of 24 species of fish successfully established in estuaries worldwide via ballast water. This suggests that indicators of success and invasiveness are likely to be species and site specific. Wonham *et al.* (2000) argue for extra attention to biological features that aid survival throughout the three stages of invasion, namely (i) dispersal, (ii) establishment and (iii) introduction, which will vary depending on the environment, species and also life stage. Thus, the development of more species and site-specific regulations and research is required for any conclusive framework of characteristic biological features of successful invasive species to be formed (Hayes and Barry, 2008). However, this poses considerable challenges given the current variety of environments, their associated species and vectors present.

The identification of management priority species should be based upon risk assessment concerning previous invasion success, ability to disperse to location, climate

match and ecological, socio-economic and health impacts of the species (Hayes and Sliwa, 2003). In addition, Williams and Grosholz (2008) suggest ecological impacts attributed to these species should be decided based on associated functional groups, *e.g.* filter feeder or larger predator, impacts rather than species-specific interactions such as competition and predation.

While it is considered impossible to predict the success of an invasive species, climate and habitat match are consistently significantly associated with invasive behaviour across all biological groups (Williams and Grosholz, 2008). Although this helps understand invasion patterns, habitat match loses some relevance in the aquatic environments, particularly if the species is euryhaline. Biological characteristics generally associated with influencing invasive success include generalist diet, adaptive lifestyle, high reproductive potential, population dynamics, genetic variability, body size, physiological tolerances and reproductive strategy, all of which affect chances for survival throughout all three phases of dispersal (*e.g.* Williamson and Fitter 1996; Wonham *et al.* 2000; Hutchings *et al.* 2002; GISP 2004). In addition to this, phenotypic plasticity (Grosholz, 2002; Hayes and Barry, 2008), life history stage of colonizer, size of inoculant population, propagule pressure (*i.e.* total number of introduced individuals) and location specific characteristics are suggested to affect the successfulness of invasion events (Carlton, 1996a). Further understanding of characteristics associated with success, predictions concerning invasions and their consequences can be achieved via the comparison of multiple independent invasions of the same nature (Grosholz and Ruiz, 1996).

A contemporary working example of multiple independent invasions is that of the Green crab *Carcinus maenus*, which is on the IUCN's 100 World's Worst Invasive Alien Species List, and has successfully invaded regions such as Western Australia and South Africa. The occurrence of multiple independent invasions provides useful insight into invasion mechanics and resulted in the deduction that diet preference is an important factor to the success of invasions (Grosholz and Ruiz, 1996).

Despite the difficulty associated with accurately identifying factors correlated with successful invasions, the ability to predict future events and their impacts upon the receiving ecosystem are crucial to preserving ecosystem integrity and will help to focus already limited resources on management and control efforts upon issues of highest priority (Grosholz and Ruiz, 1996). Along with the identification of characteristics that are indicative of invasion success, is the recognition of circumstantial aspects to success. Carlton (1996b) recognised six scenarios in which the event of an invasion is inadvertently encouraged (Table 2.3). These scenarios highlight the impact that changes in propagule pressure and environmental factors may have on invasion success, regardless of species characteristics and help explain the occurrence of successful invasions after many years of unsuccessful inoculations (Lockwood *et al.*, 2005).

Table 2.3. Invasion scenarios and the likely responses. Taken from Carlton (1996b).

Invasion scenario	Response
Change to donor region	The donor region may undergo environmental changes, which can serve to increase abundance of certain resident species and/or induce range expansion of regional species
New donor regions	Exposure to new species from new regions due to, for example, expansion in shipping activities
Changes in recipient region	Environmental changes in recipient region that lead to altered ecological, biological, chemical or physical states and therefore changing the susceptibility of invasion
Invasion windows	Combination of circumstantial aspects favourable to the establishment of a species
Stochastic inoculation events	Release of very large number of individuals into the area, increasing reproductive potential
Dispersal vector change	Vector size, speed, and quality increase lead to an increase in inoculant species diversity, abundance and number of post transport fit individuals

Tying in with this concept of invasion windows is the existence of a mixture of biotic and abiotic factors, separate to those identified above, which also influence the success of an invasion. Perhaps the most commonly noted factor is disturbance, with

extreme natural disturbance and human alteration of natural environments making establishments increasingly possible (*e.g.* Cohen and Carlton 1998; Thresher 1999; Mcneely and Schutyser 2003; Lohrer *et al.* 2015). Lohrer *et al.* (2015) state that disturbance and diversity can influence invasive species success if resource limitation is a prime determinant of establishment and disturbance and diversity affects the availability of required resources. In support of this, Ruiz *et al.* (1999) found that over half of the introduced fish and plant species in Chesapeake Bay were associated with anthropogenic disturbance of some form.

Moreover, Smith *et al.* (1999) state that factors known to influence the success of biological invasions are propagule pressure, post-transport inoculant survival and intra and interregional differences in susceptibility to invasion. Concurring with this view, multiple studies (*e.g.* Kolar and Lodge 2001; Lockwood *et al.* 2005; Ojaveer *et al.* 2014) suggest that propagule pressure is the primary determinant of invasion success. Occhipinti-Ambrogi and Savini (2003) suggest that these regional differences in susceptibility are a direct result of different disturbance levels experienced and that the old discussion concerning ‘invasibility’ of an ecosystem should adopt a view of likelihood correlated with overall ecosystem health, as opposed to the strict measures of species abundance glorified by the typically accepted biotic resistance paradigm (Simberloff and Von Holle, 1999). It is important to note that Occhipinti-Ambrogi and Savini (2003) are not suggesting that a healthy ecosystem is not a factor in invasion success, rather they challenge the definition of what is a healthy ecosystem and lend emphasis toward universally applicable and comparable measures such as niche, and hence resource, availability in case comparisons exhibiting such varied idiosyncrasies.

The impact of anthropogenic disturbance cannot be understated, studies such as that conducted by Thresher (1999) even suggesting the implementation of precautions that coordinate disturbance with periods of low risk, *i.e.* dredging or development around time of native reproduction in order to induce quick colonisation of newly disturbed environments by natives. The basis of this recommendation stems from the traditionally accepted biotic resistance paradigm, in which it is proposed that high

native species diversity results in lower invasive success (Simberloff and Von Holle, 1999). Furthermore, the correlation between species diversity and invasive success suggested by a plethora of studies (*e.g.* Williamson and Fitter 1996; Stachowicz *et al.* 1999) is fully supported by the occurrence of extensive NIS colonisation of San Francisco Bay which is said to be in part due to a depauperate native biota (Cohen and Carlton, 1998).

The impact of disturbance upon the relationship between native species abundance and diversity and invasion success becomes apparent when considering the suggestion from Byers (2002) that disturbance can alter the environment and its selection pressures to such an extent that even well adapted native species are essentially experiencing a novel environment in similar ways an invasive species would. This proposition underlines a shift in resource availability, which Lohrer *et al.* (2015) stated as crucial to colonisation, that purportedly occurs as a result of the change in community dynamics and niche availability from disturbance events. This helps explain cases of successful invasion in regions that are otherwise considered healthy, *i.e.* highly diverse and abundant, but experience, like most of the modern marine environments, elevated levels of anthropogenic activities and the disturbance associated with them. It is important to note, that other environmental factors such as sea surface temperature, rising sea levels, increasing atmospheric carbon dioxide and ocean acidification will disturb and significantly impact coastal habitats in the coming decades, making them potentially more susceptible to invasions by NIS (Bindoff *et al.*, 2007).

All of these factors mentioned essentially alter circumstantial aspects of the environment that may or may not be favourable to certain species and potentially results in small windows of invasion (Mcneely *et al.*, 2003). The various idiosyncrasies associated with each invasion, *e.g.* species, transportation, environment, provide a substantial challenge for future management focused on predictive and preventative measures, however, progress is being made in formulating a framework focused on propagule pressure and disturbance/overall health of the ecosystem that is applicable and successful (Kolar and Lodge, 2001). The path holding the most promise at present

is to attempt to predict future invasive species based on the organisms physical tolerance range, hence the identification of likely sites of inoculation can be achieved via remote sensing technology and Geographic Information Systems (Williams and Grosholz, 2008).

2.8. Summary

In conclusion, NIS are becoming increasingly common throughout aquatic habitats worldwide. Their dispersal relies upon the existence of multiple dynamic anthropogenic vectors, intentional and unintentional results of human activity, to circumvent naturally occurring biogeographic barriers and successfully establish themselves in regions otherwise unavailable. The majority of aquatic NIS establishments are primarily attributed to shipping activities, such as ballast water discharge and bio-fouling, with substantial contributions also coming from aquaculture, the aquarium trade and canals and waterways. As focal points of human activity and resource use, estuaries face increasing pressure from inadvertently introduced species that reflect trade driven increases in marine activity.

Invasive species are among the top threats facing aquatic ecosystems globally and are associated with a diverse range of socio-economic, biological/ecological and health impacts upon the system they inhabit. These impacts typically affect key ecosystem goods and services provided by estuaries, from which they derive their value. Efforts to curb the internationally increasing invasion rate and its associated impacts have been largely unsuccessful due to lack of resources, coherency in management approaches and knowledge concerning the species, ecosystem and invasion characteristics. The standardisation of data systems, availability of taxonomic expertise, early detection and monitoring, multi vector management and adoption of a single authority approach are all suggested as necessities in successful assessment and management of NIS.

Lack of knowledge concerning the culpability of vectors and the characteristics conducive to success has prompted suggestion that high risk species be identified using criteria involving climate similarity, history of previous invasions, ability to be transported to a region and level of harm, *e.g.* economic or ecological, associated with them. Holistic vector management is advised for the management and control of NIS, as many species are compatible with more than one vector. In addition, the identification of high-risk species and regions would enhance the effectiveness of resource allocation and management of these species.

As shipping activities are the predominant vector of dispersal worldwide, particular emphasis has been placed on developing appropriate protocols to minimise its impacts. Treatment methods, *e.g.* chemical or thermal treatment, are currently in their infancy in terms of applicability due to safety, economic and efficiency issues. Thus, most recommendations on ballast water management revolve around increasing traceability of activities, with emphasis upon using risk assessment strategies in order to reduce delays and economic downfalls associated with uniformly applying treatment protocols, whilst also preventing unwanted introductions.

Chapter 3: Spatial and temporal distribution of *Acentrogobius pflaumi* in the Swan-Canning Estuary

3.1. Abstract

Given the rapid establishment of *Acentrogobius pflaumi* in non-native systems previously, concern understandably arose for the health of the Swan-Canning Estuary and its native flora and fauna following the appearance of *A. pflaumi*. Extensive sampling was undertaken between January 2014 and September 2015 throughout 20 near shore, shallow water (< 2 m) sites and 16 offshore, deep water (2-17 m) sites within the Swan-Canning Estuary with the aim of determining the spatial and temporal distribution of *A. pflaumi* and other native gobiid species within this system. The results indicated that gobiid richness was greater in the deep water regions, however, abundance of the gobiid species was higher in the shallow regions. Despite collecting 880 samples throughout 21 sampling occasions, *A. pflaumi* did not occur in any shallow water region. *Acentrogobius pflaumi* was the most abundant gobiid species in the deeper water regions, comprising >50% of the total gobiid abundance throughout 704 samples. Multivariate statistical analyses yielded significant regional differences in gobiid abundance, richness and assemblages, with Lower Melville water and Middle Swan Estuary being most different. This difference was primarily due to the abundance of *A. pflaumi*, which dominated samples from all time periods in this region and overall composing ~97% of gobiid samples. Other species of notable abundance, *Arenigobius bifrenatus* and *Pseudogobius olorum*, co-occurred with *A. pflaumi* in meaningful abundances on some occasions during sampling. The density of *A. pflaumi* decreased with distance from the estuary mouth while the opposite was true for *A. bifrenatus* and *P. olorum*. Thus, *A. pflaumi* appears restricted to the mostly marine conditions displayed by deep water regions closer to the estuary mouth.

3.2. Introduction

Estuaries are located at the interface between freshwater and marine environments and are thus influenced by freshwater discharge from the catchment and wave and tidal actions from nearshore coastal waters (McLusky and Elliott, 2007; Potter *et al.*, 2010). As a result, their physico-chemical environment can change over multiple spatial and temporal scales (Chuwen *et al.*, 2009). While such environments are stressful for stenohaline marine and freshwater faunas, a number of species are adapted to survive, and in fact thrive, in the dynamic estuarine environment (Elliott and Quintino, 2007; Tweedley *et al.*, *in press*).

The permanently-open estuaries of south-western Australia, like the Swan-Canning Estuary, are able to be separated into a number of distinct regions on the basis of morphology and water quality parameters, such as salinity, temperature and turbidity (*e.g.* Potter *et al.*, 1990; Potter and Hyndes, 1999; Veale *et al.*, 2014). These differences in environmental variables are mirrored by corresponding changes in ichthyofaunal composition (*e.g.* Loneragan *et al.*, 1989; Loneragan and Potter, 1990; Gill and Potter, 1993). Thus, the upper reaches are colonised predominantly by species that spawn within the estuary (solely estuarine species), while the entrance channel and lower reaches are predominantly colonised by marine species which spawn outside of the estuary and whose juveniles recruit to the estuary and utilise it as a nursery area (marine estuarine-opportunists; *e.g.* Prince and Potter, 1983; Gill and Potter, 1993; Valesini *et al.*, 2009, 2014). Although this separation of particular suites of fish into different regions of the estuary is primarily driven by their response to abiotic environmental variables (*e.g.* benthic habitat type and water quality), biotic variables, such as competition among species for resources across one or more of three axes, *i.e.* food, space and time (Ross, 1986), also influence distribution.

Non-indigenous species (NIS), *i.e.* species with natal environments alternative to those they currently inhabit, are recognised as a pervasive and significant threat to aquatic habitats globally (*e.g.* Carlton, 1999; Thresher, 1999; Bax *et al.*, 2003; Molnar *et al.*, 2008; Williams and Grosholz, 2008; Chapter 2.2). This is particularly true for

estuaries and nearby coastal waters, as anthropogenic modifications, such as ports and their associated shipping activities, have resulted in some of these systems harbouring very large numbers of NIS, *e.g.* San Francisco Bay, USA, and Port Phillip Bay, Australia (Cohen and Carlton, 1998; Hewitt *et al.*, 2004). Once established, NIS can have deleterious impacts upon a number of ecosystem services, including, but not limited to, fishery productivity, the extent and quality of nursery habitats and the filtering and detoxification services provided by suspension feeders, submerged vegetation and wetlands (Worm *et al.*, 2006). Thus, NIS can reduce the ability of environment and fisheries management agencies to effectively maintain the health and sustainability of these ecosystems (Robinson, 2001; Simberloff, 2003; Crowder and Norse, 2008)

As the deleterious effects of NIS in estuarine and coastal environments are well documented (*e.g.* Jude *et al.*, 1992; Ricciardi *et al.*, 1998; Sánchez-Paz, 2010) and the fact that representatives of the Gobiidae, due to their biological and ecological characteristics, are among the most prolific and successful of the non-indigenous fish species (Wonham *et al.*, 2000), concerns arise when new non-indigenous goby species are detected. This occurred with the detection of the Striped Sandgoby *Acentrogobius pflaumi*, a native of Japan and Korea, in Port Phillip Bay near Melbourne, Australia and the Waitemata and Whangapoua Harbours near Auckland in New Zealand (Lockett and Gomon, 2001; Francis *et al.*, 2003). Particularly alarming was the explosive colonisation of *A. pflaumi* in the former location, as the species was first recorded in 1996 but just one year later had become the most widely distributed and among the most abundant fish species recorded in surveys (Lockett and Gomon, 2001). In Western Australia *A. pflaumi* was first recorded in Cockburn Sound in 2005 (Mead-Hunter, 2005) and had spread to the Swan-Canning Estuary later in that same year (Maddern and Morrison, 2009). Given the rapid proliferation of *A. pflaumi* in Port Phillip Bay, the overall aim of this chapter is to determine the spatial and temporal distribution and abundance of *A. pflaumi* in the Swan-Canning Estuary. More specifically, this will involve:

1. Comparing the overall abundance and distribution of *A. pflaumi* with other native goby species in the shallow, nearshore and deeper, offshore waters of the Swan-Canning Estuary.
2. Determining if and how the abundance and distribution of *A. pflaumi* and other native goby species changes over a 21 month period between January 2014 and September 2015.
3. Compare the abundance of *A. pflaumi* in the Swan-Canning estuary, in relation to native goby species, to other established *A. pflaumi* populations nationally and internationally.

3.3. Materials and methods

3.3.1. Site study

The Swan-Canning Estuary (Figure 1.1; 3.1), located in south-western Australia (32.055°S, 115.735°E), is part of the broader Swan-Avon catchment that drains an area of ~121,000 km² (Peters and Donohue, 2001). This region experiences a Mediterranean climate, with highly seasonal rainfall and a microtidal tidal range, *i.e.* diurnal tides of ~0.5m in height (Hodgkin and Hesp, 1998). The estuary, which is ~50 km long and covers an area of ~55 km², is a drowned river valley system, permanently connected to the Indian Ocean via a narrow entrance channel that opens into two lagoonal basins and the tidal portions of the Swan and Canning Rivers (Figure 3.1). The majority of the estuary is shallow, *i.e.* <2 m in depth, however, it does reach a maximum depth of ~20 m in the entrance channel. Due to ~70% of the rainfall occurring between May and September (Hodgkin and Hesp, 1998), the estuary experiences marked seasonal variations in environmental conditions, with stable, relatively high salinities occurring throughout much of the estuary during summer, but following substantial freshwater discharge during winter a marked longitudinal salinity gradient can be present (Kanandjembo *et al.*, 2001; Tweedley *et al.*, 2015a).

The sedimentary environment of the Swan-Canning Estuary is heterogeneous, with differences in both the organic (particulate organic matter) and inorganic (sediment

grain size) components of the sediment among water depths and locations in the estuary (Bennett, 2014). In general, composition of the sediment in the deeper offshore areas contain a higher percentage of particulate organic matter and finer inorganic particles (*i.e.* those <125 μm – fine mud) than corresponding shallow, nearshore waters. Regardless of water depth, the substrate of downstream reaches contains sediment the largest proportion of 125-249 μm and is likely sand transported in from the nearshore marine environment (Wildsmith *et al.*, 2005; Tweedley, 2010). The proportion of the larger sediment grain sizes was found to increase in an upstream direction, as these terrestrially derived particles were likely deposited first as water velocity decreased progressively downstream (Bennett, 2014).

Seagrasses, such as *Zostera* sp., *Heterozostera* sp. and *Halophila ovalis* are present in the estuary, with the first two species occurring in the entrance channel and the last forming extensive beds in shallow parts of Melville Water (Valesini *et al.*, 2009; Department of Water, 2014). In general only small seagrass beds are present in the Canning Estuary and upstream of the Narrows Bridge (Department of Water, 2014).

The estuary flows through the capital city of Perth, which supports ~78% of the Western Australian population (Australian Bureau of Statistics, 2015). Both the estuary and its catchment have been highly modified by anthropogenic activities (Commonwealth of Australia, 2002), subsequently resulting in the development or exacerbation of multiple stressors, such as increased delivery of sediments and nutrients, in addition to changes to salinity and hydrological regime, including periodic hypoxia (Stephens and Imberger, 1996; Tweedley and Hallett, 2014; Tweedley *et al.*, 2014b). However, despite these perturbations, the estuary still holds substantial value to the community for its aesthetic, commercial, environmental and cultural importance (Department of the Environment, 2015).

3.3.2. *Sampling regime*

Data on the abundance of *Acentrogobius pflaumii* and other goby species were obtained from an established sampling regime designed to determine the spatial and temporal distribution of the Western School Prawn (*Metapenaeus dalli*) in the Swan-Canning Estuary (Tweedley *et al.*, 2014a, 2015b). Sampling was conducted at 20 shallow, nearshore sites (water depth < 2 m) and at 16 deeper, offshore sites (water depth 2-17 m) in five regions, namely, Lower Melville water (LM), Upper Melville water (UM), Lower Canning Estuary (LC), Upper Canning Estuary (UC) and the Middle Swan Estuary (MS; Figure 3.1). Note, no deep water sites were sampled in Upper Canning Estuary due to accessibility issues.

Two replicate trawls were conducted at each site on each sampling occasion, with sampling undertaken during the night every 28 days on the new moon phase (*i.e.* when the portion of the moon illuminated was < 10%) between October 2013 and September 2015. A hand trawl net that was 4 m wide and comprised of 9 mm mesh was employed to sample the nearshore waters. This net was dragged for 200 m and, accounting for the fact that the net was on average 2.85 m wide during trawling (Broadley, 2014), covered an area of 570 m². The offshore waters were sampled using an otter trawl, which was 2.6 m wide and constructed from 25 mm mesh, with a 9 mm cod end. The net was towed using a boat at a speed of ~1.6 knots for five minutes covering a distance of ~250 m and thus an area of ~650 m².

At the end of each trawl the catch of fish and prawns was sorted, with all fish identified and returned alive to the water as quickly as possible, with the exception of any introduced species. In accordance with our scientific licence from the Department of Fisheries Western Australia and our Animal Ethics permits, any introduced species, including specimens of *A. pflaumii*, were euthanized in an ice slurry, transported to Murdoch University for biological analysis (see Chapter 4) and then appropriately disposed of.

Although the sampling regime for *M. dalli* was initiated in October 2013 it was not until January 2014 that the individuals of *A. pflaumii* were able to be reliably

recognised in the field and thus only data collected after this date has been employed in this study. Note also that, due to mechanical difficulties with the boat used to tow the otter trawl, not all sites were sampled in December 2014 and thus the limited data collected during this time was excluded from the overall data set.

Salinity and water temperature were obtained from the Department of Water's Water information reporting tool (DOW, 2015). Regular Department of Water sampling sites within each of the established sampling regions (Fig. 3.1) were identified and the relevant data imported in all months related to the sampling periods. Results were filtered by water depth, only the values corresponding to the minimum and maximum depths in each site throughout only deep water regions were utilised and those sites lacking all of the required data excluded. The resultant averaged monthly values were then plotted to indicate seasonal variability in salinity and temperature between water depths throughout the course of sampling.

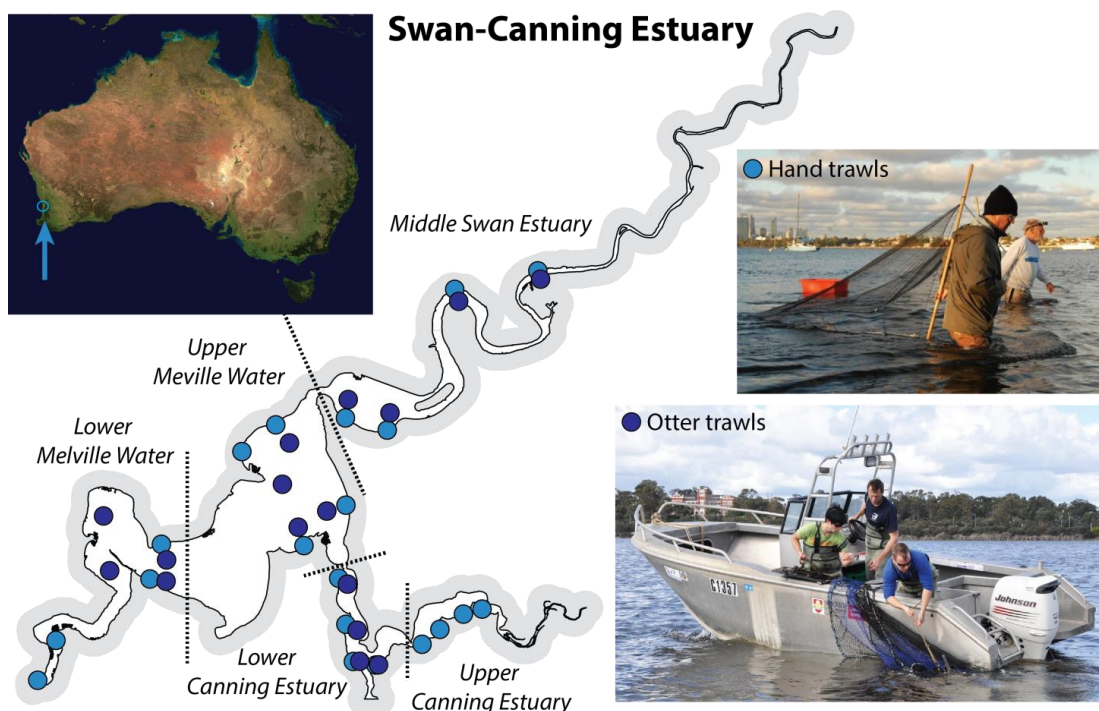


Figure 3.1. The location of shallow (●) and deep (●) sampling sites within the five regions of the Swan-Canning Estuary sampled using hand and otter trawls, respectively, every 28 days between October 2013 and September 2015.

3.3.3. Statistical analyses

All statistical analyses were undertaken using PRIMER v7 (multivariate statistics software package; Clarke *et al.*, 2014), with the PERMANOVA+ add on (Anderson *et al.*, 2008). Note, as the main focus of this chapter was to determine the spatial and temporal distribution of all goby species, with emphasis upon *A. pflaumi*, and that this species was not recorded in any of the 880 samples, covering > 500,000 m² of substratum in the nearshore waters over a 21 month period (see Table 3.1), all statistical analyses were conducted on data obtained from 704 samples collected from the deeper, offshore waters of the Swan-Canning Estuary. Furthermore, as this study focused solely on gobiid fauna, the data for all other fish species was not analysed, however a species list is provided in Appendix 3.1.

Univariate analysis

The abundance data for each goby species in each individual sample was converted to the number of fish per 500 m² and the species richness (number of species) and total density of gobies calculated for each replicate sample using the DIVERSE routine. The data for each of the two dependent variables (*i.e.* gobiid richness and density) were examined separately to determine the extent to which any transformations were required to meet the test assumptions of homogenous dispersion among *a priori* groups. This was achieved by calculating the extent of the linear relationship (slope) between the log_e (mean) and log_e (standard deviation) of each variable among all groups and comparing them to the criteria described in Clarke and Warwick (2001) to select the appropriate form of transformation. This analysis demonstrated that density required a square-root transformation, while species richness did not require transformation.

The transformed data for both variables were used to construct separate Euclidean distance matrices and subjected to a two-way Permutational Analysis of Variance (PERMANOVA; Anderson *et al.*, 2008). The aim of this analysis was to determine whether the values for each variable differed among five regions and 21

sampling occasions. The null hypothesis of no significant differences among groups was rejected if the significance level (P) was < 0.05 , and the relative influence of each term in the model was quantified using the components of variation. The main source of significant differences detected by PERMANOVA were identified by examining plots of the marginal means of the dependent variable, back transformed where necessary, with associated 95% confidence intervals.

Multivariate analysis

The abundance of each gobiid species recorded in each replicate sample were subjected to a pre-treatment shown to be effective for estuarine fish community data (Clarke *et al.*, 2013). This involved firstly subjecting the data to dispersion weighting to down-weight the effects of those species whose numbers exhibited erratic differences among replicate samples due to schooling (Clarke *et al.*, 2006), followed by a square-root transformation to down-weight the contributions of species with consistently high values (across replicates within a group) in relation to those with consistently low values. The resultant pre-treated data were then used to construct a Bray-Curtis similarity matrix, which was subjected to the same two-way PERMANOVA design as described above, though now with multivariate data. Pairwise tests for selected terms in the model were also conducted using a pairwise PERMANOVA test.

To visually explore the results of the two-way PERMANOVA, the pre-treated replicate data for each region on each sampling occasion were averaged and used to create a Bray-Curtis similarity matrix. This matrix was, in turn, subjected to non-metric Multi-dimensional Scaling (nMDS; Clarke, 1993) to produce an ordination plot. A shade plot was produced from the same pre-treated and averaged gobiid faunal data for each region in each sampling occasion. This plot was used to visualise the trends exhibited by the transformed abundances of the various goby species among regions and sampling occasions. The resultant plot is a simple visualisation of the frequency matrix, where a white space for a species demonstrates that the taxon was never collected, while the intensity of shading from grey-scale shading is linearly proportional

to the abundance of that species (Clarke *et al.*, 2014; Tweedley *et al.*, 2015c). Species (*y* axis) exhibiting similar patterns of abundance across the ‘samples’ (*x* axis) were ordered in the shade plot by the results of a group-average hierarchical agglomerative cluster analysis (also shown) employing a Bray-Curtis similarity matrix. Samples were arranged in chronological order within a region, and the regions ordered by location along the longitudinal axis in the estuary from approximately most downstream to most upstream.

3.4. Results

3.4.1. Salinity and water temperature

Mean monthly water temperatures, calculated for both the surface and bottom of the water column of each of four regions of the Swan-Canning Estuary exhibited a sinusoidal pattern between January 2014 and September 2015. In all regions and water depths, water temperatures peaked in either January or February and declined to their lowest values during July (Fig. 3.2.a, b). The highest mean water temperature (~ 27 °C) occurred in the Middle Swan (both water depths) during January of both years. The lowest mean monthly water temperature (~ 14 °C) occurred in the surface water of all regions during July 2014 (Fig. 3.2.a). During the winter months temperatures at the bottom of the water column were slightly greater than the corresponding values at the surface (*cf.* Fig. 3.2.a, b).

Mean monthly salinity values varied among months, regions and water depths. Means occurring in surface waters also followed a sinusoidal pattern in each region, peaking between January to March (~ 37) and falling to their lowest values in August and September (~ 7 ; Fig. 3.2.c). Although salinity changed markedly throughout the year in each region, the pattern of differences among region remained consistent. Thus, the highest values (in each month) were typically always recorded in Lower Melville Water, followed by Upper Melville Water and the lowest values in the Middle Swan Estuary (Fig. 3.2.c). While mean monthly surface salinities were relatively consistent during the warm summer months of both years, salinities during winter in 2014 (*i.e.* ~ 7 in the Middle Swan Estuary) declined further than during the same time in 2015 (*i.e.* ~ 15 in the Middle Swan Estuary; Fig 3.2.c).

The monthly pattern of salinity at the bottom of the water column differed markedly among regions (Fig. 3.2.d). Thus, while salinities in the Middle Swan Estuary underwent pronounced changes which mirrored those in the surface waters, *i.e.* being greatest in summer (~ 37) and lowest in winter (~ 18), this was not the case in the regions in Melville Water where salinities were much more consistent (~ 34 - 37 ; Fig. 3.2d).

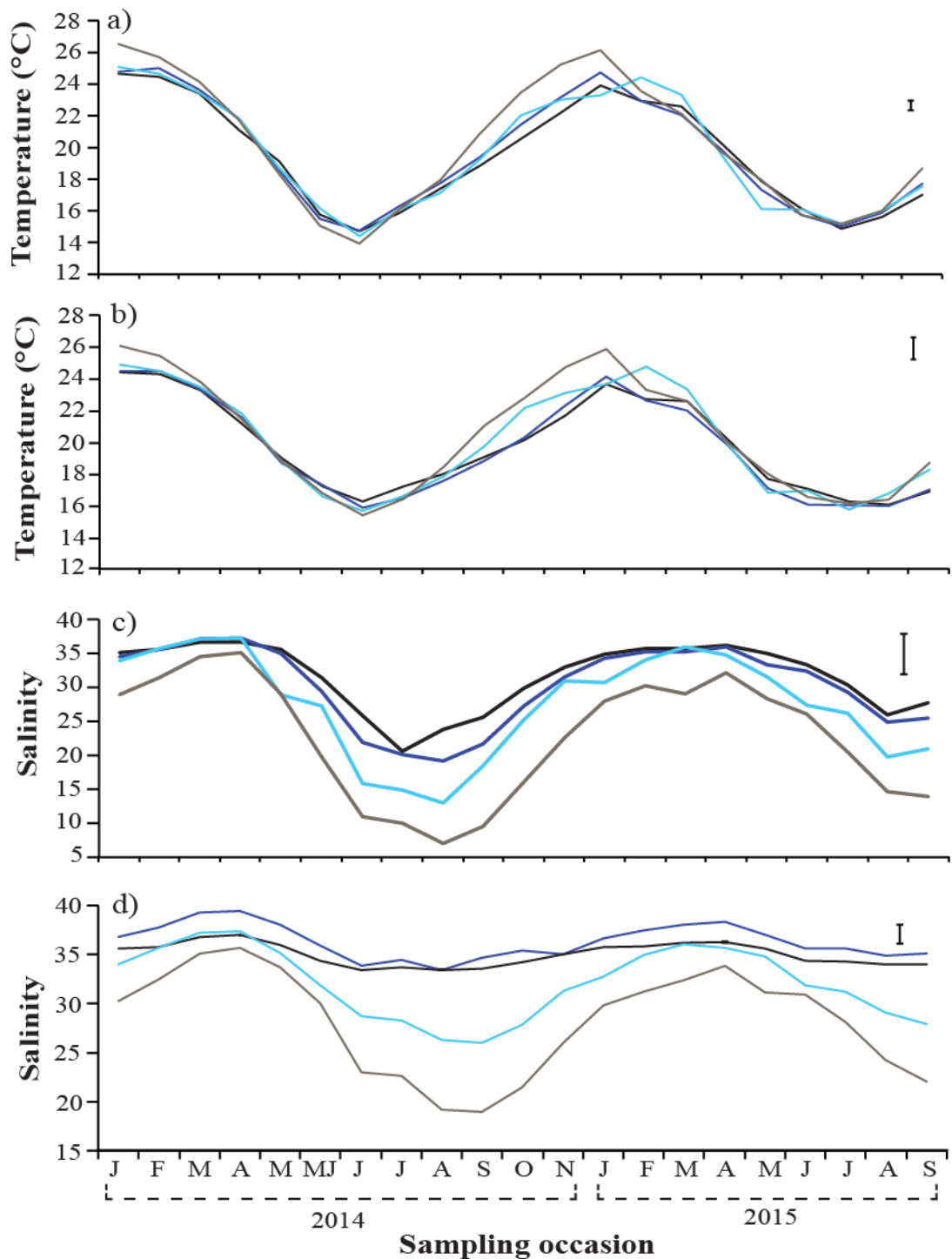


Figure 3.2. Mean monthly surface (a) and bottom (b) water temperature (°C) and surface (c) and bottom (d) salinities throughout the four regions of the Swan-Canning Estuary between January 2014 and September 2015. ● = Lower Melville Water, ● = Upper Melville Water, ● = Lower Canning Estuary and ● = Middle Swan Estuary. For the sake of clarity, the average \pm 1 standard error value has been presented for each plot.

3.4.2. Contribution of gobiids in the shallow and deeper waters

A total of 272,390 fish, representing 60 species (Appendix 1), were recorded at the 36 sites sampled every 28 days in the Swan-Canning Estuary between January 2014 and September 2015. The vast majority of those fish, *i.e.* 250,824 fish (92%), were recorded at the 20 sites in the shallow, nearshore waters. Members of the Gobiidae comprised four of the 38 species and ~2% of the individual fish in shallow waters. Although at least three of the four gobiid species were present in each of the five regions, the average density of gobiids differed markedly, with ~7 and ~6 individuals 500 m⁻² being recorded in the Upper Canning Estuary and Middle Swan Estuary, respectively, but only 0.3 individuals 500 m⁻² caught at the sites in Upper Melville Water. Such was the abundance of the gobies in the first two regions they contained 74% of all gobiid individuals recorded in the shallow waters (Table 3.1.a).

Among the four goby species in the shallow waters *Favonigobius punctatus* and *Arenigobius bifrenatus* were the most abundant, comprising 39 and 36% of the total number of gobies respectively, while *Pseudogobius olorum* and *Favonigobius lateralis* were considerably less abundant, representing 15 and 10% respectively (Table 3.1.a). The percentage contributions of the species in the various regions differed considerably. For example, *F. lateralis* comprised 82% of the gobiid fauna in Lower Melville Water, but declined in abundance in an upstream direction representing 0.07% in the Upper Canning Estuary and not being recorded in the Middle Swan Estuary (Table 3.1.a). In contrast *A. bifrenatus* was by far the most abundant goby in the Upper Canning Estuary region, representing 71% of all the gobiid fish, made a relatively low contribution (*i.e.* <15%) in the Lower Canning Estuary, Upper Melville Water, Middle Swan Estuary and being absent entirely from Lower Melville Water. *Favonigobius punctatus* was the dominant goby species in Upper Melville Water, Lower Canning Estuary and Middle Swan Estuary representing between 42 and 87% of all gobies in those regions (Table 3.1.a).

Table 3.1. Life cycle guilds (LG), rankings by abundance (R), mean densities of gobiid fish 500m⁻² (\bar{X}), standard error (SE) and percentage contribution to total catch (%) of the goby species caught in the different regions of the (a) shallow and (b) deep waters of the Swan-Canning Estuary between January 2014 and September 2015. Total number of samples and species, individuals and density of gobiids are also provided. Introduced species highlighted are in bold and species contributing >25% of total goby catch in each region are highlighted grey. Life cycle guilds for gobiid species are allocated on the basis of being solely estuarine (E), estuarine and marine (EM), estuarine and freshwater (EF) and marine straggler (MS). *F. pun.* = *Favonigobius punctatus*, *A. bif.* = *Arenigobius bifrenatus*, *P. olo.* = *Pseudogobius olorum*, *F. lat.* = *Favonigobius lateralis*, *A. pfl.* = *Acentrogobius pflaumi*, *C. dep.* = *Callogobius depressus*, *T. tri.* = *Tridentiger trigenocephalus* and *C. muc.* = *Callogobius mucosus*.

(a) Shallow		Total				Lower Melville				Upper Melville				Lower Canning				Middle Swan				Upper Canning			
Species	LG	R	\bar{X}	SE	%	R	\bar{X}	SE	%	R	\bar{X}	SE	%	R	\bar{X}	SE	%	R	\bar{X}	SE	%	R	\bar{X}	SE	%
<i>F. pun.</i>	E	1	1.30	0.23	38.67	3	0.06	0.03	4.21	1	0.27	0.10	87.30	1	1.07	0.28	42.18	1	3.36	1.02	59.53	2	1.76	0.47	24.91
<i>A. bif.</i>	E	2	1.21	0.31	36.02					3	0.01	>0.01	3.17	4	0.23	0.16	9.04	3	0.83	0.36	14.73	1	5.00	1.56	70.81
<i>P. olo.</i>	EF	3	0.50	0.08	14.89	2	0.10	0.05	7.66	2	0.03	0.02	9.52	2	0.63	0.24	24.86	2	1.45	0.23	25.74	3	0.30	0.10	4.21
<i>F. lat.</i>	EM	4	0.35	0.139	10.42	1	1.15	0.23	88.12					3	0.61	0.57	23.92					4	0.01	0.01	0.07
Tot. # samples			880					176				176				176				176				176	
Tot. # species			4					3				3				4				3				4	
Tot. # goby			3,325					261				63				526				1,117				1,353	
Tot. density			3.37					1.30				0.31				2.53				5.65				7.07	

(b) Deep		Total				Lower Melville				Upper Melville				Lower Canning				Middle Swan							
Species	LG	R	\bar{X}	SE	%	R	\bar{X}	SE	%	R	\bar{X}	SE	%	R	\bar{X}	SE	%	R	\bar{X}	SE	%	R	\bar{X}	SE	%
<i>A. pfl.</i>	?	1	1.55	0.21	54.98	1	4.23	0.80	97.58	2	0.22	0.05	31.37	2	0.314	0.07	17.94	4	<0.01	<0.01	0.24				
<i>A. bif.</i>	E	2	0.80	0.08	28.32	3	0.02	0.01	0.50	1	0.22	0.06	31.82	1	1.116	0.20	63.88	1	1.10	0.26	57.28				
<i>P. olo.</i>	EF	3	0.40	0.06	13.83	2	0.07	0.03	1.72	3	0.18	0.05	24.96	3	0.192	0.07	11.00	2	0.80	0.22	41.05				
<i>C. dep.</i>	MS	4	0.06	0.020	2.06	4	0.01	<0.01	0.10	4	0.06	0.03	8.73	4	0.109	0.07	6.22								
<i>F. pun.</i>	E	5	0.02	<0.01	0.55					5	0.02	0.01	3.12	6	0.004	0.04	0.24	3	0.02	0.01	1.19				
<i>T. tri.</i>	MS	6	0.01	<0.01	0.15									5	0.013	<0.01	0.72								
<i>C. muc.</i>	MS	7	0.01	<0.01	0.05													4	<0.01	<0.01	0.24				
<i>F. lat.</i>	EM	8	0.01	<0.01	0.05	4	0.01	0.004	0.10																
Tot. # samples			704					176				176				176				176				176	
Tot. # species			8					5				5				6				5				5	
Tot. # goby			1,988					991				160				418				419				419	
Tot. density			2.87					4.33				0.70				1.75				1.92				1.92	

A total of 21,566 fish from 48 species were recorded at the 16 sites in the deeper, offshore waters, of which ~12% of the individuals and ~17% of the species (8) were gobiids. Between five and six of the eight goby species recorded in the deeper waters were recorded in each of the regions, however, the total densities of gobies differed markedly (Table 3.1.b). The highest densities were recorded in Lower Melville Water, *i.e.* ~4 gobies 500m⁻², and the lowest in Upper Melville Water, *i.e.* < 1 goby 500m⁻², with intermediate densities recorded in the Middle Swan Estuary and Lower Canning Estuary (Table 3.1.b).

Of the eight goby species, three, namely *Acentrogobius pflaumii* (55%), *A. bifrenatus* (28%) and *P. olorum* (14%), comprised just over 97% of all gobies recorded (Table 3.1.b). The most abundant of these species *A. pflaumii* overwhelmingly dominated the goby fauna in Lower Melville Water and remarkably represented ~98% of all gobiids (Table 3.1.b). The contribution of this species to the fauna declined progressively in an upstream direction comprising 31, 18 and 0.2% of the individuals in Upper Melville Water, Lower Canning Estuary and Middle Swan Estuary, respectively. Instead, those three regions, and particularly the last two, were dominated by *A. bifrenatus* and *P. olorum* (Table 3.1.b). The remaining five species, including another non-indigenous species *Tridentiger trigonocephalus*, were found at between one and three regions, but cumulatively made relatively small contributions to the total goby fauna in all cases, *i.e.* <10 and usually <5% (Table 3.1.b).

In total, more gobiid species were recorded in the deeper offshore waters than shallower waters, with all four of the species found in shallow waters also being captured in the corresponding offshore sites. Four of the species, however, were only ever recorded in the deeper waters, namely *A. pflaumii*, *T. trigonocephalus*, *C. depressus* and *C. mucosus*. In fact such was the dominance of *A. pflaumii*, it had the highest average abundance of any gobiid in either the shallow or deep waters (Table 3.1.a, b). Among the species which occurred in both water depths, the densities of all were greater in the shallow waters and this was particularly true for *F. punctatus* and *F. lateralis* (Table 3.1a, b).

Note that as the main species of interest for this study, *A. pflaumii*, was only recorded in the deeper water, despite 880 samples being collected from the shallow waters over an 18 month period, all subsequent description and analyses focuses on data for the goby fauna in those deeper waters only.

3.4.4. Spatial distribution of Acentrogobius pflaumii and other abundant co-occurring gobiids

Over the 18 month duration of the sampling program individuals of *A. pflaumii* were found at 12 of the 16 deep water sites. Abundance was highest at the most downstream sites, *i.e.* Mosman and Freshwater Bay, followed by Dalkeith and Point Walter and declined in an upstream direction (Fig. 3.3.a). Three of the four sites where this species was not recorded were all upstream of the Narrows Bridge. The native gobies *A. bifrenatus* and *P. olorum* were recorded at 14 of the 16 sites, with those ones upstream, *i.e.* Maylands and Garratt Road Bridge, harbouring the greatest densities of these two species, respectively (Fig. 3.3.b, c). In the case of *A. bifrenatus* the densities of this species declined in a downstream direction from Maylands on the Swan axis of the estuary and from Rossmoyne on the Canning axis, with this species not being found at two of the four lower point sites and in very low abundances at the remaining two sites (Fig. 3.3.b). Patterns of abundance were less clear cut with *P. olorum*, but notable hotspots of abundance included Pelican Point and Deep Water Point (Fig 3.3.c).

3.4.5. Gobiid richness and density

The average number of goby species collected in each otter trawl sample (gobiid richness) was shown by PERMANOVA to differ among sampling occasions, regions and the interaction between these two main effects (Table 3.2.a). The pseudo-*F* values indicated that region was the most influential term in the model, followed by sampling occasion. The number of gobiid species typically ranged from 0.5-1.5, however, there were few clear trends among either main effect (Fig. 3.4.a). The fact that the regional pattern of richness differed markedly in the various sampling occasions helps explain

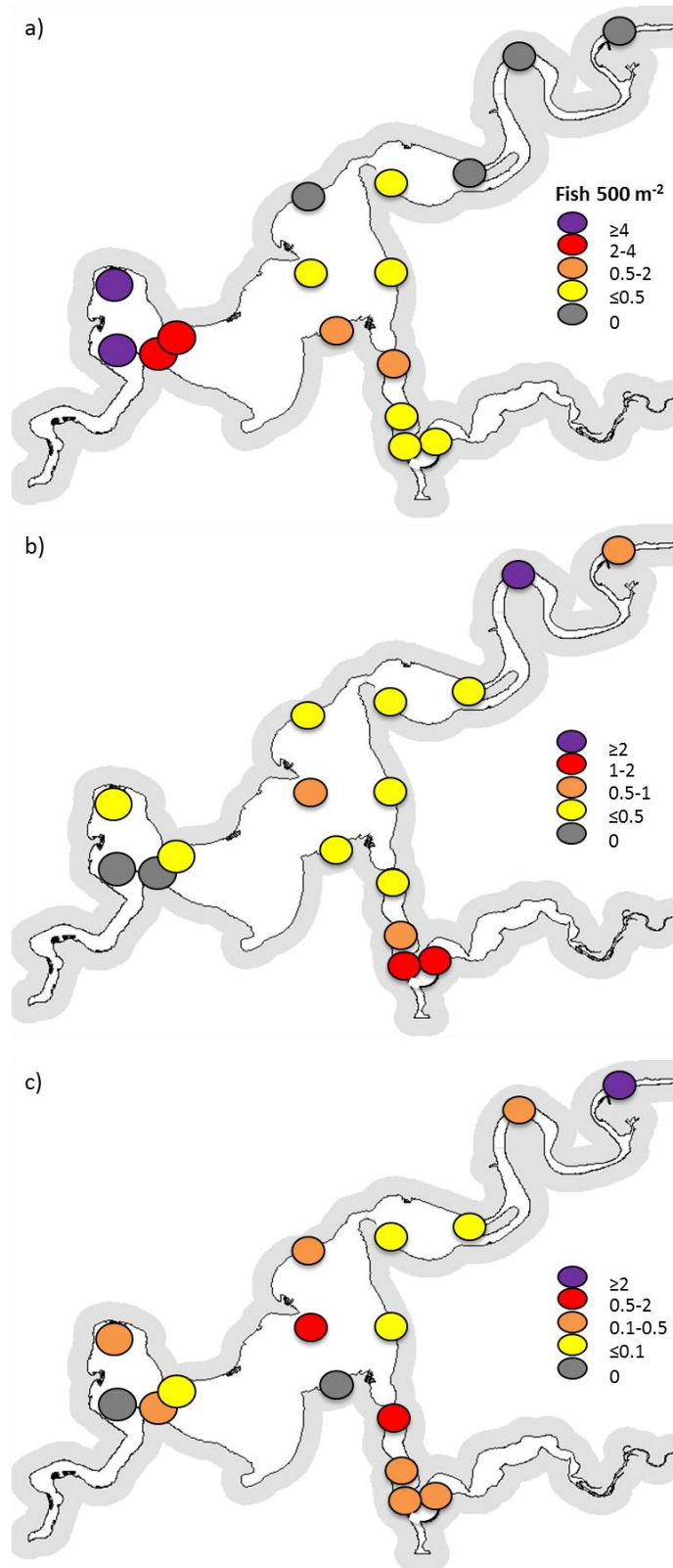


Figure 3.3. Average density 500 m⁻² of the three most abundant deep water gobiid species, (a) *Acentrogobius pflaumi*, (b) *Arenigobius bifrenatus* and (c) *Pseudogobius olorum*, at the 16 deep water sites of the Swan-Canning Estuary sampled ‘monthly’ between January 2014 and September 2015.

the significant Sampling period x Region interaction term (Table 3.2.a). For example, gobiid richness was greatest in each of the four regions during at least two of the sampling occasions (Fig. 3.4.a).

PERMANOVA also demonstrated that the density of gobies (fish 500 m⁻²) differed among sampling occasions, regions and the interaction between these two main effects (Table 3.2.b). Among these terms in the model, region exerted the greatest influence on the variability and was shown to be the most influential. Typically, gobiid density ranged from 0-5 fish 500 m⁻², however, there was a pronounced increase in the number of gobies recorded in Lower Melville Water (up to ~38 fish 500 m⁻²) between July and October 2014 (Fig. 3.4.b). It was likely the presence of large densities in this region was responsible for the significant region effect and also the significant differences among sampling occasions. The large differences between the densities of gobies in Lower Melville Water and the other regions on these sampling occasions compared to the limited differences at other times, *e.g.* January to September 2015, are responsible for the significant Sampling occasion x Region interaction term.

Table 3.2. Degrees of freedom (df), Mean squares (MS), Pseudo-*F* values and Significance levels (*P*) for two-way PERMANOVA tests on average a) gobiid richness and b) gobiid density (fish 500 m⁻²), recorded at the 16 deep water sites in the four regions of the Swan-Canning Estuary sampled ‘monthly’ between January 2014 and September 2015.

	df	MS	Pseudo- <i>F</i>	<i>P</i>
(a) gobiid richness				
Sampling occasion	20	1.84	3.98	0.001
Region	3	2.55	5.51	0.003
Sampling occasion x Region	60	0.85	1.84	0.002
Residual	588	0.46		
(b) gobiid density				
Sampling occasion	20	9.98	7.36	0.001
Region	3	23.45	17.30	0.001
Sampling occasion x Region	60	4.68	3.45	0.001
Residual	588	1.36		

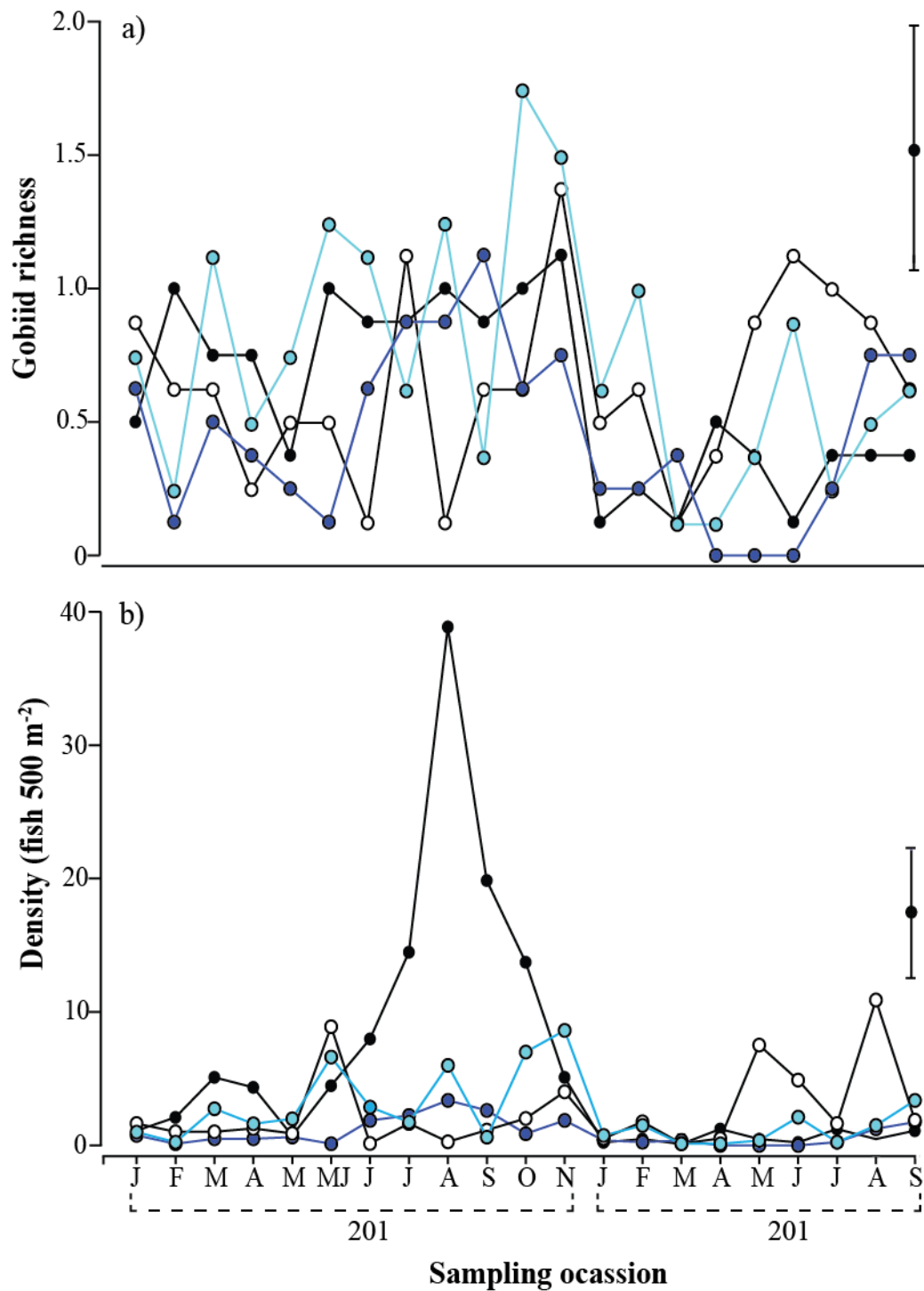


Figure 3.4. Mean a) gobiid richness and b) gobiid density (fish 500 m⁻²), recorded at the 16 deep water sites in the four regions of the Swan-Canning Estuary sampled ‘monthly’ between January 2014 and September 2015. ● = Lower Melville Water, ● = Upper Melville Water, ● = Lower Canning Estuary and ● = Middle Swan Estuary. For the sake of clarity, the average ± 95% confidence intervals have been presented for each plot.

3.4.6. Gobiid faunal composition

PERMANOVA, demonstrated that the gobiid faunal composition differed among sampling occasions, regions and the interaction between these two main effects (Table 3.3). As indicated by the magnitude of the pseudo- F value (~ 51), Region explained by far the largest portion of the variance in faunal composition than either Sampling occasion (~ 5) or the Sampling occasion x Region interaction (~ 3 ; Table 3.3). Thus, region has been made the focus of subsequent analyses, although the influence of the other significant terms in the PERMANOVA model have been considered when describing trends in the abundance of the various goby species.

Table 3.3. Mean squares (MS), degrees of freedom (df), Pseudo- F values and significance levels (P) for a two-way PERMANOVA test on the densities of the various goby species recorded at the 16 deep water sites in the four regions of the Swan-Canning Estuary sampled ‘monthly’ between January 2014 and September 2015.

	df	MS	Pseudo- F	P
Sampling occasion	21	1604.6	4.55	0.001
Region	3	17908	50.77	0.001
Sampling occasion x Region	63	1021.8	2.90	0.001
Residual	616	352.74		

The results of a pairwise PERMANOVA, demonstrated that the gobiid fauna of each of the four deep water regions was distinct from that recorded at all other deep water regions ($P = < 5\%$; Table 3.4). The largest difference was observed between Lower Melville Water and the Middle Swan Estuary ($t = 10.64$) and the former region with the Lower Canning Estuary and Upper Melville Water ($t = 8.96$ and 8.10 , respectively). The magnitude of the differences in gobiid fauna between Lower Melville Water and the other more upstream regions is reflected on the nMDS plot, where the points representing this region on each sampling occasion are situated in a broad group on the left hand side of the plot and well separated from those in the Middle Swan and Lower Canning estuaries (Fig. 3.5).

Table 3.4. PERMANOVA t values for pairwise comparison of the gobiid faunal composition between the four regions. Note that a significant difference was detected for each pairwise comparison ($P < 5\%$). LM = Lower Melville Water, UM = Upper Melville Water, LC = Lower Canning Estuary and MS = Middle Swan Estuary.

	LM	UM	LC
UM	8.10		
LC	8.96	4.45	
MS	10.64	4.82	2.67

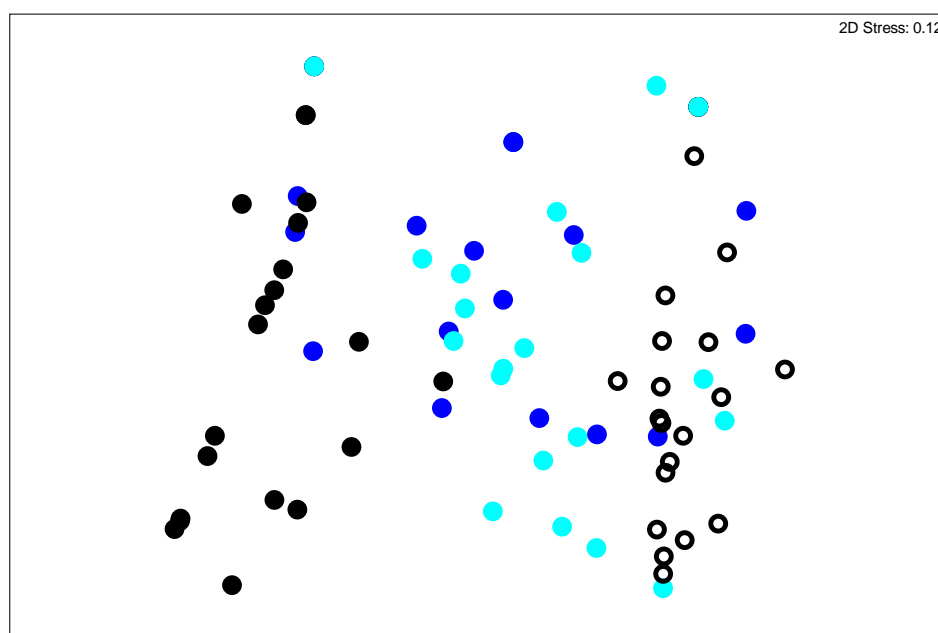


Figure 3.5. Non-metric Multi-dimensional scaling ordination plot derived from Bray-Curtis similarity matrix of the average square-transformed densities of the various goby species recorded in each of the four regions of the Swan-Canning Estuary in each of the 21 ‘monthly’ sampling occasions between January 2014 and September 2015. ● = Lower Melville Water, ● = Upper Melville Water, ● = Lower Canning Estuary and ○ = Middle Swan Estuary.

The distinctness of the gobiid fauna of Lower Melville Water was due to the overwhelming domination of *A. pflaumi* in samples from this region on all 21 sampling occasions, whereas both the frequency of occurrence and density of this species declined progressively upstream, with individuals recorded on only one occasion in Upper Melville Water (Fig. 3.7). Such was the dominance of *A. pflaumi* that, while four other species of goby were recorded in Lower Melville Water, only a single goby species, *i.e.* *P. olorum*, was recorded on more than two sampling occasions and even

then in low densities. In contrast, *P. olorum* and another goby found in very low numbers in Lower Melville Water, *i.e.* *A. bifrenatus*, were both very abundant in both the Lower Canning and Middle Swan estuaries (Fig. 3.6).

Although *A. pflaumii* was abundant in Upper Melville Water, its densities were relatively consistent among the sampling occasions and did not undergo the marked increase during the winter and early spring of 2014, which was likely responsible for the significant Sampling occasion x Region interaction (Table 3.3). Moreover, while the densities of co-occurring species such as *A. bifrenatus* and *P. olorum* in Upper Melville Water were not as high as they were in the Lower Canning and Middle Swan estuaries, they were substantially greater than those recorded in Lower Melville Water.

The next largest differences in gobiid fauna among regions involve comparisons between Upper Melville Water vs Middle Swan Estuary and Lower Canning Estuary ($t = 4.82$ and 4.45 , respectively). This was due to densities of *A. pflaumii* declining progressively upstream and far larger densities of *A. bifrenatus* and *P. olorum* in the upper reaches (Fig.3.7). Relatively minor differences ($t = 2.67$) were detected between Middle Swan Estuary and Lower Canning Estuary, which largely reflected the increase in the frequency of occurrence and density of *P. olorum* in the former region (Table 3.3; Fig. 3.6).

Trends in species composition among sampling periods were far less marked than the regional trends, however, there were some notable changes in density of particular species. For example, in addition to the very substantial increase in the density of *A. pflaumii* in Lower Melville Water in winter and early spring of 2014, there was also an increase in the abundance of *A. bifrenatus* and *P. olorum* at the same time in the Lower Canning Estuary (Fig. 3.6). Densities of the latter species also increased in the Middle Swan Estuary during the winter of 2015.

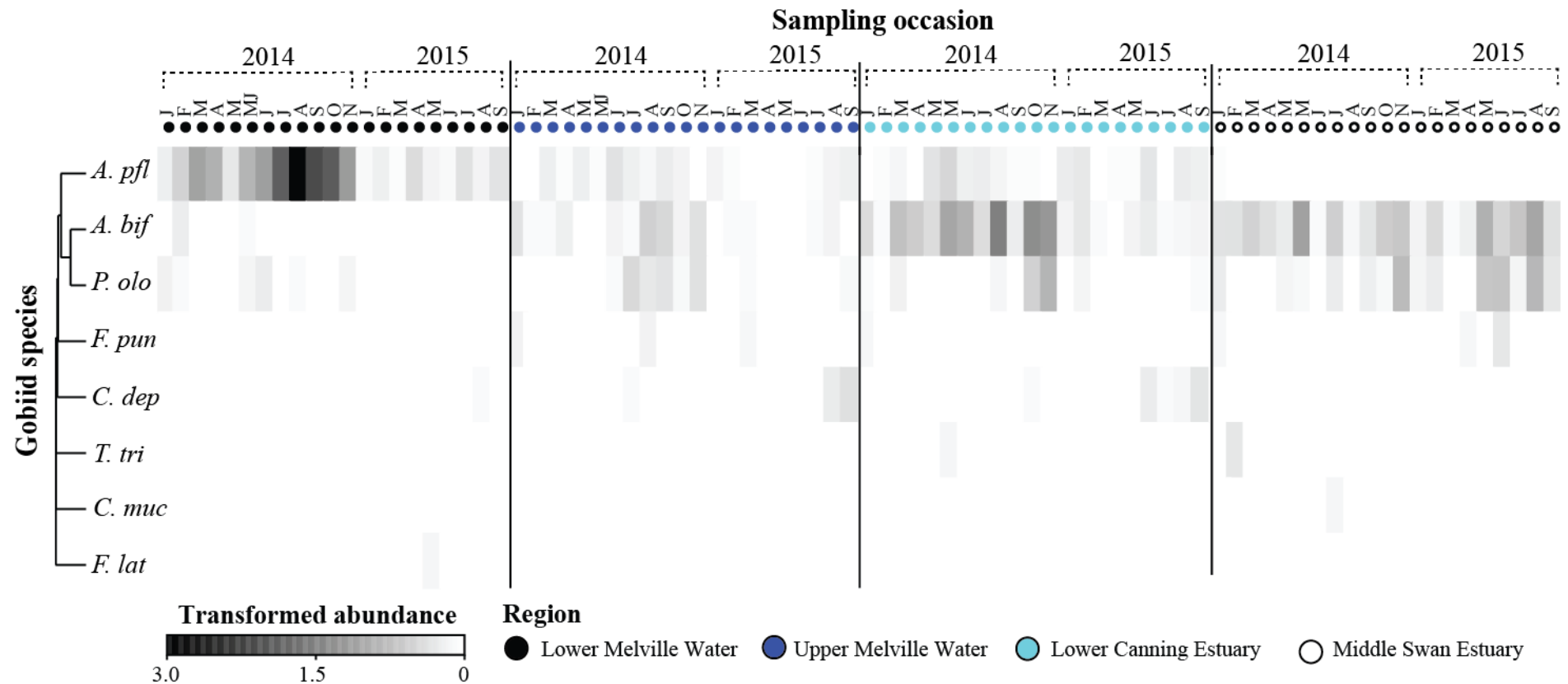


Figure 3.6. Shade plot of the square-root transformed densities (fish 500 m⁻²) of the eight gobiid species recorded in each of the four regions of the Swan-Canning Estuary in each of the 21 ‘monthly’ sampling occasions between January 2014 and September 2015. Grey scale represents the square-root transformed densities of each species (fish 500 m⁻²). Regions (x axis) ordered from closest to estuary mouth (left) to furthest from estuary mouth (right) and the 21 ‘monthly’ samples within each of the regions in chronological order. Species (y axis) ordered using the results of a group-average hierarchical agglomerative cluster dendrogram constructed from a Bray-Curtis similarity matrix, which groups together species with similar patterns of abundance across the ‘samples’(x axis). *A. pfl.* = *Acentrogobius pflaumi*, *A. bif.* = *Arenigobius bifrenatus*, *P. olo.* = *Pseudogobius olorum*, *F. pun.* = *Favonigobius punctatus*, *C. dep.* = *Callogobius depressus*, *T. tri.* = *Tridentiger trigenocephalus*, *C. mus.* = *Callogobius mucosus* and *F. lat* = *Favonigobius lateralis*.

3.5. Discussion

Trends in gobiid fauna among water depths

Data from 1,584 samples, comprising 272,390 fish representing 60 species, from the shallow and deep waters of the Swan-Canning Estuary, collected over a 21 ‘month’ period between January 2014 and September 2015, provided a sound basis for elucidating trends in the spatial and temporal distribution of goby species during that period. Comparisons of the data from the nearshore, shallow (< 2m deep) and offshore, deeper (2-17 m deep) waters collected at the same sites, at the same time and using broadly similar methods (see Gill and Potter, 1993) indicated the gobiid richness was significantly higher in all deeper waters and overall gobiid densities in the shallow waters.

The increased species richness of the deeper waters reflects the fact that, despite 880 samples being collected, four of the eight goby species, *i.e.* *Acentrogobius pflaumii*, *Callogobius depressus*, *Tridentiger trigonocephalus* and *Callogobius mucosus* were only recorded in the deeper waters (Table 3.1.a, b). It is thus relevant that none of these species were collected during a comprehensive study of the fish fauna of the shallow waters of five estuaries in south-western Australia, including the Swan-Canning (Valesini *et al.*, 2009, 2014). Given that *C. depressus*, *T. trigonocephalus* and *C. mucosus* are regarded as marine straggler species their presence in only the deeper waters may reflect the reduced seasonal variation in salinities in the deeper waters. Moreover, as the composition of the sediment varies markedly between the sandy shallow and muddy deeper environments (Bennett, 2014) the presence of these four species in the deeper waters may be due to sediment preference (*e.g.* Nip and Wong, 2010; Hewitt and McDonald, 2013; White *et al.*, 2014).

The greater densities of gobiids in the shallow than deeper water mirrors the previous studies of the Swan-Canning Estuary ichthyofaunal assemblages (*e.g.* Loneragan *et al.*, 1989; Gill and Potter, 1993). As mentioned by the authors of the latter study, this difference was unlikely due to differences in mesh size from 25 mm in the otter trawl to 3 mm in the hand trawl, as dense accumulations of weed and

gelatinous sediment block the net and facilitate the collection of small individuals and both fish and crustaceans (Poh, unpublished data). Instead, it is more likely a reflection of the preference of species such as *Favonigobius punctatus* and *Favonigobius lateralis* for coarser sediments and perhaps also the reduced number of larger piscivorous predators, e.g. Flathead such as *Platycephalus westraliae* (Coulson *et al.*, 2015), small Mulloway *Argyrosomus japonicus* (Taylor *et al.*, 2006) and Black Bream (Chuwen *et al.*, 2007).

Regional differences in gobiid fauna in deeper waters

As *A. pflaumi*, the focus species of this study, was only recorded in deeper water, detailed comparisons of the spatial and temporal trends in gobiid fauna were only undertaken for the deeper waters. Goby faunal composition was shown to differ among regions, sampling occasions and the interaction between these two variables, with region being by far the most influential. As with other studies of the fish fauna of the Swan-Canning Estuary distinct faunal assemblages were recorded in each of the regions (e.g. Prince *et al.*, 1982; Loneragan *et al.*, 1987; Loneragan and Potter, 1990; Gill and Potter, 1993; Humphries and Potter, 1993).

The lower most reaches, Lower Melville Water, contained the most distinct gobiid assemblage, due to the overwhelming dominance of *A. pflaumi*. This species represented ~98% of all goby individuals collected, with very minor contributions from *P. olorum* (1.7%) and *A. bifrenatus* (0.5%). The dominant abundance of *A. pflaumi* in the Swan-Canning Estuary is consistent with gobiid faunas within its native range, such as the Jilong River Estuary in China (Nan *et al.*, 2009) and elsewhere in its non-native range, e.g. Port Phillip Bay, in south-eastern Australia (Lockett and Gomon, 2001). This dominance could be due to the fact that these ‘open water’ silty habitats in Melville Water were not previously colonised, to any substantial extent, by native goby species. This hypothesis is supported by the fact that Maddern and Morrison (2009) found no other gobiid species utilising this habitat in the Swan River Estuary. Furthermore, although Gill and Potter (1993) recorded 26,232 gobies during their survey of the Swan-

Canning Estuary, only 135 individuals were recorded from the deeper water, the majority of which were *A. bifrenatus*.

Despite the dominance of *A. pflaumi* in Lower Melville Water, the average density of this species in this region, *i.e.* ~ 4 fish 500m^{-2} , is substantially lower than the densities recorded in their native populations, which range from 5-10 fish 500m^{-2} (Nan *et al.*, 2009). It should be noted, however, the large numbers of *A. pflaumi*, *i.e.* between 12 and 34 fish 500m^{-2} , were recorded between June and October 2014 in Lower Melville Water. Thus, the populations of this species may be highly variable in abundance and seeded by recruitment from within estuaries and nearby coastal waters.

The presence of *A. pflaumi* declined in an upstream direction, while there was an increase in the abundance of the two native gobies *A. bifrenatus* and *P. olorum*. In Upper Melville Water, all three species represented between 24 and 31% of the total number of gobies. This finding contrasts with Francis *et al.* (2003) as these authors found that although *A. bifrenatus* and *A. pflaumi* occupied similar habitats in two harbours in New Zealand, the populations of both species did not overlap and were only collected in the same seine-net sample on a single occasion. Thus, the population of *A. pflaumi* in the Swan-Canning Estuary does overlap with distribution of native species and thus competition may be occurring. The reason as to why the distribution of these three species overlap in the upper but not lower parts of Melville Water may be the result of the reproductive biology of the *A. bifrenatus* and *P. olorum*, which spawn in the upper parts of the estuary and thus tend to be most abundant in those waters (Gill and Potter, 1993; Gill *et al.*, 1996; Potter and Hyndes, 1999). It is thus relevant that these two species dominated the fauna of the Middle Swan Estuary, while *A. bifrenatus* dominated that in the Lower Canning Estuary.

Ecology of Acentrogobius pflaumi

Contrary to the established population of *A. pflaumi* in Port Phillip Bay in Victoria, which, shortly after being first detected, became the most widespread and one of the most abundant fish species in this marine embayment (Lockett and Gomon,

2001). The *A. pflaumii* population in the Swan-Canning Estuary appears to be restricted, in terms of density, to sites within close proximity to the lower most reaches. Given that research on the atherinid and goby species present in south-western Australian estuaries has demonstrated that the members of these families partition themselves throughout estuaries spatially based on water quality parameters, such as salinity (Prince and Potter, 1983; Humphries and Potter, 1993; Potter and Hyndes, 1999; Prince *et al.*, 1982), it is likely salinity may be responsible for restricting the range of *A. pflaumii*.

Salinities, in the surface waters of the Swan-Canning Estuary, are consistently greatest in Lower Melville Water due to the tidal movement of marine waters into the estuary from the nearby coastal areas. Furthermore, while salinities in these most downstream areas declined to ~20 during winter, salinities at the bottom of the water column only decreased to ~33 and were analogous to salinities recorded in Port Phillip Bay (Murray and Parslow, 1997). The tendency of *A. pflaumii* to prefer 'marine' waters has also been recorded in its native areas of Korea and Japan, where it inhabits coastal embayments and the lower reaches of estuaries (*e.g.* Horinouchi and Sano, 2001; Kanou *et al.*, 2004; Horinouchi, 2008; Nan *et al.*, 2009; Wang *et al.*, 2015).

The decreasing densities of *A. pflaumii* longitudinally in the estuary and the lack of penetration to sites upstream of Perth Water provide an indication that this species prefers areas where the salinity is close to that of full strength seawater. When compared to some of the native gobies, such as the estuarine & freshwater *P. olorum* and estuarine & marine *F. lateralis* which have both been recorded in salinities < 1 and up to 76 and 50, respectively (Hoeksema *et al.*, 2006a; Tweedley, 2011; Potter *et al.*, 2015b), *A. pflaumii* could be considered relatively stenohaline. However, at this stage, it is not known whether the lack of occurrence in waters with a reduced salinity is due to a physiological inability to osmoregulate in salinities less than seawater or due to differences in sediment composition, food availability, habitat and/or biotic interactions with other species. Thus, there is substantial merit in conducting laboratory experiments to determine both the gradual and acute salinity tolerances of *A. pflaumii* and further explore habitat preferences.

Despite 880 hand trawls being conducted over the course of the 21 ‘months’, individuals of *A. pflaumii* were never recorded in the nearshore, shallow waters, including at sites located adjacent to deep sites where this species was known to occur in substantial numbers. As gobies are benthic species and thus in regular association with the substratum, the occurrence of *A. pflaumii* in the deeper, but not shallow waters of Lower Melville Water may also be due to the sediment composition. The substratum of the deeper waters of this region are characterised, as are much of the deeper waters of the microtidal estuaries in south-western Australia (Tweedley, 2011; Tweedley *et al.*, 2012; Bennett, 2014; Tweedley and Hallett, 2014), by greater proportions of particulate organic matter and small inorganic particles (*i.e.* those with grain sizes $< 125 \mu\text{m}$). This is a result of reduced current velocities and increased salinity in the basins of these estuaries, which aids in flocculation and deposition (Ryan *et al.*, 2003; Tweedley *et al.*, *in press*) and can result in up to 80% of the fine sediments that are transported into the estuary becoming ‘trapped’ (Patchineelam *et al.*, 1999; Roy *et al.*, 2001).

Acentrogobius pflaumii is known to inhabit bare and vegetated soft sand, muddy sand and muddy sediments (Yanagisawa, 1978; Horinouchi and Sano, 2000, 2001; Lockett and Gomon, 2001; Horinouchi, 2008). It is known to prefer, however, softer substrates found in deeper waters (*i.e.* $> 5\text{m}$; Yanagisawa, 1978; Lockett and Gomon, 2001; Horinouchi, 2008), which would explain the absence of *A. pflaumii* in shallow nearshore waters with coarser sediment sizes. Further evidence of the depth preference of *A. pflaumii* is provided by the fact that it was rarely recorded in the shallow waters of Port Phillip Bay (Lockett and Gomon, 1999, 2001).

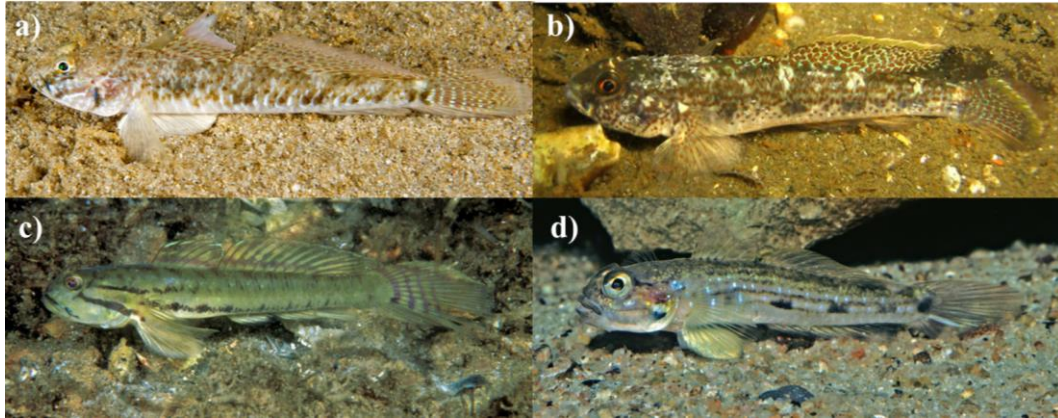
Field observations have determined that, in soft sediment, *A. pflaumii* is able to partially bury itself (Horinouchi, 2008) and that it also cohabits burrows with species of alpheid shrimps (Yanagisawa, 1978; Maddern and Morrison, 2009), which are abundant in the sediments of Melville Water (Poh, unpublished data). While, the mechanical capacity of *A. pflaumii* to bury itself is unknown, the fact that this species was not recorded in adjacent shallow waters may be due to a lack of ability to bury in the coarser sediment and/or the absence of alpheid shrimp in these waters (Wildsmith *et al.*,

2011). It is thus relevant that most fishes that exhibit burying behaviours avoid sediments with larger grain sizes (e.g. Moles and Norcross, 1995; Phelan *et al.*, 2001; Stoner and Titgen, 2003), presumably due to the increased difficulty and energetic requirement of burial.

While the shallow sediments are coarser, Bennett (2014) demonstrated that, in the deeper water, sediment grain size increases in areas upstream of Melville Water, presumably due to decreasing current velocity and the deposition of the larger terrigenous particles. This may explain why, although salinities in these areas of the Swan-Canning Estuary exceeded 32 in late summer and early autumn of both 2014 and 2015, only three *A. pflaumi* were ever recorded in these areas. It is also relevant that another benthic species exhibiting burying tendencies, the Western School Prawn, *Metapenaeus dalli*, was shown to occur in greater densities at sites in Melville Water than the Middle Swan Estuary and the results from laboratory experiments suggested that *M. dalli* exhibited a preference for the finer sediment from Melville Water. Moreover, the act of burying took twice as long at sediments from the Middle Swan Estuary due to the coarser grain sizes and gelatinous nature of the sediment, thus putting the prawns at a greater risk of predation (Bennett, 2014).

One final consideration could be the coloration of *A. pflaumi*. Whereas species such as *F. lateralis* and *F. punctatus* are well camouflaged against the coarse yellow sandy substrates of the shallow waters, this is not the case for species such as *A. bifrenatus* and *A. pflaumi*, whose colorations are more suited to darker, muddier sediment (Fig. 3.8). Frequenting the shallow sandy habitats may make these latter two species far easier to spot and thus at a greater risk of predation, especially as gobies swim at relatively low speeds. For example, in a study of the swimming speeds of 35 fishes, benthic species such as the blenny *Zoarces viviparous* and goby *Pomatoschistus minutus* were able to swim at 0.8 and 1.0 km/h, compared to piscivorous fishes, such as the mackerel *Scomber scombrus* and salmon *Salmo salar*, which are recorded at 10.9 and 11.6 km/h, respectively (Nursall, 1962).

Figure 3.7. Photographs detailing the colouration of dominant shallow (a) *Favonigobius lateralis*, (b) *Favonigobius punctatus*, (c) *Arenigobius bifrenatus* and (d) *Acentrogobius pflaumii*. Photographs taken from <http://www.fishesofaustralia.net.au>.



3.6. Conclusion

The non-indigenous species *Acentrogobius pflaumii*, while not present in the nearshore, shallow waters, was the most abundant gobiid species in the offshore, deeper water, representing 55% of all gobiids recorded. The density and percentage contribution of this species to the gobiid fauna in the deeper waters declined progressively in an upstream direction from Lower Melville Water (~98%) to the Middle Swan Estuary (0.2%). Contrary to work in New Zealand the spatial distribution of *A. pflaumii* was shown to overlap with that of *A. bifrenatus* (an introduced species in that country) and also *P. olorum* in the upper parts of Melville Water, and to a far lesser extent in the Lower Canning Estuary. Instead this latter region is dominated by *A. bifrenatus*, which together with *P. olorum* dominated the gobiid fauna of the Middle Swan Estuary. Thus, there are marked regional differences in goby composition. This may aid resource partitioning within the estuary, thus reducing competition. In the Swan-Canning Estuary, as in other systems, *A. pflaumii* displays a preference for deeper waters with soft sediments and in regions where salinities are stable and close to that of full strength seawater. While the current data are based on a substantial number of samples, detailed statistical analysis between the *in situ* abundance of *A. pflaumii* and environmental variables, such as salinity, are required together with laboratory

experiments concerning accessory factors such as sediment preference. Rigorous assessment of environmental preferences will allow us to identify future areas at risk of colonisation by *A. pflaumii*, both nationally and internationally.

3.7. Appendix

Appendix 1: List of fish species recorded during hand trawling in the nearshore, shallow waters and otter trawling in the offshore, deeper waters of the Swan-Canning Estuary on 21 sampling occasions between January 2014 and September 2015. x denotes a species was present.

Scientific Name	Family	Shallow	Deep
<i>Ostorhinchus rueppellii</i>	APOGONIDAE	x	x
<i>Atherinomorus vaigiensis</i>	ATHERINIDAE	x	x
<i>Parablennius postocolomaculatus</i>	BLENNIIDAE		x
Blenniidae sp.	BLENNIIDAE	x	
<i>Dactylopus dactylopus</i>	CALLIONYMIDAE		x
<i>Eocallionymus papilio</i>	CALLIONYMIDAE		x
<i>Pseudocalliurichthys goodladi</i>	CALLIONYMIDAE		x
<i>Pseudocaranx dentex</i>	CARANGIDAE	x	x
<i>Nematolosa vlaminghi</i>	CLUPIDAE	x	x
<i>Spratelloides robustus</i>	CLUPIDAE	x	
<i>Hyperlophus vittatus</i>	CLUPIDAE	x	
<i>Paraplagusia bilineata</i>	CYNOGLOSSIDAE		x
<i>Engraulis australis</i>	ENGRAULIDAE	x	x
<i>Enoplosus armatus</i>	ENOPLOSIDAE	x	
<i>Gerres subfasciatus</i>	GERREIDAE	x	x
<i>Arenigobius bifrenatus</i>	GOBIIDAE	x	x
<i>Pseudogobius olorum</i>	GOBIIDAE	x	x
<i>Favonigobius lateralis</i>	GOBIIDAE	x	x
<i>Acentrogobius pflaumii</i>	GOBIIDAE		x
<i>Tridentiger trigonocephalus</i>	GOBIIDAE		x
<i>Callogobius depressus</i>	GOBIIDAE		x
<i>Callogobius mucosus</i>	GOBIIDAE		x
<i>Favonigobius punctatus</i>	GOBIIDAE	x	x
<i>Hyporhamphus melanochir</i>	HEMIRAMPHIDAE	x	
Labridae sp.	LABRIDAE		x
<i>Lesueurina platycephala</i>	LEPTOSCOPIDAE		x
Monacanthidae spp.	MONACANTHIDAE		x
<i>Acanthaluteres brownii</i>	MONACANTHIDAE	x	
<i>Aldrichetta forsteri</i>	MUGILIDAE	x	x
<i>Mugil cephalus</i>	MUGILIDAE	x	x
<i>Parupeneus spilurus</i>	MULLIDAE		x
<i>Melanotaenia australis</i>	MYLIOBATIDAE		x
<i>Pentapodus vitta</i>	NEMIPTERIDAE		x
<i>Haletta semifasciata</i>	ODACIDAE	x	
<i>Neoodax balteatus</i>	ODACIDAE	x	
Ostraciidae spp.	OSTRACIIDAE		x
<i>Pseudorhombus jenynsii</i>	PARALICHTHYIDAE	x	x
<i>Pegasus volitans</i>	PEGASIDAE		x
<i>Parapercis haackei</i>	PINGUIPEDIDAE		x
<i>Platycephalus endrachtensis</i>	PLATYCEPHALIDAE	x	x
<i>Platycephalus laevigatus</i>	PLATYCEPHALIDAE	x	x
<i>Ammotretis rostratus</i>	PLEURONECTIDAE		x
<i>Cnidoglanis macrocephalus</i>	PLOTOSIDAE	x	x
<i>Plotosus lineatus</i>	PLOTOSIDAE	x	
<i>Gambusia holbrooki</i>	POECILIDAE	x	
<i>Argyrosomus japonicus</i>	SCIAENIDAE		x
<i>Sillago schomburgkii</i>	SILLAGINIDAE		x
<i>Sillago bassensis</i>	SILLAGINIDAE	x	x
<i>Sillago maculata</i>	SILLAGINIDAE	x	x

Appendix 1 continued: List of fish species recorded during hand trawling in the nearshore, shallow waters and otter trawling in the offshore, deeper waters of the Swan-Canning Estuary on 21 sampling occasions between January 2014 and September 2015. x denotes a species was present.

Species name	Family	Shallow	Deep
<i>Acanthopagrus butcheri</i>	SPARIDAE	x	x
<i>Rhabdosargus sarba</i>	SPARIDAE	x	x
<i>Pugnaso curtirostris</i>	SYNGNATHIDAE	x	x
<i>Stigmatopora argus</i>	SYNGNATHIDAE	x	x
<i>Hippocampus angustus</i>	SYNGNATHIDAE	x	x
<i>Fistularia commersonii</i>	SYNGNATHIDAE	x	
<i>Amniataba caudavittata</i>	TERAPONTIDAE	x	x
<i>Pelates octolineatus</i>	TERAPONTIDAE	x	x
<i>Pelsartia humeralis</i>	TERAPONTIDAE	x	
<i>Gymnapistes marmoratus</i>	TETRAROGIDAE		x
<i>Torquigener pleurogramma</i>	TETRAODONTIDAE	x	x

Chapter 4: Biological characteristics of *Acentrogobius pflaumi* in the Swan-Canning Estuary

4.1 Abstract

Biological information provides the basis for appropriate management. Marginal increment analysis on whole otoliths of *Acentrogobius pflaumi* demonstrated that opaque zones present are formed annually. Although *A. pflaumi* was shown to live for just under 4 years, the age composition of the population in the Swan-Canning Estuary was dominated by 1+ individuals, indicating a high natural mortality rate. The maximum size of 96 mm attained by *A. pflaumi* in the Swan-Canning Estuary is larger than that recorded in other native and non-native populations. The rapid growth exhibited by *A. pflaumi* in the Swan-Canning Estuary, as demonstrated by the high von Bertalanffy growth coefficient (k) values of 2.74 and 2.06 for females and males respectively, was reflected in females and males attaining >87% of their L_{∞} 's of 77.3 and 72.9 mm within the first year of life. The results from gonadosomatic indices, prevalences of gonads at different stages of development and histological examination of gonads suggest that *A. pflaumi* is able to spawn throughout most of the year within the Swan-Canning Estuary, but exhibits a peak in spawning from November to February. Oocyte diameter frequencies from the ovaries of five mature females during the spawning period revealed two discrete modes of oocytes, previtellogenic and yolk granule oocytes, demonstrating determinate fecundity. Given the duration of the spawning period, it is assumed that multiple batches of eggs are released during that period within the Swan-Canning Estuary. *Acentrogobius pflaumi* mature at the end of the end of their first year of life and at a length ~ 65mm. Juvenile *A. pflaumi* were notably absent from all samples, however 0+ individuals began recruiting to the population by March at a size of ~40 mm. The operational sex ratio of *A. pflaumi* was heavily female biased and thus is considered a population characteristic and implies that they mate in pairs, limited by the availability of males.

4.2. Introduction

4.2.1. Ageing

Age information plays a crucial role in fish biological investigations as it is directly related to important life history characteristics, such as growth rate, mortality and productivity (Campana, 2001). Fisheries scientists require these parameters, amongst other, to assess and sustainably manage the stocks of commercially and recreationally important species (King and McFarlane, 2003). In addition to recreationally and commercially important species, biological information for newly exploited or invasive species is of highest interest to management bodies as they seek to regulate the impacts on the former species by the latter (Simberloff, 2003). As life history characteristics are the underlying determinants of population responses to environmental forcing (King and McFarlane, 2003), the synthesis of life history characteristics for invasive species, in particular, holds immense value as it can be used to formulate effective control strategies and also enables predictive modelling concerning the rate and spread of invasion (*e.g.* Sakai *et al.*, 2001; Simberloff, 2003; GISP, 2004).

The determination of age in fish species relies on the identification of periodically formed growth increments contained in calcified structures, such as spines, opercula, scales, cleithra, vertebra, sphenoids, dorsal scutes and otoliths (*e.g.* Erickson, 1983; Horppila and Nyberg, 1999; Sipe and Chittenden, 2002; Francis *et al.*, 2007; Jackson *et al.*, 2007; Phelps *et al.*, 2007; Muir *et al.*, 2008). While all of the above structures can provide an avenue for ageing, numerous comparison studies have found otoliths to be superior to other calcified structures for age determination (*e.g.* Ihde and Chittenden, 2002; Sipe and Chittenden, 2002; Khan *et al.*, 2013; van der Meulen *et al.*, 2013; Klein *et al.*, 2014; Braaten *et al.*, 2015). Thus otoliths are regarded by fisheries

scientists as the single most important biological structure with unrivalled chronological capabilities (Begg *et al.*, 2005; Campana and Thorrold, 2001) and are used widely for fish ageing purposes (*e.g.* Begg *et al.*, 2005; Khan *et al.*, 2013; Wells *et al.*, 2013; Shih *et al.*, 2014; Bottari *et al.*, 2015; Cavole and Haimovici, 2015)

The dominance of otoliths in age and growth investigations stems from several factors. Perhaps the most important is the stable allometry maintained by otoliths and body size (Boehlert, 1985; Popper *et al.*, 2005), making them highly desirable as indicators of age and, subsequently, growth. The formation of growth zones in otoliths occurs through the rhythmically alternating depositions of calcium carbonate, in the form of aragonite, and various other trace elements (Boehlert, 1985; Woydack and Morales-nin, 2011). The difference in the density in which the calcium carbonate is deposited on the otoliths at different times of year gives the appearance of opaque (dark) and translucent (light) bands, representing periods of slow (late autumn to early spring) and fast growth (mid-spring to mid-autumn), respectively. Some of the biological processes that control otolith growth are related to the biochemical processes that regulate somatic growth and therefore both otolith and somatic growth may react in similar ways to biological events (Mugiya and Tanaka, 1992). Temperature plays an important role in the growth of ectotherms such as fish, with temporal and spatial variation in this factor demonstrated to influence otolith growth (*e.g.* Walther *et al.*, 2010; Coulson *et al.*, 2014; Morrongiello and Thresher, 2014; Rountrey *et al.*, 2014). However, otolith growth is also suggested to be influenced by other physico-chemical processes independent of somatic growth (Neuman *et al.*, 2001). As a result, otoliths grow in a fashion representative of somatic growth and continue to grow after somatic growth has effectively ceased, which makes them invaluable for ageing purposes. The effective ‘uncoupling’ of otolith growth from somatic growth is a well-documented

phenomenon (*e.g.* Neuman *et al.*, 2001; Fey, 2005, 2006; Takasuka *et al.*, 2008) and is a prominent factor in their wide applicability in ageing studies as it helps avoid issues associated with cessation of growth in other calcified structures, such as scales, which may be resorbed during and regenerated after times of extreme physiological stress (Bereiter-Hahn and Zylberberg, 1993)

4.2.2. Age validation

Before counts of growth bands in otoliths can be used for ageing, the periodicity of their formation first needs to be validated. One of the most accurate ways to validate the age of fish is the release and recapture of marked (*i.e.* chemical or temperature mark) and known age fish (*e.g.* Mc Dougall, 2004; Ross *et al.*, 2005). Other accepted validation practices include defined length modes, radiocarbon and radiochemical dating, natural date-specific markers, captive rearing and marginal increment analysis (Campana, 2001). Trends presented by length-class modes can be used to validate the number of age cohorts present within a population, given that growth is uniform and the population consists of a series of distinct age groups at any one time (*e.g.* Mayo *et al.*, 1979; Gill *et al.*, 1996). Issues with this method lie in the overlapping of length classes due to fast growth, asymptotic growth, recruitment pulses and size selective mortality, thus invalidating the assumption that a discrete age cohort is being tracked (*e.g.* Gibson and Ezzi, 1981; Meekan and Fortier, 1996; Shemonaev and Kirilenko, 2009). Radiocarbon and radiochemical dating, and natural date-specific markers all rely upon a biologically significant event (*e.g.* *El Nino* induced growth disruption, atomic bomb testing in 1950's) that in some way leave an identifiable and consistent chemical signature in the otoliths across relevant members of the species that allow the age to be back dated from the point of capture. However, while radiocarbon dating has been used recently as a method of age validation (*e.g.* Armsworthy and Campana, 2010; Andrews

et al., 2011; Morin *et al.*, 2013), the applicability of this technique is decreasing over time as it relies upon long-lived fish species (*i.e.* alive during 1950's) inhabiting the region of testing and thus accumulating detectable levels of C¹⁴ (Piner and Wischniowski, 2004).

Although captive rearing and tag and release approaches are effective and accurate, they are expensive and time consuming. In contrast, methods such as marginal increment analysis are inexpensive and quick to undertake, and are thus usually the preferred method of age validation in many studies (*e.g.* Piercy *et al.*, 2010; Kemp *et al.*, 2013; Coulson *et al.*, 2014; Smith, 2014). Marginal increment analysis is based on the level of completion for the outermost growth band as a proportion of the width of the previously formed growth band in samples collected throughout the year. The use of marginal increment analysis assists in determining the timing of zone formation, with a single gradual rise and rapid decline in the increment width in a 12 month period, confirming the annual formation of the growth zones.

As noted earlier (see Chapter 1.7), members of the gobiidae exhibit variable interspecies longevity, from as little as 59 days, for the adorned dwarfgoby (*Eviotta silligatai*), to as long as 13 years, for the yellow clown goby (*Gobiodon okinawae*) (Randall and Delbeek, 2009). Such varied longevity has led to ageing of gobiids being conducted using both daily growth rings for shorter lived species (*e.g.* Iglesias *et al.*, 1997; Hernaman *et al.*, 2000; Iida *et al.*, 2010; Winterbottom *et al.*, 2011) and annual growth rings for longer lived species (*e.g.* Kovačić, 2006; Gumus and Kurt, 2009; Adriat *et al.*, 2013). While, in some studies, sagittal otoliths have been polished or ground to produce transverse sections in order to count growth zones (*e.g.* Hernaman *et al.*, 2000; Sokołowska and Fey, 2011; Grant, 2013), others have counted annually and daily formed bands in whole otoliths (*e.g.* La Mesa 2001; Gumus and Kurt, 2009; Iida

et al., 2010). Validation of the ageing procedures of gobiids has included the use of chemical (*i.e.* strontium chloride) staining of otoliths of fish held in aquaria (Iglesias *et al.*, 1997; Hernaman *et al.*, 2000), otolith length and weight relationships (İlkyaz *et al.*, 2011), length frequency analysis (Sponaugle and Cowen, 1994; Gill *et al.*, 1996) and marginal increment analysis (Gumus and Kurt, 2009; Hajji *et al.*, 2013). Gobiid species, with typically abbreviated lifecycles, display a tendency to possess impressive growth capabilities (*e.g.* Gill and Potter 1993; Depczynski and Bellwood 2005). Indeed, Hernaman and Munday (2005) noted that contrary to most other reef fishes, which tend to reach asymptotic size early in life and then persist at that size for some time, the gobiid species they investigated experienced relatively little, if any time at asymptotic size before death. The compensation for shorter lifespans with accelerated growth is considered somewhat characteristic of gobiid species, particularly smaller species. For example, some *Eviota sp.* have life spans < 100 days and display rapid linear growth throughout the entirety of their lives (Depczynski and Bellwood, 2006), while others such as *P. olorum* may attain von Bertalanffy growth coefficients (k) of > 7 year⁻¹ (Gill and Potter, 1993). This is in stark contrast to a vast array of teleosts which, as a result of their comparative longevity, display low growth coefficients, such as the Western Blue Groper *Achoerodus gouldii*, which can attain ages of 70 years and has a k value of 0.1 year⁻¹ (Coulson *et al.*, 2009).

4.2.3. Reproduction

Reproductive biology studies of fishes are designed to examine the gonadal development of a species throughout a biological year and in doing so provide information regarding the timing and duration of its spawning period and the length and age at which maturity is first attained, all of which are key management criteria (West,

1990; Kjesbu *et al.*, 2003; Murua and Saborido-Rey, 2003; Lowerre-Barbieri *et al.*, 2011).

Key to determining reproductive timing is the assessment of gonad development, which can be done through oocyte diameter frequencies, mean monthly gonad weights or gonadosomatic indices and histological and macroscopic assessment. Oocyte size measurement is the most labour intensive method of analysis, but does provide a precise level of development of oocytes as well as indicating spawning frequency based upon the maximum oocyte diameter (*e.g.* Alejo-Plata *et al.*, 2011) or average oocyte diameter (*e.g.* Branco *et al.*, 2013). Gonad weights by themselves can often be misleading as gonad size increases with fish size, but which can be overcome by calculating gonad weight based on a standardised length of fish (West, 1990; Coulson *et al.*, 2010). Gonadosomatic indices (GSI) are another commonly used tool in which monthly changes in gonad weight as a proportion of the total body weight are employed to determine both the timing and duration of spawning. Most assessments of reproductive cycles in fishes are also often done in conjunction with histological and macroscopic analysis of the gonads (*e.g.* Coulson *et al.*, 2009; Alejo-Plata *et al.*, 2011; Klibansky and Scharf, 2015). Macroscopic assessment (*i.e.* visually determining gonad development by examining progressive physical changes in pigment and size) provides a rapid method for assigning developmental stages to individual fish, although it is open to individual interpretation and relies upon the assessors experience to accurately differentiate between stages (*e.g.* Ferreira *et al.*, 2006; Honji *et al.*, 2006; Klibansky and Scharf, 2015). Histological assessment, however, enables the precise determination of the progression through oogenesis and spermatogenesis via the identification of germ cell types and estimation of their relative abundances (Kjesbu *et al.*, 2003). As histological examination of gonads is expensive and time consuming, it is most

commonly used as a validation tool in combination with the other methods mentioned above (Kjesbu *et al.*, 2003).

Most gobiid fishes utilise a reproductive strategy where males are responsible for significant parental investment (Miller, 1984). The reproductive strategies of most gobiid species can be divided into five stages: establishment of a territory, nest preparation, courtship behaviour, spawning, and parental care of the eggs (Reese, 1964). Members of the Gobiidae exhibit the full range of reproductive strategies, including semelparity (*e.g.* *Crystallogobius linearis*; Caputo *et al.*, 2003), abbreviated iteroparity (*e.g.* *Aphia minuta*; Caputo *et al.*, 2001) and prolonged iteroparity (*e.g.* *Zosterisessor ophiocephalu*; Miller, 1984; Adriat *et al.*, 2013). Furthermore, fecundity varies substantially between species of this family, with gobiid species such as *Eviota lacrimae* producing <100 eggs (Sunobe and Nakazono, 1995) and other species such as *Awaous guamensis* producing >500,000 eggs (Ha and Kinzie, 1996). The majority of gobiid species display multiple spawning-event breeding seasons during the spring and summer months, with smaller, shorter lived gobiids tending to possess protracted or even biannual spawning seasons (*e.g.* Gill and Potter, 1993; La Mesa, 2001; Mazzoldi and Rasotto, 2001; La Mesa *et al.*, 2005), although annual reproductive cycles are much more common in most gobiids (*e.g.* Fouda *et al.*, 1993; Sapota, 2004; Kovacic, 2007; Hwang and Baek, 2013). While there is inevitably variability in such a speciose family of fishes, gobiids tend to attain sexual maturity at a relatively early age (*e.g.* Kon and Yoshino, 2002; Caputo *et al.*, 2003; La Mesa *et al.*, 2005; Depczynski and Bellwood, 2006), an adaption thought to be a reflection of the substantial predation pressure on smaller fishes (Caputo *et al.*, 2003; Hernaman and Munday, 2005).

Native gobiid species within the Swan-Canning Estuary display marked segregation throughout the estuary in terms of spatial and temporal distribution (See

Chapter 3.4) that reflect their life-cycle characteristics, with marine species restricted to the lower estuary and estuarine-spawning species occurring largely in the upper estuary (Gill and Potter, 1993). Despite the paucity of general biological data on most native gobiid species in the Swan-Canning Estuary, some information is available concerning the distribution tendencies and spawning habits of the most abundant gobiids. For example, the Bridled Goby *Arenigobius bifrenatus* and the Bluespot Goby *Pseudogobius olorum* both reside predominantly within the upper reaches of the saline tributaries in the Swan-Canning Estuary and, as indicated by the dominance of their larvae during spring and summer periods, spawn and complete their lifecycle within this region (Gill and Potter, 1993; Gill *et al.*, 1996; Potter and Hyndes, 1999; Valesini *et al.*, 2009). Both species guard their demersal eggs (Potter and Hyndes, 1999), which *A. bifrenatus* deposits in constructed burrows it additionally utilises for the purposes of food gathering and protection (Gill and Potter, 1993; Usmar, 2003). While the exact spawning duration of *A. bifrenatus* is unknown, *P. olorum* exhibits bi-annual spawning tendencies with multiple recruitment phases throughout its short (*i.e.* < 12 month) lifespan (Gill *et al.*, 1996).

4.2.4. Aims

The establishment of the gobiid *Acentrogobius pflaunii*, for which relatively little life history information is known, in the Swan-Canning Estuary has provided the unique opportunity to investigate the biology of a species outside its typical distribution and in an environment where there are several other native gobiid species already present. Thus, the aims of this chapter are to:

- 1) Establish appropriate protocols for ageing *A. pflaumi* using increments in their otoliths and to validate the periodicity of the formation of such increments.
- 2) Determine the growth rates for female and male *A. pflaumi*.
- 3) Determine whether *A. pflaumi* spawns in the Swan-Canning Estuary and, if so, the timing and duration of reproduction and the length and ages at maturity for females and males.
- 4) Establish criteria to aid macro- and microscopic assessment and staging of gonads of *A. pflaumi*.
- 5) Compare the biological traits of *A. pflaumi* to those other goby species globally, with particular reference to native species in the Swan-Canning Estuary, when possible.

4.3. Materials and Methods

4.3.1. Study site

See chapter 3.3.1.

4.3.2 Sampling methods and fish measurements

Due to absence in the shallow regions in the Swan-Canning Estuary (See Chapter 3.4.2), all 432 *Acentrogobius pflaumi* utilized in the age and growth investigations were collected using otter trawls in 16 deep water sites (See Chapter 3.3.2 for full details) in the Swan-Canning Estuary between July (2014) and September (2015). Samples were euthanized in an ice slurry and transported to Murdoch University for biological analysis.

The total length (TL) and total weight (TW) of each *A. pflaumii* was measured to the nearest 1 mm and 0.1 g, respectively. Both sagittal otoliths were removed, cleaned and dried before being stored in gelatine capsules within labelled seed envelopes.

4.3.3. Age determination and validation

Paired sagittal otoliths from each individual were viewed concurrently to minimise the impact of naturally occurring otolith imperfections upon ageing accuracy. Otoliths were placed proximal surface up in a small, black glass dish, covered in immersion oil (type B) and photographed under reflected light using a Leica DFC425 digital camera attached to a Leica MZ7₅ dissecting microscope. The number of opaque zones clearly delineated from the primordium were then identified (Fig. 4.1) and recorded for each otolith employing Leica application suite image viewing software (LAS v. 3.6). All otoliths were also independently read by a second experienced otolith reader (Peter Coulson). The level of precision between the counts recorded by the two readers was assessed using the coefficient of variation (*CV sensu stricto* Chang (1983)). The resultant *CV* value of 3.3 % is well below the 5% considered acceptable by Campana (2001), demonstrating that there was a high level of agreement between the counts of the two readers for the otoliths of *A. pflaumii*.

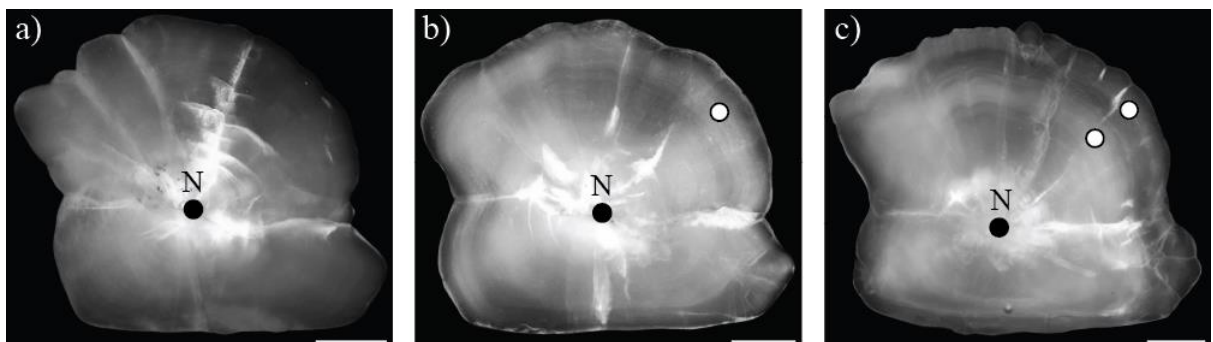


Figure 4.1. *Acentrogobius pflaumii* otoliths with a) zero, b) one and c) two opaque zones. Black dot in all images denotes the position of the nucleus (N), white dots in b and c denote delineated opaque zones. Scale = 0.5mm.

Marginal increment analysis was performed in order to identify the periodicity of the formation of the opaque bands in the otoliths of *A. pflaumii*. Marginal increment analysis, *i.e.* the proportional characterisation of the growth increments state of completion (Campana, 2001), was performed for every otolith with at least one opaque zone, with all measurements taken along the same axis (*i.e.* horizontally, towards the ventral edge, in alignment with the primordium) on the same digital images employed when counting the number of opaque zones. The marginal increment (MI) on each otolith, *i.e.* the distance between the outer edge of the outermost opaque zone and the otolith periphery, was expressed as a proportion of either the distance between the primordium and the outer edge of the single opaque zone, when one such zone was present, or of the distance between the outer edges of the two outermost opaque zones when two or more such zones were present.

4.3.4. Growth determination

The ages of *A. pflaumii* were estimated using the date of capture, an average “birthdate” (approximate mid-point of spawning period) of 1st December, the number of opaque zones in its otolith and the timing of formation of those zones. von Bertalanffy growth curves were fitted separately to the lengths at ages of female and male *A. pflaumii* in R (v. 3.1.1.) using a non-linear ‘growth’ function in the package ‘fish methods’ (Nelson, 2015). Individuals that could not be sexed were excluded from the dataset. The von Bertalanffy growth equation is $L_t = L_\infty [1 - \exp^{-k(t-t_0)}]$, where L_t is the total length (mm) at age t (years), L_∞ is the mean asymptotic length, k is the growth coefficient (year^{-1}) and t_0 is the theoretical age (years) at which the fish would have zero length. Due, largely, to the lack of small individuals (*i.e.* < 40 mm), traditional von Bertalanffy growth curves could not be fitted to the lengths at age of females or males. *Acentrogobius pflaumii* are known to settle at a length of 5-8 mm after a pelagic larval

phase of 30 days (Lockett and Gomon, 1999; Kanou *et al.*, 2004). Thus, von Bertalanffy growth curves, with t_0 fixed at 0.08 at 30 days were fitted to the lengths at age of females and males. A likelihood-ratio test (Cerrato, 1990) was used to compare the growth of the females and males of *A. pflaunii*.

4.3.5. Reproductive biology

The ovaries and testes of female and male *A. pflaunii* were removed and weighed to the nearest 0.0001g wet weight. Gonads of all individuals were examined using a dissecting microscope, sexed and allocated to one of eight stages of development in accordance with Laevastu's criteria (1965), Where: I = virgin; II = resting adult/immature virgin; III = developing; IV = maturing; V = mature; VI = spawning; VII = spent; VIII = degenerating/recovering. Stage V and VI are pooled later for the sake of clarifying spawning/spawning capable periods.

A subsample of gonads at each macroscopically identified developmental stage in each month were placed in Bouin's solution for a minimum of 24hr, dehydrated in an ascending series of ethanol concentrations and embedded in paraffin wax. Transverse sections of the mid-region of each gonad were taken, stained with Mallory's trichrome and then mounted on glass microscope slides to be viewed using a compound microscope with a mounted camera. Photographs of all slides were taken and viewed using Leica application suite image viewing software (LAS v.3.6). Germ cell stages present were identified and used to develop species specific developmental stages based on established gobiidae structures and general teleost developmental patterns (*e.g.* Hole, 1981; Caputo *et al.*, 2001; Kjesbu *et al.*, 2003; La Mesa *et al.*, 2005; Lowerre-Barbieri *et al.*, 2011; Paschoalini *et al.*, 2013; Cho *et al.*, 2014). The frequencies of gonads at each stage of development in each month was used to help identify the timing and duration of spawning for *A. pflaunii* in the Swan-Canning Estuary. In some instances,

where consecutive months in which sample sizes were small (*i.e.* < 10 fish), months were pooled in order to clarify trends if otherwise difficult to determine.

The Gonadosomatic Index (GSI) was calculated for all sexed individuals using the following equation, $(GW/TW) \times 100$, where GW = gonad weight and TW = total body weight. The resultant values were then plotted as pooled monthly averages for each sex to demonstrate the annual reproductive cycle.

As the majority of female and male *A. pflaumi* caught during the spawning period, which were 45 - 94 mm and 46 - 96 mm respectively, possessed or had possessed maturing gonads (*i.e.* stages III-VIII), all fish collected during this study were used for determining the timing and duration of spawning.

4.4. Results

4.4.1. Marginal increment analysis

The mean monthly marginal increments (MI) for whole otoliths of *A. pflaumi* with one opaque zone increased from low levels in February (Late summer) to a maximum in June (early winter), after which the mean monthly MI decreased rapidly to low levels in September to November (early to mid-spring) (Fig. 4.2). Overall, a similar trend was exhibited for *A. pflaumi* with 2-3 opaque zones, to that describe for *A. pflaumi* with one opaque zone, in that the mean marginal increment reached a maximum in June, after which it declined rapidly to low levels. The presence of a single pronounced rise and decline throughout the year displayed by fish with 2-3 growth zones, and to lesser extent the fish with one opaque zone, represents the pronounced sinusoidal cycle required to substantiate the formation of a single opaque zone per year. Therefore, the number of opaque zones in *A. pflaumi* otoliths can be used to calculate the age of individuals of this species.

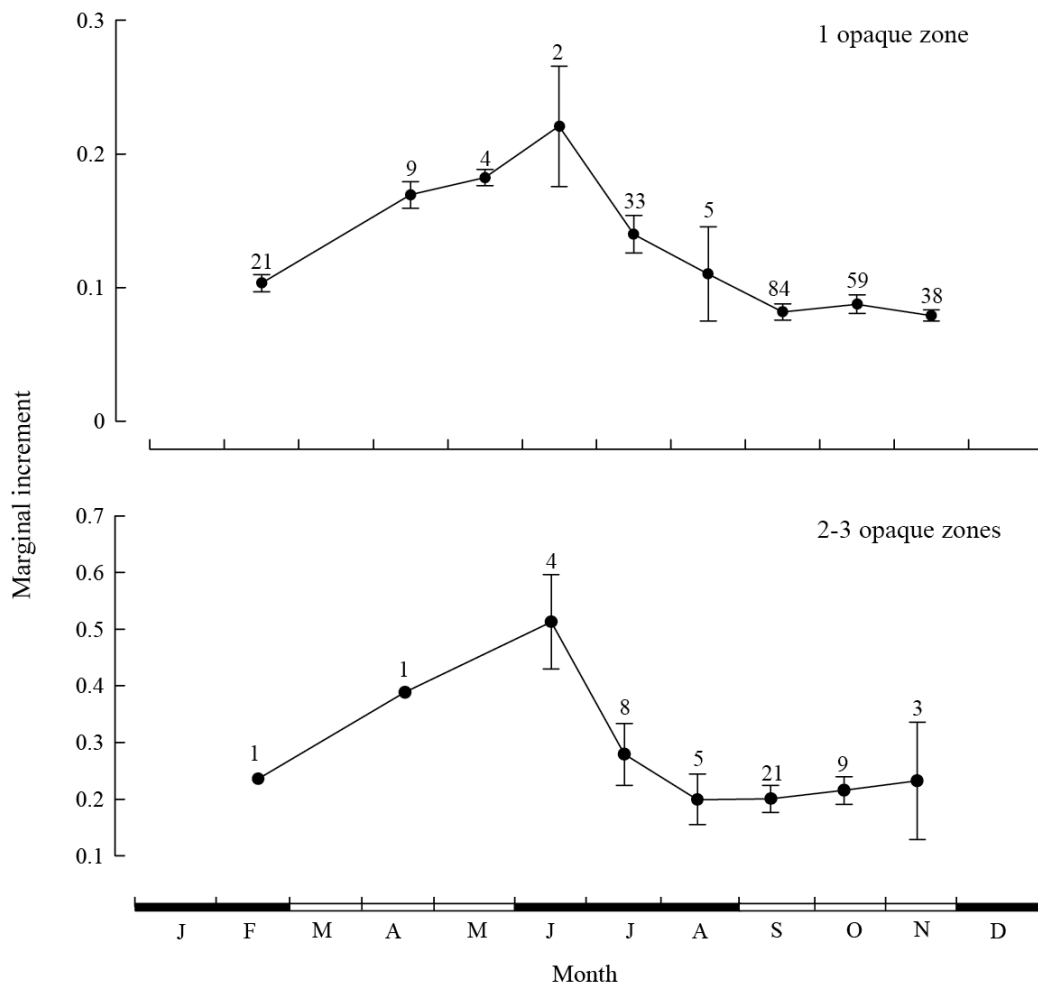


Figure 4.2. Mean monthly marginal increments \pm 1 SE for *Acentrogobius pflaumii* whole otoliths with with a) one and b) two to three opaque zones. On the x-axis in this Figure and Figure 7, closed rectangles refer to summer and winter months and the open rectangles to autumn and spring months.

4.4.2. Length and age frequencies

The 277 female *A. pflaumii* collected from the Swan-Canning Estuary ranged in length from 45 - 94 mm and the 127 males ranged in length from 46 - 96 mm (Fig. 4.3). The 14 fish, whose sex could not be determined, ranged in length from 45 - 60 mm. Females were substantially more abundant than males, both overall, and in all length classes up to 80 mm, above which males became more abundant in all length classes. Females (*i.e.* 114 individuals) were most abundant in the 61-65 and 66-70 mm length

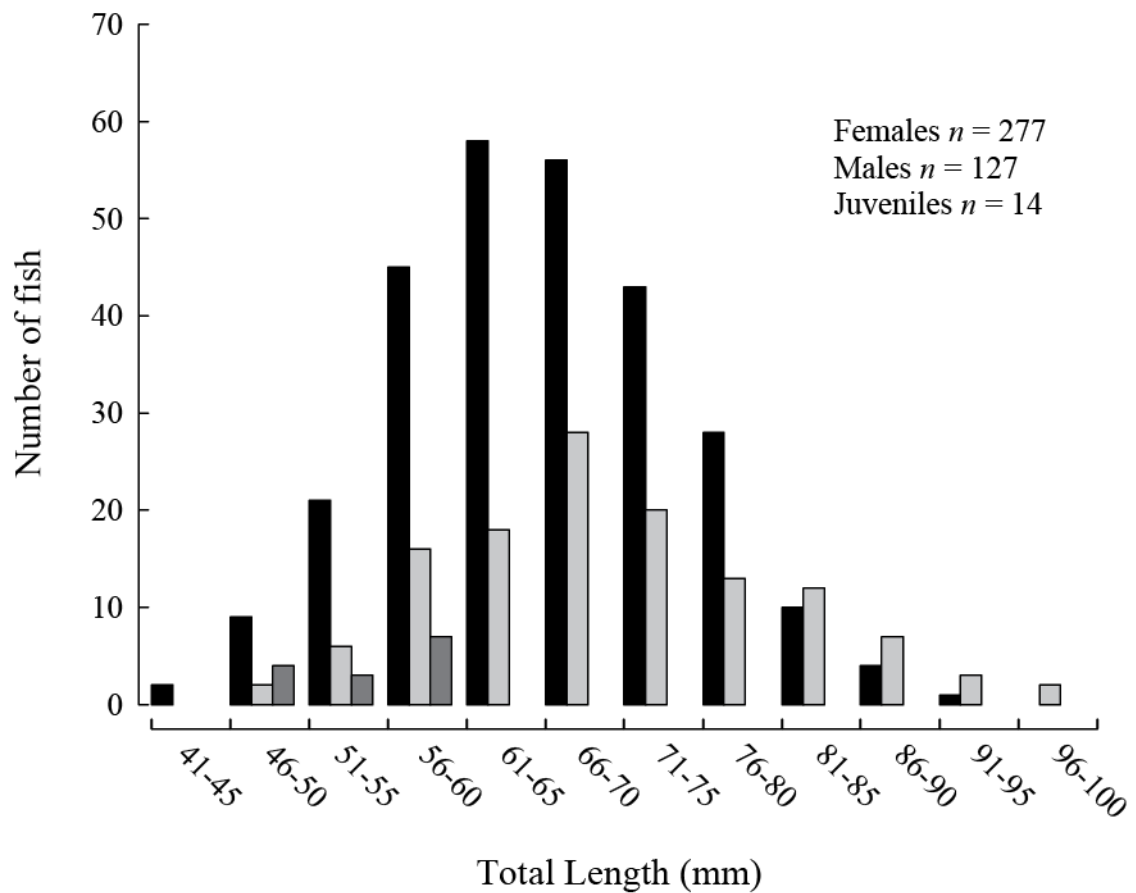


Figure 4.3. Length frequency distributions for female (black bars), male (grey bars) and unsexed (dark grey bars) *Acentrogobius pflaumii* from the Swan-Canning Estuary.

classes (Fig. 4.2). The modal length class for male *A. pflaumii* was 66-70 mm, although relatively uniform frequencies were exhibited by males throughout the length classes between 56 and 75 mm. The average length of all females and males was 66.3 ± 0.5 and 70.4 ± 0.9 mm, respectively (Fig. 4.3).

All 432 individuals obtained during sampling, except one with malformed otoliths, were aged. Of these, 408 individuals were able to be assigned a sex and thus useable in this investigation. The individuals that could be confidently sexed ranged from 0.3 to 3.9 years in age (Fig. 4.4; Fig. 4.6). Samples collected of *Acentrogobius pflaumii* population were dominated by 1+ individuals of both sexes. Female *A. pflaumii* dominated both the 0+ and 1+ age classes with 92 and 147 individuals,

respectively, compared to 32 and 60 individuals, respectively, for males (Fig. 4.3). In the 0+ and 1+ age classes, the ratios of females to males of 2.8:1 and 2.4:1, respectively, were significantly different from parity (both $P < 0.001$, $\chi = 29.0$ and $\chi = 36.6$, respectively). The ratio of females and males in the 2+ age class (1.2:1) was close to parity ($P = 0.5$ $\chi = 0.5$), while of the six fish in 3+ age class, only one fish was female (Fig. 4.4). The overall sex ratio of 2.2 females to 1.0 male is significantly different from parity ($P < 0.001$ $\chi = 55.1$)

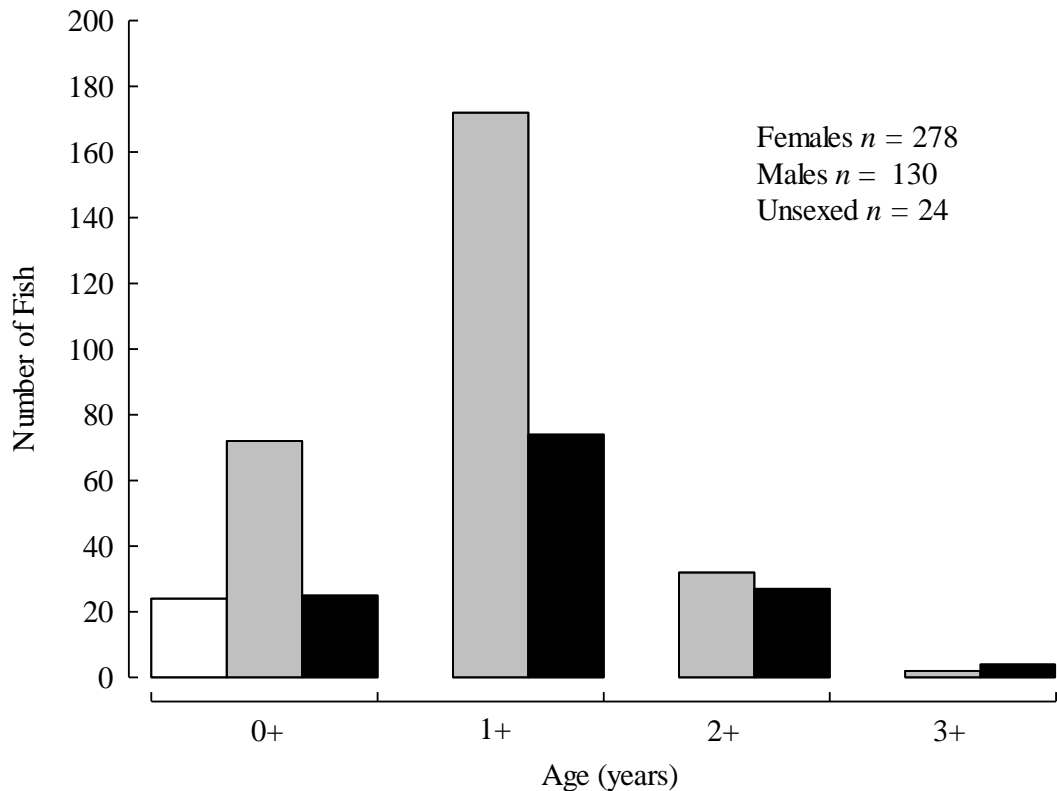


Figure 4.4. Age frequency distributions for female (grey bars), male (black bars) and unsexed (white bars) *Acentrogobius pflaumii* from the Swan-Canning Estuary.

4.4.3. Monthly length frequency distributions

Female *A. pflaumii* in the 0+ cohort were first present in March-May and ranged between 40-49 mm in length (Fig. 4.5). Males in the 0+ cohort, ranging between 50 and

69 mm in length, were first seen in June-July when the abundant female 0+ cohort ranged in length between 45 and 74 mm. The length range of the 0+ cohorts for females and males increased in August-September to range in length from 40-74 mm and 50-74 mm, respectively (Fig. 4.5). The lengths of the 0+ cohort for both sexes did not increase in the following months of October and November, as would be expected for a species that would typically attain a length close to its maximum length by the end of their first year of life. The length range of the 1+ cohort for females and males in February measured between 50 and 74 mm and 65 and 74 mm, respectively, increased marginally over the next 10 months to range in length from 50-84 mm and 45-79 mm, respectively, in November (Fig. 4.5). Due to little or no increase in length in female and male *A. pflaumi* (see later) after the first year of life and even less after the second year of life, the lengths of the 2+ and 3+ cohorts for both sexes caught in some months of the year overlapped with those of the younger age cohorts (Fig. 4.5).

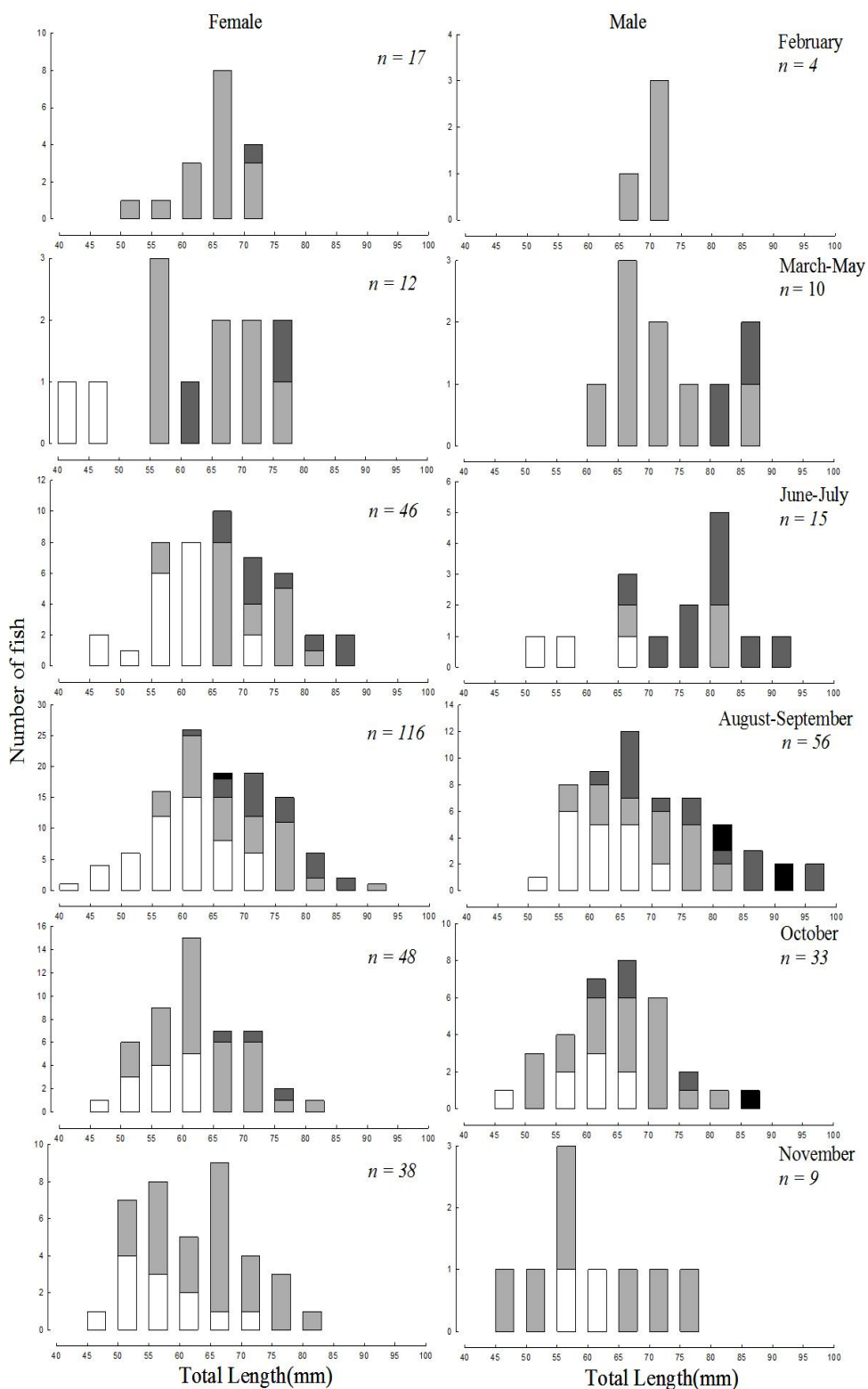


Figure 4.5. Length frequency histograms of 0+ (white bars), 1+ (grey bars), 2+ (dark grey bars) and 3+ (black bars) male and female *Acentrogobius pflaumi* in the Swan-Canning Estuary between July 2014 and August 2015.

4.4.4. Growth

With t_0 fixed at 0.08, the von Bertalanffy growth curves for females and males provided a good fit to the lengths at age for each sex. Although, there was considerable variation in length at age for both sexes, as demonstrated by the large deviations from zero of the standardized residuals (Fig. 4.6.b, d) and the low r^2 values (Table 4.1), there is a relatively even spread of points for the standardized residuals either side of zero indicating the von Bertalanffy growth curves are providing an adequate description of the growth of females and males (Fig. 4.6.a, c). The likelihood ratio test demonstrated that there was a significant difference ($P < 0.001$) between the growth of female and male *A. pflaumii*. Based on the von Bertalanffy growth curves, at 1 year of age, females and males, on average, were 65 mm in length, while at 2 and 3 years of age females were 69 mm and males were 74 mm in length. The growth coefficient (k) values for the von Bertalanffy growth curves for each sex, with t_0 fixed to zero, indicates that both sexes exhibit rapid growth early in life to attain a length close to their L_∞ by the end of their first year of life (Fig. 4.6a, d). A slightly higher k value of 2.74 for females compared to 2.06 for males, suggests marginally faster growth for the former sex.

Table 4.1. von Bertalanffy growth parameters L_∞ , k and t_0 for individual length at age data for *Acentrogobius pflaumii* in the Swan-Canning Estuary. Note, t_0 has been fixed at 0.08, representing an age of 30 days, which is the duration of pelagic phase of *A. pflaumii*, after which fish settle at a length of 5-8 mm in other environments (Lockett and Gomon, 1999; Kanou *et al.*, 2004).

Sex		L_∞	k	t_0	r^2	n
Females	Estimate	69.32	2.74	0.08	0.16	277
	Upper	70.77	3.21			
	Lower	67.94	2.38			
Males	Estimate	74.98	2.06	0.08	0.23	128
	Upper	77.65	2.55			
	Lower	72.56	1.69			

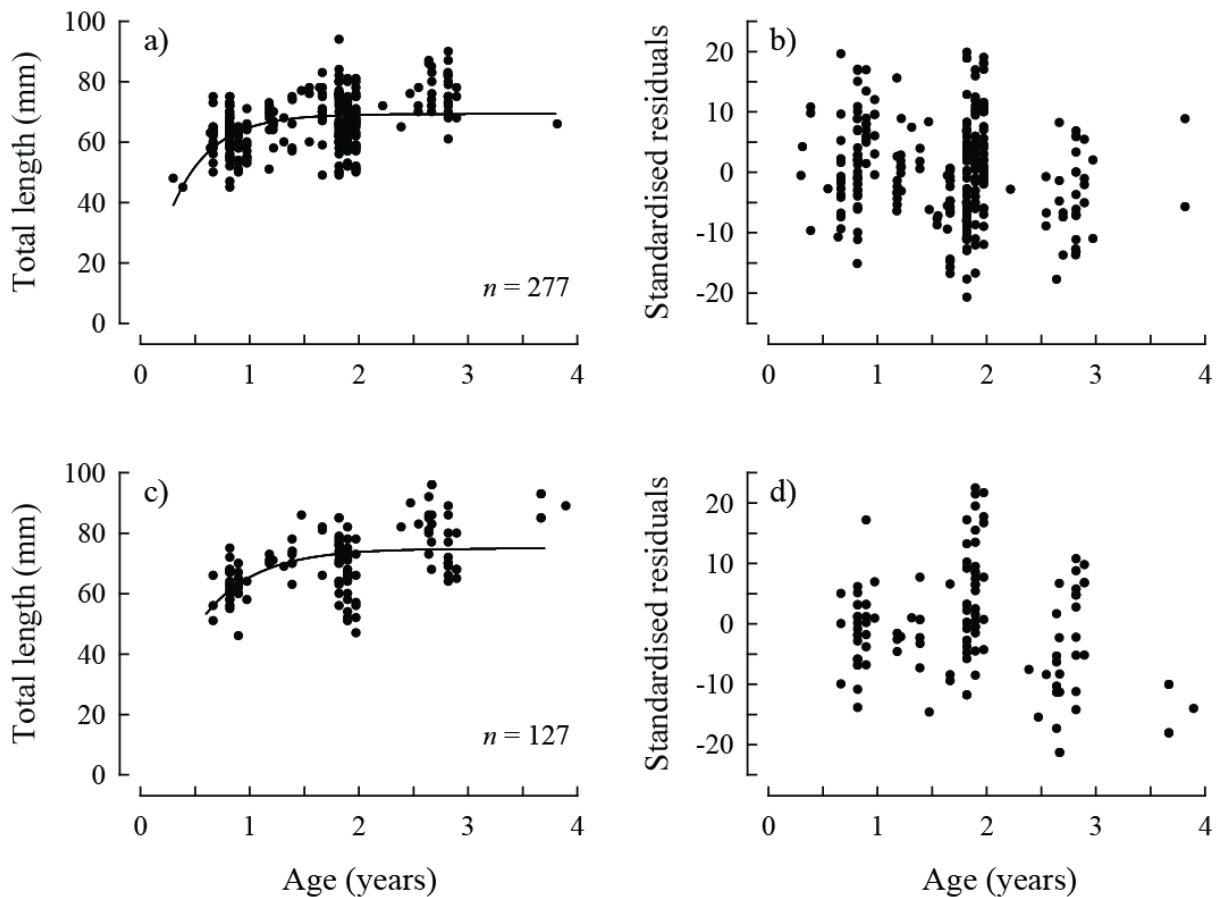


Figure 4.6. von Bertalanffy growth curves fitted to the individual lengths at age of a) female and c) male *Acentrogobius pflaumii*. The standardised residuals for individual length at age data for b) females and d) males are provided.

4.4.5. Timing and duration of spawning and maturation of Acentrogobius pflaumii in the Swan-Canning Estuary

Mean monthly gonadosomatic indices (GSI) for female *A. pflaumii* rose sharply from 1.0 in September to a peak of 4.9 in October and remained elevated in November before declining to 4.2 in February and abruptly decreasing to 1.3 in March (Fig. 4.7). The GSI for females remained low (< 3.0) between April and June. The trend in GSIs for males was similar to that for females, but less pronounced. Male GSIs increased steadily between August and November and attained a maximum value of 1.1 in February, after which the GSI declined rapidly to 0.2 in March and remained at a similarly low level through to June (Fig. 4.7).

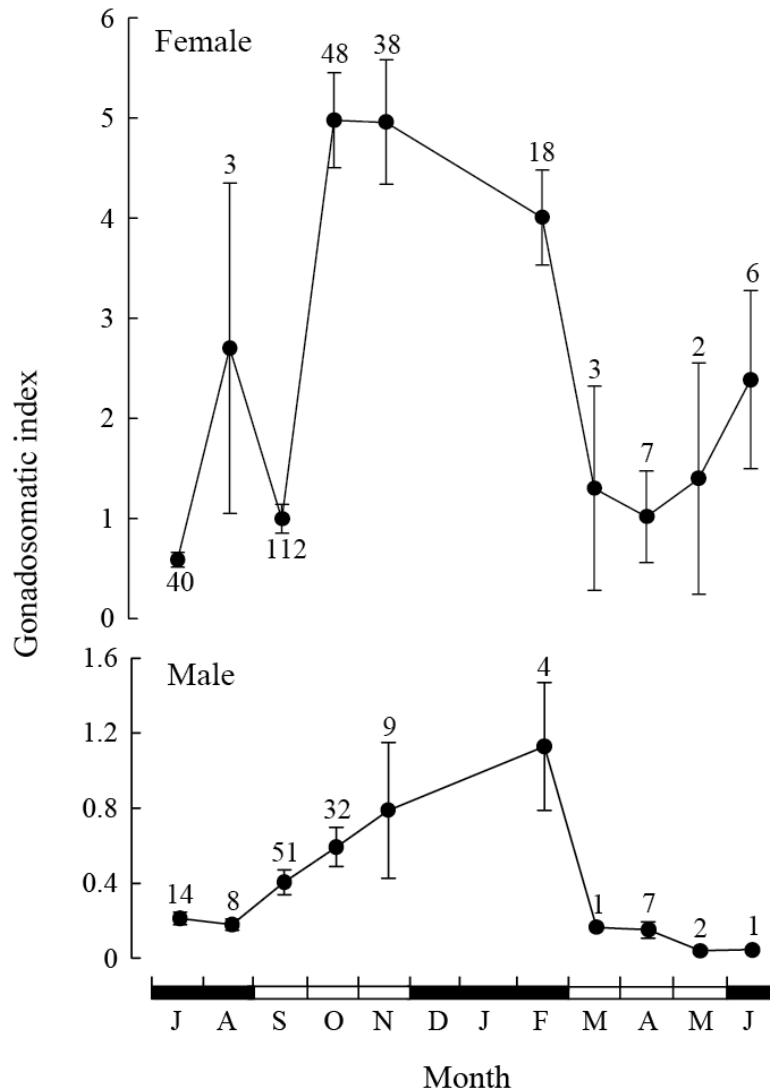


Figure 4.7. Mean monthly gonadosomatic indices \pm 1 SE for male and female *Acentrogobius pflaumii* in the Swan-Canning Estuary.

Macroscopic and histological descriptions of the gonads of female and male *A. pflaumii* are detailed in Tables 4.2 and 4.3, respectively.

In July, female *A. pflaumii* possessed stage II (immature), III (developing) and IV (maturing) ovaries. Of the three females caught in August, two possessed stage IV ovaries (maturing) and the other possessed stage V/VI (mature/spawning) ovaries (Fig. 4.8). Female *A. pflaumii* with stage III, IV and V/VI ovaries were present between

September and November, with the frequency of those with stage V/VI ovaries ranging between 9 and 66%. The peak period of stage V/VI ovaries was in October. Females with stage IV ovaries were present in February, in addition to stage VII (spent) and VIII (recovering spent) ovaries, which were also recorded in March (Fig. 4.8). Although the sample sizes are small in the following months, the data shows that mature (stage V/VI) females are present up until May and resting (stage II) females and those in early stages (III and IV) of maturation are present in June (Fig. 4.8).

The trends exhibited in sequential months by the prevalence of the different stages of teste development of male *A. pflaumi* were similar to those just described for females, with an increasing frequency in the occurrence of stage IV (maturing) testes between July and September and an increasing occurrence of stage V/VI (mature/Spawning) testes between September and November (Fig. 4.8). Although the samples sizes for males in those months between February and June were low, they indicate gonadal senescence in males had commenced by February and gonadal recrudescence has begun in April, May and June (Fig. 4.8).

The trends displayed by the prevalence of the different stages in gonadal development of female and male *A. pflaumi*, particularly those between July and November, indicate that all females and males, within the length ranges sampled in this study, progress through to maturity. This is consistent with the fact that during the main spawning period of October to February, all females and males possessed gonads at stages III (developing) to VIII (recovering spent) (Fig.4.8).

The majority, if not all, females and males caught during the peak spawning period of ~ October to February possessed mature gonads (stages III – VIII). Thus all fish with ages and lengths > 10 months old and 45 mm, respectively, were mature.

Table 4.2. Description of the macroscopic and histological features of the ovaries of female *Acentrogobius pflaumii* throughout stages of development. AF – atretic follicle; EVO – Early vitellogenic oocyte; LU – Lumen; N - Nucleus; P1 – early pre-vitellogenic oocyte; P2 – late pre-vitellogenic oocyte; O – oogonia; OD – oil droplets; OW – Ovarian membrane; VO – Vitellogenic oocyte; YG – yolk granule oocyte ; ZP – Zona pellucida. Note: no histological samples were available for stages III and VII.

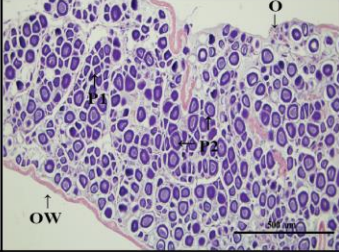
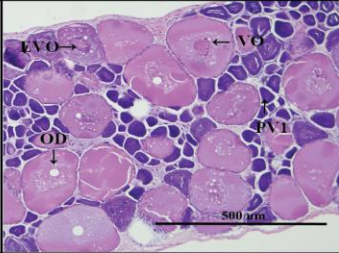

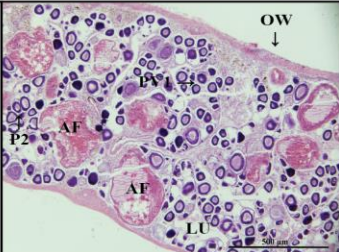
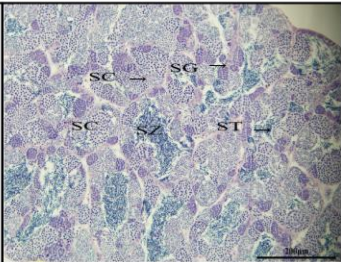
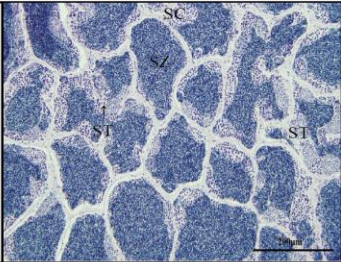
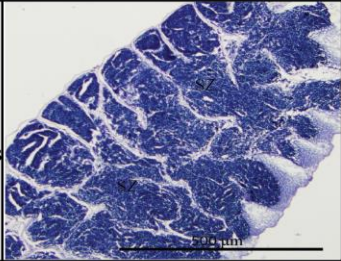
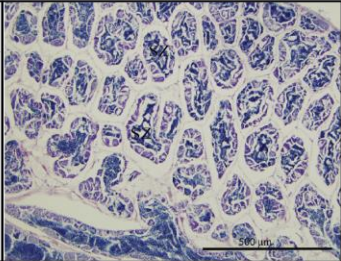
Macroscopic stage	Macroscopic characteristics	Histological characteristics	Histological section
Stage II - <i>Immature</i>	Ovaries small and translucent to opaque in colour, eggs not visible.	Oogonia, initial perinucleolar oocytes and some advanced perinucleolar oocytes present. Oocytes similar in size, abundant and highly organized.	
Stage III - <i>Developing</i>	Ovaries opaque, light orange/yellow or occasionally rose coloured and slightly larger in size than previous stages. Small clusters of eggs just visible, typically starting development towards the ends of each lobe		
Stage IV - <i>Maturing</i>	Ovaries dark orange to light yellow in colour, occupying ~ 1/3 of ventral cavity. Eggs larger and visible to the naked eye, becoming tightly packed	Previtellogenic oocytes still common and vitellogenic oocytes start to appear. oil droplets accumulating	
Stage V /VI- <i>Mature/ Spawning</i>	Ovaries distinctly yellow in colour or occasionally green, occupying 80-90% of ventral cavity. Eggs large, tightly packed, easily discernible to the naked eye, occasionally observed forming yolk plates just prior to spawning. Gonad membrane fragile	Ovaries are tightly packed yolk granule oocytes. Small number of previtellogenic oocytes and occasional cortical alveoli oocyte present.	
Stage VII- <i>Spent</i>	Ovaries opaque orange to rose in colour and ~ 1/3 of the size of stage V ovaries, flaccid in appearance. Mature eggs still visible. Gonad membrane weak and thin.		
Stage VIII - <i>Recovering/ Degenerating</i>	Ovaries orange and translucent in colour, flaccid with a thicker weak membrane. Remnant eggs visible in the form of orange flecks throughout ovaries. Occasional pockets of smaller undeveloped eggs.	Atretic oocytes and scar tissue present. Primary and advanced perinucleolar oocytes common, pre-vitellogenic oocytes sometimes present. Ovary walls thick and oocytes disorganized, some small gaps in lumen	

Table 4.3. Description of the macroscopic and histological features of the testes of male *Acentrogobius pflaumii* testes throughout stages of development. SC – spermatocyte; SG – spermatogonia; ST – Spermatid; SZ – Spermatozoa. Note: no histological samples were available for stages II and VIII

Macroscopic stage	Macroscopic characteristics	Histological characteristics	Histological section
Stage II - <i>Immature</i>	Testes long, thin and strand like, translucent to opaque in colour with a tendency to be “cloudy” in nature. Lobe-like structures start to occur		
Stage III - <i>Developing</i>	Testes typically opaque or translucent not noticeably larger, seminiferous tubules display more organization and clarity. Sperm duct occasionally visible and well developed.	All spermatogenic cell stages present (e.g. spermatogonia, spermatocytes, spermatids and spermatozoa). Primary spermatogonia less common, secondary spermatogonia more so. Spermatocyt and spermatids are the dominant germ stage and spermatozoa increase	
Stage IV - <i>Maturing</i>	Testes small, translucent with white structures or entirely white in colour. Seminiferous tubules and duct more pronounced, tubules occurring at higher densities	Reduced number of spermatogonia. Spermatocytes and spermatids still abundant. Spermatozoa now most abundant cell stage, however there are between seminiferous tubules, which are clearer and not tightly packed as of yet.	
Stage V /VI- <i>Mature/ Spawning</i>	Testes completely white, noticeably larger and longer, ~full length of cavity , occupy 1/3 of ventral cavity. Sperm duct clear, well developed and segmented sperm packets visible. Testes possess scalloped lobe edges and occasionally enlarged capillaries.	Seminiferous tubules enlarged and densely packed with spermatozoa. Some spermatids and spermatocytes may be present. gaps in lobules showing if spawning and lobe membranes less pronounced.	
Stage VII- <i>Spent</i>	Testes white, strandlike and flaccid. Enlarged capillary bundles common	Notable gaps in seminiferous tubules, absence of spermatozoa due to spawning. some spermatogonia occurring in small numbers at periphery, some scar tissue.	
Stage VIII - <i>Recovering/ Degenerating</i>	Range from translucent to opaque, very small and flaccid. No structures visible. Membrane now brittle		

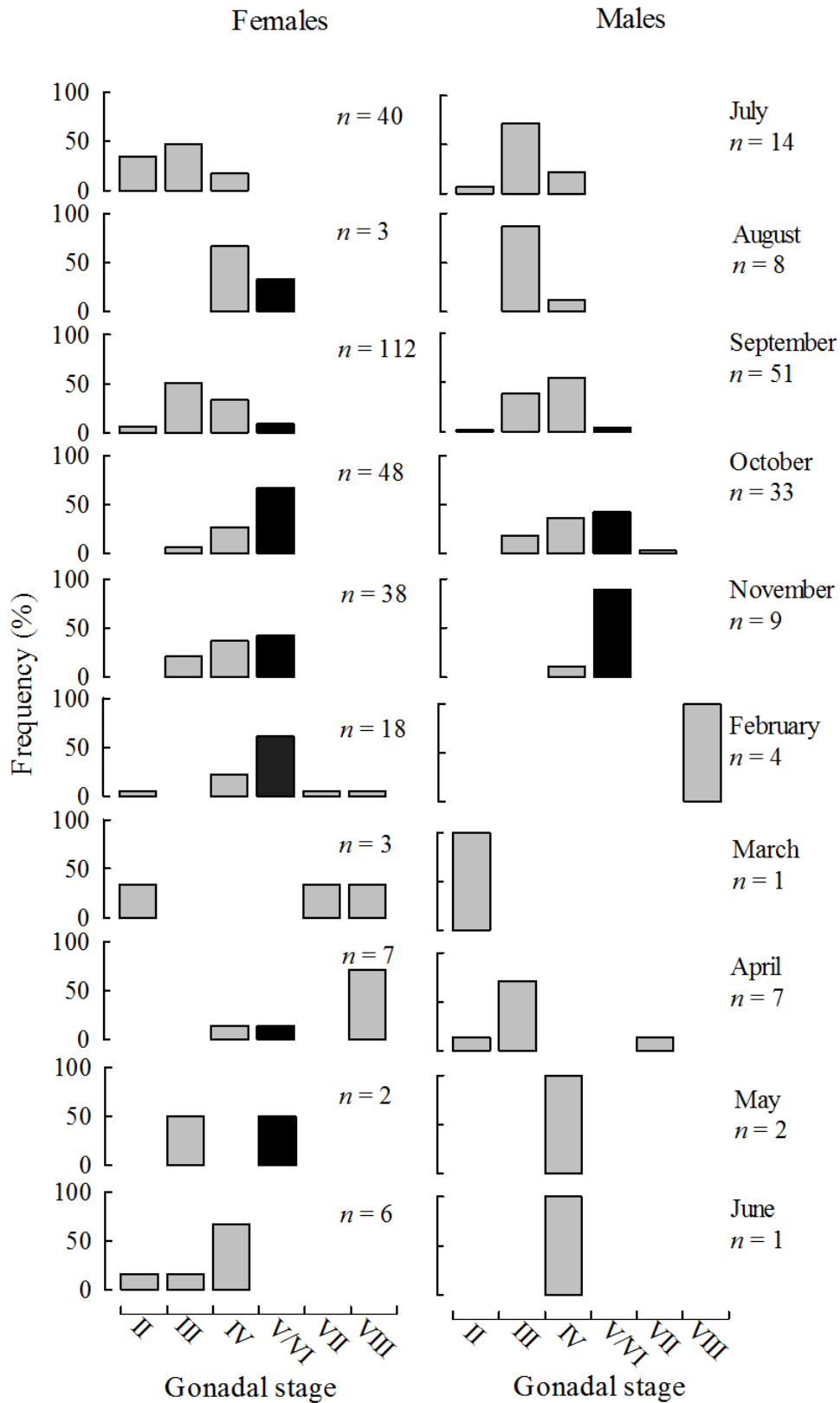


Figure 4.8. Monthly percentage frequencies of occurrence of gonadal stages in female and male *Acentrogobius pflaumi* in the Swan-Canning Estuary. Black histograms represent stage V/VI (mature/spawning) gonads.

4.4.6. Oocyte diameter frequency distributions of Acentrogobius pflaumii

The oocyte diameter frequencies for mature ovaries (stage V/VI) from five female *A. pflaumii* that ranged in length from 67 to 85 mm caught during the spawning period were conspicuously discontinuous (Fig. 4.9). The previtellogenic oocytes (*i.e.* chromatin nucleolar and perinucleolar oocytes) in each case produced a prominent modal class between 41 and 100 μm . No oocytes were measured with diameters between 141 and 320 μm . Yolk granule stage oocytes ranged in diameter from 321 to 480 μm (Fig. 4.9).

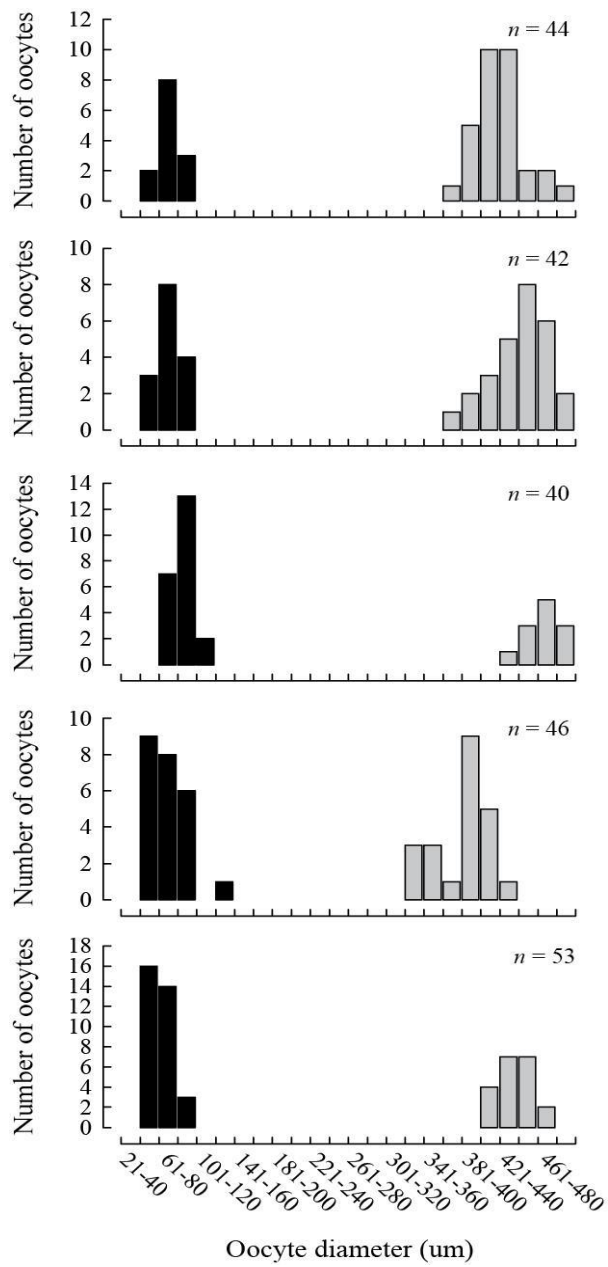


Figure 4.9. Oocyte diameter frequency distributions for ovaries of five mature *Acentrogobius pflaumi*. Previtellogenic (*i.e.* chromatin nucleolar and perinucleolar) oocytes, black bars; Yolk granule stage oocytes, grey bars. *n* = number of oocytes measured.

4.5. Discussion

4.5.1. Age validation, length and age composition and growth

The mean monthly marginal increments for otoliths of *Acentrogobius pflaumi* with 1 and 2-3 opaque zones exhibit a single pronounced rise and decline during a 12 month period (Fig. 4.2), demonstrating that the opaque zones in their otoliths form annually and can thus be used for ageing purposes. The marginal increments for the two otolith groups exhibited a significant increase throughout April-June. Immediately after this period, a substantial decrease in the marginal increment occurred in both otolith groups, suggesting that the opaque zones become delineated during July and the new translucent zone begins forming during the end of winter (July-August) as growth starts to increase with increasing water temperature (Figs. 3.2.a, b). This is somewhat atypical, as opaque zones in the otoliths of most fish in south-western Australia become delineated in late spring to early summer, e.g. black bream *Acanthopagrus butcheri* (Sarre and Potter, 2000), sea mullet *Mugil cephalus* (Smith and Deguara, 2003) and yellow-finned whiting *Sillago schomburgkii* (Hyndes and Potter, 1997). Validation of growth band formation in gobiid species has previously been undertaken using chemical marking of otoliths of fish held in aquaria (e.g. Hernaman *et al.*, 2000; Iida *et al.*, 2010) and marginal increment analysis (e.g. Gumus and Kurt 2009; Sokołowska and Fey 2011; Hajji *et al.* 2013). These results demonstrate a viable method for age validation in species, such as the estuarine goby *Pseudogobius olorum* which also occurs in the Swan-Canning Estuary (Gill *et al.*, 1996), for which other ageing techniques, such as length frequency analysis could not be used, or in those species whose otoliths do not exhibit annually formed growth zones (e.g. Iglesias *et al.*, 1997; Hernaman *et al.*, 2000; Taillebois *et al.*, 2012).

In the Swan-Canning Estuary, female and male *A. pflaumi* attain maximum lengths of 94 and 96 mm, respectively. These maximum sizes are much larger than

those of 72 and 80 mm for *A. pflaumi* in their native habitats in South Korea and Japan, respectively, and also than that of 75 mm in New Zealand, where *A. pflaumi* is an invasive species (Masuda *et al.*, 1984; Francis *et al.*, 2003; Baeck *et al.*, 2004). However, it must be noted that *A. pflaumi* have been shown to attain a maximum size of 109 mm in waters in China (Wang *et al.*, 2015). The lack of smaller individuals caught during this study suggests that either the sampling methods were not capable of capturing *A. pflaumi* with lengths < 40 mm, or that small *A. pflaumi* are not present in the lower reaches of the Swan-Canning Estuary. However, as otter trawling did catch other small fish and prawns (*i.e.* < 40 mm), and hand trawling in shallow waters did not catch any *A. pflaumi* (see Chapter 3.4.1), it is most likely that the small individuals were not present in the habitat sampled during this study. Interestingly, like this study, no small *A. pflaumi* were caught in the established population in New Zealand (Francis *et al.*, 2003), although small individuals of *A. pflaumi* were caught during another study in the Jiulong River Estuary, China, in which surface and mid-water trawls were employed to sample larval fish composition. The findings from this study indicated that *A. pflaumi* was the most abundant fish species overall, composing 28 and 40% of the surface and mid-water fish, respectively. Furthermore, the *A. pflaumi* captured ranged from 12-86 mm (Nan *et al.*, 2009), suggesting *A. pflaumi* has a more mobile lifestyle than was previously expected for a small, territorial benthic fish. As such, this could potentially explain the absence of small *A. pflaumi* in the present study in which the sampling regime was solely designed to target the benthos, therefore possibly inadvertently missing the small individuals of *A. pflaumi*. Further possible explanation is the tendency of larval *A. pflaumi* to settle in seagrass beds in native regions (Horinouchi and Sano, 2001; Kanou *et al.*, 2004). The lack of suitable equivalent habitat in Swan-Canning Estuary and the existence of extensive seagrass beds just to the south

of this Estuary in Cockburn Sound, where *A. pflaumi* was first identified (Maddern and Morrison, 2009) may provide an alternate habitat for the early juveniles *A. pflaumi*. Thus, in order to accurately describe the growth of *A. pflaumi* throughout their life and, in particular, during the early stages, a similar sampling method or alternative sampling regions may need to be considered.

The population of *A. pflaumi* in the Swan-Canning Estuary sampled during this study was dominated by 1+ individuals and, to a lesser extent, 0+ individuals, with very few individuals > 1 year old present (Fig. 4.5). Gobiid species allocate a significant amount of energy into reproduction, not only for the production of eggs and sperm, but also nest building and guarding (*e.g.* Kvarnemo *et al.*, 1998; Lindström, 1998; Olsson *et al.*, 2009). The rapid decline in the abundance of 2 and 3+ individuals of *A. pflaumi* suggests that the energy cost of two spawning periods (*i.e.* at the end of the first and second years of life) result in high natural mortality rates which is consistent with the high post-reproductive mortality described for other Gobiid species, greatly reducing the abundance of older fish (Jude *et al.*, 1998; Caputo *et al.*, 2003; La Mesa *et al.*, 2005).

Based on the von Bertalanffy growth curves, at one year of age, females and males, on average, attain a length of ~ 65 mm, which is equivalent to ~94 and 87% of the estimates of L_{∞} for each sex, respectively. The rapid growth displayed by *A. pflaumi* in the Swan-Canning Estuary is consistent with this species in Gwangyang Bay, South Korea, and with other longer-lived (max. age >2 years) gobiid species, such as *Gobius vitattus*, which are known to obtain >50% of their maximum size within the first year of life (Baeck *et al.*, 2004; Kovačić, 2006). Additionally, five species of short-lived (max age <16 months) gobiid species were found to reach 2/3 maximum size within half of their expected maximum lifespan (Hernaman and Munday, 2005). The

rapid growth exhibited by *A. pflaumi* and other Gobiid species within the first year of the life in order to attain a large body size quickly is thought to positively affect reproductive output because of the direct effects of body size on fecundity and competition over resources, such as food, nest sites and mates (Hernaman and Munday, 2005).

There is a large variation in the length at age for females and males of *A. pflaumi* in the Swan-Canning Estuary. The large variation in growth, in part, can be attributed to the fact that *A. pflaumi* possibly spawns over a very extended period (*i.e.* August to February) that enable fish within the same cohort to be spawned, potentially, up to seven months apart, and thus commencing life and growth when environmental conditions could be very different. For example, individuals spawned late winter and early spring (*i.e.* August-September) will have the entire following summer in which to grow before entering the next winter. In contrast, fish spawned in late summer (*i.e.* February), but within the same spawning period, have only a few months of warm conditions in which to grow before entering winter. Large variability in the length at age of the 0+ cohort, dependant on time of spawning and the nature of the immediate growth period, is a known phenomenon. For example, in Kuibyshev Reservoir, Russia, the round goby *Neogobius melanostomus* exhibits a protracted spawning season which results in individuals of the 0+ cohort that were spawned in May (late spring) attaining a size of 111mm in one year, compared to only 42mm for those spawned in July (mid-summer) (Shemonaev and Kirilenko, 2009).

As no specimens <40 mm and <0.3 years old were obtained throughout the sampling period, the description of growth early in life was not possible and the resultant t_0 estimates, without fixing t_0 , highly negative and unrealistic. Thus, t_0 was set at a length of 8 mm, the length at which *A. pflaumi* are known to settle after a pelagic

larval phase of 30 days (Lockett and Gomon, 1999; Kanou *et al.*, 2004) in order to produce biologically meaningful von Bertalanffy growth curves. While the information yielded by the growth curves should be treated with some amount of caution, the resultant curves suggested that, on average, males obtained a larger size than females resulting in a higher L_{∞} estimates for males (77.35 mm) than females (72.9mm). However, females exhibited faster growth than males as demonstrated by the higher k value of 2.191 year⁻¹ compared to 1.81 year⁻¹ (Table 4.1). Sex-based differences in growth rate and size has been noted in several goby species and has been attributed to differences in the cost of gamete production, parental care, mating systems and sexual selection (Hernaman and Munday, 2005). The larger maximum sizes displayed by male *A. pflaumi* is a typical feature of most gobiid species, such as the transparent goby *Aphia minuta*, grass goby *Zosterisessor ophiocephalus* and round goby *Neogobius melanostomus* (La Mesa, 1999; Gumus and Kurt, 2009; Hajji *et al.*, 2013). As gobiids are typically polygynous, the larger size attained by males is thought to aid in attracting multiple female mates and in the parental care activities, such as guarding and fanning nests (Miller, 1984; Lindström and Hellström, 1993; Hernaman and Munday, 2005). Furthermore, the parental care provided by male gobiids is so intrinsically linked to reproductive success that some species display sexual selection towards males that display high levels of parental care (*e.g.* Pampoulie *et al.*, 2004; Lindström *et al.*, 2006), hence possibly explaining the predisposition for larger males than females in gobiid species. Findings by Hernaman and Munday (2005) support this view, where three monogamous gobiid species studied displayed no substantial difference in size between sexes, supposedly due to the lack of interspecific competition for mates and nesting sites characteristic of these species.

Rapid growth in small fishes in general is suggested to be facilitated by a tendency to transition in preference for dietary items ontogenetically, thus utilising alternative and more beneficial food sources (McCormick, 1998). Indeed, *A. pflaumi* has repetitively displayed a tendency to progress from consuming amphipods to polychaetes with increasing size (e.g. Kanou *et al.*, 2004; Horinouchi, 2008). However, the rapid growth displayed by *A. pflaumi* is not unique. Recent studies on smaller gobiid species indicate rapid, linear growth patterns (e.g. Kritzer, 2002; Hernaman and Munday, 2005; Winterbottom *et al.*, 2011), several of which possess higher growth rates than *A. pflaumi*, such as *Eviotta epiphanies* ($k = 7.68$), *Gobius vittatus* ($k = 2.76$) and *P. olorum* ($k = >7$) (Grant, 2013; Kovacic, 2007; Gill *et al.*, 1996). Larger, longer-lived gobiid species display a tendency to possess comparatively slower growth rates (i.e. $k = <1$). For example, the round goby *N. melanostomus* and the yellowfin goby *Acanthogobius flavimanus* attain lengths of 236 and 432 mm respectively and growth rates (k) of 0.11 and 0.31 respectively (Kwak *et al.*, 2009; Grul'a *et al.*, 2012). It should be noted, however, that *A. pflaumi* possesses a growth rate greater than some goby species which are both smaller and shorter lived, such as the transparent goby *Aphia minuta* which lives for less than 8 months and attains a maximum size of 44 mm with a growth rate (k) of 1.68 (Iglesias *et al.*, 1997). Thus, while there is evidently variability in the relationship between growth rate and size in gobiid species, it seems that *A. pflaumi* possesses a much higher growth rate than several other gobies, with the exception of the smallest and shortest lived species. However, the paucity of validated data concerning growth rate in gobiid species in addition to difficulties conforming typical von Bertalanffy growth models to the shorter lived species (e.g. *Eviotta sigillata*; Depczynski and Bellwood 2006) makes this difficult to confirm.

4.5.2. Time and duration of spawning and sex ratios

The trends exhibited by the GSI and prevalence of fish with mature/spawning (stage V/VI) gonads, particularly females, demonstrate that *A. pflaumi* in the Swan-Canning Estuary is capable of spawning over 10 months of the year from late winter (August) to late autumn (May) (Fig. 4.8). However, the high prevalence of females with stage V/VI (mature/spawning) ovaries in November, December and February, as well as the first occurrence of females with stage VII and VIII (spent and recovering) in February indicate that spawning takes place mainly between November (mid-spring) and February (late summer). In its native habitat of Gwangyang Bay, South Korea, *A. pflaumi* spawns over a similar time period, from April (early Spring) to June (early Summer) (Baeck *et al.*, 2004), which is consistent with this environment being at a similar latitude north (*i.e.* 34°N), as the Swan-Canning Estuary is south (*i.e.* 32°S) of the equator, and thus changes in environmental conditions occurring on the same seasonal time scale. The commencement of spawning in late winter, when water temperature is close to its minimum is consistent with the trends proposed by Lam (1983) for fish species in temperate regions.

The timing of the spawning period displayed by *A. pflaumi* in the Swan-Canning Estuary is also consistent with other native species in this estuary such as yellowtail flathead *Playcephalus westraliae* (Coulson, 2008), the western gobbleguts *Apogon rueppelli* (Chrystal *et al.*, 1985), the atherinids *Atherinosoma presbyteroides*, *Allanetta mugiloides*, *Atherinosoma wallacei* (Prince and Potter 1983) and the estuarine goby *P. olorum* (Gill *et al.*, 1996). Fish fauna of south-western Australian estuaries typically spawn during late spring and summer as the conditions present during this time, *i.e.* limited tidal action and negligible freshwater input, result in a highly stable environment conducive to spawning and, presumably, growth and survival of progeny

(*e.g.* Potter and Hyndes, 1999). Furthermore, most fish species in estuarine environments in this region display adaptations to reduce the impact of atypical water movements during this time period by encouraging the retention of eggs and larvae within the estuary (Tweedley *et al.*, *in press*). This is achieved via various methods, such as the production of demersal or benthic eggs, *e.g.* the sparid *Acanthopagrus butcheri* and the tetrapontid *Amniataba caudavittata*, egg guarding, *e.g.* the gobiids *A. bifrenatus* and *P. olorum* and mouth brooding, *e.g.* the cardinalfish *Apogon rueppelli* (Chrystal *et al.*, 1985; Gill *et al.*, 1996; Potter and Hyndes, 1999).

The maximum mean monthly GSI for female *A. pflaumi* in the Swan-Canning Estuary is approximately four times greater than that of their males. The large differences in the mass of the ovaries and testes suggest that this species is spawning in pairs, which would be consistent with the reproductive strategy of gobiids (*e.g.* Hernaman and Munday, 2005) and other marine fish species, such as those of certain serranids (Sadovy, 1996; Marino *et al.*, 2001; Moore *et al.*, 2007). The reduction in energy allocated to reproduction in male gobiids is thought to stem from a reduced requirement for sperm due to increased fertilisation rates that accompany laying eggs in nests. For example, the round goby *N. melanosomus*, successfully fertilizes ~95% of eggs in the nest, which may be upwards of 10,000 (Charlebois *et al.*, 1997a). It should also be noted that in South Korea, *A. pflaumi* exhibited a much higher mean GSI value of 13.8 during peak spawning times (Baek *et al.*, 2004), thus suggesting further reproductive potential for *A. pflaumi* in the Swan-Canning Estuary.

The distribution of oocyte diameters in the mature ovaries of five female *A. pflaumi* exhibited two discrete groups of oocytes, *i.e.* previtellogenic and yolk granule oocytes, and no intermediate oocyte stages (*e.g.* cortical alveoli). The histological sections of those ovaries revealed that yolk granule oocytes were tightly packed within

the ovary, with very few pre-vitellogenic oocytes present. The presence of a major interval in the oocyte size classes provides evidence to support the conclusion that *A. pflaumii* exhibits determinate fecundity (Hunter and Macewicz, 1985). It is unknown, however, whether all eggs are released in a single batch or if multiple batches are released throughout the spawning period. *Acentrogobius pflaumii* in Gwangyang Bay, South Korea, were suggested to spawn only once per year due to observations of consistently homogenous oocyte sizes (Baeck *et al.*, 2004). Considering that the spawning period of *A. pflaumii* in the Swan-Canning Estuary potentially extends over 10 months, it would be highly unlikely that *A. pflaumii* releases a single batch, but instead releases multiple batches throughout the spawning season. Such serial batch spawning occurs in other goby species, such as the round goby *N. melanostomus*, which may produce a new batch of eggs up to 6 times, or every 20 days, during its protracted spawning season (Jude *et al.*, 1998).

Female *A. pflaumii* in the Swan-Canning Estuary occurred more than twice as frequently as males overall, a trend which was also exhibited by this species in its natural distribution within Kwangyang Bay, South Korea, *i.e.* 1:0.59 (Baeck *et al.*, 2004). Other gobiid species also exhibit skewed operational sex ratios (OSR), both male biased (*e.g.* round goby *N. melanostomus*; Gutowsky and Fox, 2011) and female biased (*e.g.* Red Sea goby *Silhouettea aegyptia* and garbled goby *Pomatoschistus marmoratus*; Fouda *et al.*, 1993). The female biased OSR displayed by *A. pflaumii* in the Swan-Canning Estuary suggests that males do not have to compete for females and that the greater presence of females may have resulted from stronger selection pressures for optimising the effectiveness of the role of male *A. pflaumii* in spawning (Kvarnemo and Ahnesjo, 1996; Kokko and Monaghan, 2001). In contrast, male biased sex ratios are exhibited by *N. melanostomus* in which there is an aggressive size based hierarchy of female courtship and nest guarding (Jude *et al.*, 1998; Gutowsky and Fox, 2011).

4.5.3. Conclusion

In summary, marginal increment analysis confirmed the annual periodicity of opaque band formation in the otoliths *A. pflaumii*. As a result, the otoliths of *A. pflaumii* can successfully be used to age this species. Subsequent investigation into the age composition revealed that the *A. pflaumii* population in the Swan-Canning Estuary was dominated by 1+ individuals, but also contained individuals over three years in age, however the rarity of these individuals suggests a high mortality and population turnover rate consistent with other goby species. Female and male *A. pflaumii* attain 94 and 87% of the L_{∞} in their first year of life, suggesting *A. pflaumii* exhibits impressive growth for its size. Indeed, only small, exceptionally short lived gobiid species exhibit higher growth rates. However, growth during subsequent years was negligible, suggesting a shift in energy allocation from somatic growth to reproduction after the first year of life. This is consistent with the observed maturation within the first year of life displayed by the South Korean population of *A. pflaumii*.

The mean monthly GSI values and prevalence of females and males with gonads at different stages of development identified November-February as the main spawning period for *A. pflaumii*, although the spawning period is protracted possibly occurring over 10 months of the year. Oocyte diameter frequencies of mature females indicate that *Acentrogobius pflaumii* exhibits determinate fecundity. Considering the possible protracted spawning season, it is thus proposed that *A. pflaumii* in the Swan-Canning Estuary spawns more than once during the season, which contrasts with *A. pflaumii* in its native habitat. Females and males mature by the end of their first year of life, when on average, they are 65 mm in length. If future investigations into *A. pflaumii* take place, it is recommended that alternative sampling methods, such as surface and mid-water towing be employed in addition to otter trawling in order to obtain juvenile fish, which are required to increase the understanding of life history characteristics.

Chapter 5: Conclusions

5.1. Summary

Non-indigenous species can have potentially significant deleterious impacts on the ecosystems in which they become established. In order to reduce the impact of these species on the native flora and fauna, management agencies require biological and ecological information on new arrivals in order to formulate appropriate objectives and management plans for mitigating and reducing their impacts. The establishment of the Striped Sandgoby *Acentrogobius pflaumi* in the Swan-Canning Estuary in southwestern Australia follows previous successful establishments in Port Phillip Bay in Victoria (Australia) and Waitemata and Whangapoua Harbours in Auckland (New Zealand). Given that this species, once established, proliferated throughout Port Phillip Bay, becoming the most abundant fish species, there is an urgent need to determine the extent of its population in the Swan-Canning Estuary, identify the native species it may compete with and understand its biological characteristics to help determine its threat and likelihood to colonise other systems.

An extensive sampling program was undertaken on 21 ‘monthly’ occasions between January 2014 and September 2015 at 20 nearshore, shallow sites (< 2 m deep) and at 16 offshore, deeper sites (2-17 m deep) in the Swan-Canning Estuary to elucidate the spatial and temporal patterns of abundance and distribution *A. pflaumi* (Chapter 3). The results demonstrate that this species does not occur in the sandy shallow waters of the estuary, inhabited by native gobiid species such as *Favonigobius punctatus* and *Favonigobius lateralis*. Substantial numbers of *A. pflaumi*, however, were recorded from the deeper waters, where it comprised 55% of the total number of gobies. This indicates that *A. pflaumi* prefers soft sediments, with greater proportions of particulate organic matter and small inorganic grain sizes.

Densities of *A. pflaumi* declined progressively in an upstream direction, thus while this species dominated the gobiid fauna to a remarkable extent in Lower Melville Water (~98%), its contributions declined to 31%, 17% and 0.2% in Upper Melville

Water, Lower Canning Estuary and Middle Swan Estuary, respectively. This spatial pattern indicates that *A. pflaumii* exhibits a preference for waters with a salinity close to that of full strength sea water. The spatial distribution of *A. pflaumii* overlaps with that of two native species of gobiid, *Arenigobius bifrenatus* and *Pseudogobius olorum*, particularly in Upper Melville Water and the Lower Canning Estuary and it remains to be seen whether it will come to dominate the fauna in these regions as it does in Lower Melville Water.

Biological analysis of individuals of *A. pflaumii* obtained between July 2014 and September 2015 determined the size and age compositions, growth and reproductive biology of this species (Chapter 4). Size and age compositions, calculated from counts of annually formed opaque zones in whole otoliths, determined that the oldest *A. pflaumii* collected from the Swan-Canning Estuary was 3.9 years old and 89 mm in total length and that, while the population contains 2-3+ fish, it is dominated by 1+ individuals. The relatively low abundances of individuals > 2 years old indicates a high population mortality and turn over rate presumably as a result of spawning related stress and predation. Male and female *A. pflaumii* attained > 87% of their asymptotic lengths (L_{∞}) of 74.9 and 69.3 mm respectively during the first year of life. The high instantaneous growth rate of both male and female *A. pflaumii* (k) is characteristic of smaller, shorter lived species of fish.

The results from gonadosomatic indices and histological examination of gonads suggest that *A. pflaumii* is able to spawn throughout most of the year, but exhibits a peak in spawning from November to February. The presence of mature, spawning and depleted gonads in *A. pflaumii* suggests that this species spawns within the Swan-Canning Estuary. Further studies to investigate the potential presence of pre- and postflexion larvae would be valuable for testing this hypothesis. Although there is no information concerning the nature of early life history of *A. pflaumii* in this system, young of the year (0+) began to recruit to the population at ~40 mm between March and May. High levels of variability in size-at-age were found in the population, probably a

result of the extended spawning season, possible multiple spawning episodes during the season and a high growth rate.

5.2. Management implications and future work

Competition among species is known to occur on three axes, space, time and food resources (Ross, 1986). The spatial and temporal patterns of distribution and abundance of *A. pflaumi* within the Swan-Canning Estuary determined in this thesis are of particular significance to management bodies. On the basis of these results, the main part of the *A. pflaumi* population appears to be restricted to the most downstream areas, *i.e.* Lower Melville Water, which experiences salinity levels similar to that of full strength sea water. Furthermore, it is notable that this region experiences relatively stable salinities throughout the year, suggesting that *A. pflaumi* does not have the osmoregulatory abilities of some of the native gobiid species in the system, such as *Pseudogobius olorum* and *Arenigobius bifrenatus*, which are highly euryhaline and penetrate into the upper parts of the estuary. Determining the acute and gradual salinity preferences and tolerances of *A. pflaumi*, together with co-occurring species *e.g.* *F. lateralis* and *A. bifrenatus*, would be valuable. The values obtained for *A. pflaumi* from such an experiment would elucidate whether the hypothesis that the relatively localised distribution within the Swan-Canning Estuary is the result of an inability to osmoregulate successfully in reduced salinities.

Based upon the spatial pattern of distribution within the estuary and the fact that mature, spawning and depleted gonads of *A. pflaumi* were recorded within the estuary, it is suggested that this species belongs to the estuarine & marine guild (*sensu* Potter *et al.*, 2015; Fig. 5.1). Thus, like *F. lateralis*, another estuarine & marine species, *A. pflaumi* is a species that contains populations in which the individuals can complete their life cycles within the estuary, but which are also represented by populations in marine environments, such as Cockburn Sound. As mentioned earlier, recording the location of pre- and postflexion larvae would be valuable for confirming this classification.

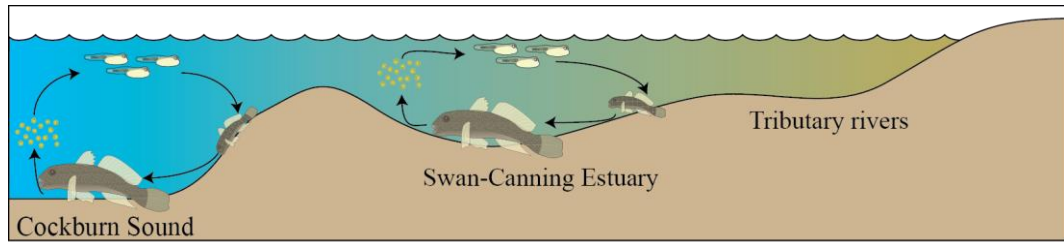


Figure 5.1. Conceptual model showing the suggested lifecycle of *Acentrogobius pflaumii* in south-western Australia. *Acentrogobius pflaumii* is considered to be an estuarine & marine species, where populations contain individuals that complete their life cycles within the estuary, but which are also represented by populations in either marine environments. Modified from Potter *et al.*, (2015).

Acentrogobius pflaumii exhibits a fast growth rate, which is faster than that of a variety of gobiids worldwide. Limited information exists on the biological characteristics of native gobiids present in the Swan-Canning Estuary. For example, age and growth information is only available for *P. olorum*, thus restricting the ability to comment on the potential for *A. pflaumii* to physically outcompete native goby species. It is relevant, however, that the protracted spawning season of *A. pflaumii*, *i.e.* potentially occurring between April - February, overlaps with that of the native gobiid species such as *P. olorum*, which spawns in spring and again in autumn months (Gill *et al.*, 1996). However, work by Gill and Potter (1993) suggests that the seven other gobiid species present within this system exhibit marked spatial segregation, which facilitate the partitioning of resources and thus reduces competition among species. Moreover, in New Zealand, distributions of *A. pflaumii* and *A. bifrenatus* within two harbours were shown not to overlap. While *A. pflaumii* dominated the gobiid faunas of Lower Melville Water to a remarkable extent, there were regions of the Swan-Canning Estuary where this non-indigenous species co-occurred in substantial numbers with *P. olorum* and *A. bifrenatus*. While, studies of the diets of the native gobies show that there is little overlap in dietary preferences (*e.g.* Gill and Potter, 1993; Humphries and Potter, 1993), it is not possible to comment on the influence of *A. pflaumii* without undertaking a study of its diet. It is thus relevant that, in its native waters, *A. pflaumii* is known to consume large quantities of polychaetes (Horinouchi and Sano, 2000; Horinouchi,

2008), which is similar to the diet of *A. bifrenatus*. Thus, it is recommended that studies of the diet of *A. pflaumii* in the Swan-Canning Estuary be undertaken along with that of *A. bifrenatus* and other species, as this would provide a clearer picture of any interspecific competition.

Based on the data in this thesis the rapid growth and extended spawning period of *A. pflaumii* appear to predispose it to overcome hostile interactions and successfully colonise new areas. Within these environments, *A. pflaumii* displays a tendency to inhabit soft sediment in the lower reaches of estuaries. As this environment is present in the vast majority of south-western Australian estuaries, other systems such as the Peel-Harvey Estuary and Leschenault Inlet may also have been colonised. It is thus recommended that *A. pflaumii* be placed on a species watch list for these areas and, if possible, presence/absence sampling be undertaken. Given that this species is not found in adjacent shallow waters, such surveys would need to sample the offshore, deeper waters using a fine otter trawl (as in this study) or using SCUBA (as in Maddern and Morrison, 2009). These methods are preferable over the traditional method of gill nets, as this method has been used regularly in the Swan-Canning Estuary in recent years, as part of an ongoing monitoring program to calculate the health of the estuary, but has failed to detect this species.

In conclusion, the invasions of multiple estuarine and coastal environments in both Australia and New Zealand demonstrate *A. pflaumii*, a native of Japan and Korea, is able to survive in ballast water and colonise foreign aquatic environments upon introduction and thus demands attention from management bodies. The biological and ecological information on *A. pflaumii* detailed in this thesis will be invaluable in assessing the threat of this species to estuarine and coastal environments in south-western Australia and, where appropriate, targeting management responses. While the overall ecological impacts of *A. pflaumii* on native gobiids were unable to be determined, the ability of this species to rapidly colonise new areas suggests that a precautionary approach to management should be utilised until its impacts are defined. Thus, preventing the spread of *A. pflaumii* into other systems should focus on

identifying areas at risk of colonisation, based on their location to existing populations and provision of suitable habitats and then minimising this risk through vector control, with a focus on managing shipping activities. Finally, in order to increase the applicability of this information for use in risk assessments and control activities it is recommended that further work be undertaken on (i) salinity preferences and tolerances, (ii) surveying nearby systems with similar habitats to the deeper waters of the downstream reaches of the Swan-Canning Estuary and (iii) determining the diet of *A. pflaumii* and comparing it to co-occurring species such as *A. bifrenatus*.

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