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A preliminary investigation of the potential impacts of the proposed Kwinana Quay development on the commercially and recreationally important fish and crab species in Cockburn Sound

Prepared for Fremantle Ports

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A preliminary investigation of the potential impacts of the proposed Kwinana Quay development on the commercially and recreationally important fish and crab species in Cockburn Sound

Prepared for the Fremantle Ports

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Non-technical summary

Cockburn Sound is the largest of the very few protected marine embayments along the lower west coast of Western Australia. It has been recognised as playing an integral role in the life history strategies of many marine species, including the highly valued snapper *Pagrus auratus* and blue swimmer crab *Portunus pelagicus*. Currently, the adult stocks of snapper in the West Coast Bioregion and blue swimmer crabs in Cockburn Sound are at depleted levels, most likely a result of high fishing pressure and below average recruitment in recent years for both species.

Due to increasing shipping operations and limited infrastructure the Port of Fremantle will soon be working at capacity. Thus, to alleviate this situation an additional harbour has been proposed in Cockburn Sound, to be located on the eastern margin north of James Point. This outer harbour development has been named 'Kwinana Quay'. As part of the requirements of the Environmental Protection Agency of Western Australia, the potential impacts of this development on the environment and marine fauna in this embayment need to be assessed. The information provided in this report will aid in the environmental impact assessment to be undertaken by GHD and Oceanica.

This report represents a preliminary investigation into the potential impacts of the Kwinana Quay development on commercially and recreationally important fish and crab species. It needs to be considered that this report does not address any broader biodiversity implications. The studies outlined in this report aimed to establish methods useful for assessment and to provide one year of sound quantitative data for future comparisons. The key points of consideration and recommendations for each of the studies in this report include:

Objective 1*. Determine the spatial extent of spawning of snapper, during their peak spawning period, in Cockburn Sound and surrounding areas and compare these findings with data collected during the spawning periods from 2001 to 2004 (Wakefield 2006).*

- There is a strong correlation between environmental parameters and reproductive cycles of snapper in Cockburn Sound.
- A strong year class has resulted from the 2007 spawning season.

The largest perceived risk to a reduction in spawning success for snapper from the Kwinana Quay development would most likely result from alterations to water circulation that would disrupt the retention of progeny in Cockburn Sound.

Objective 2*. Identify the species other than snapper that use Cockburn Sound as a spawning area.*

- Fish larvae from the ichyoplankton samples collected in Objective 1 have been preserved for identification at a later time.
- Given concerns over dredge plume induced mortality of fish larvae through gill fouling. The distribution and abundance of fish larvae in Cockburn Sound is to be used in a model to predict the potential risk associated with suspended sediment from dredging during the construction of Kwinana Quay, based on lethal concentrations established by Partridge and Michael (2008).

Objective 3*. Determine the distribution and abundance of demersal fish species, focussing on juvenile snapper, in Cockburn Sound and surrounding areas and identify any associations of fish assemblages with benthic habitat, topography and/or artificial structures.*

- BRUVs identified more species at higher abundances than traps and thus provided a better description of the fish communities in Cockburn Sound.
- There were three types of fish communities in Cockburn Sound, including those associated with seagrass, extensive areas of soft sediment (typically sand or silt) and areas comprising some form of limestone structure.
- Although a large part of Cockburn Sound comprises relatively featureless soft sediment habitat the majority of demersal fish species were found in seagrass or near naturally occurring limestone reef.
- A similar number of species were sampled at the rockwall sites compared to reef and upper slope sites (which consisted of interspersed small reef outcrops and were predominantly located on the upper slope of the topographic margin bordering the basin and eastern plateau). However, the abundances of these species at the rockwall sites were markedly lower, which suggests this artificial structure has a lower carrying capacity for the species sampled using BRUVs.
- The numbers of species sampled in the dredged areas were low and similar to those found in the relatively featureless soft sediment areas. However, the highest numbers of 0+ aged snapper were found in these areas.
- The habitats that include high relief limestone reef (not including rockwall/groyne), small interspersed reef outcrops and seagrass were associated with significantly higher numbers and abundances of fish species. Given the importance of these habitats to the fish communities and the small area they occupy in Cockburn Sound, it is highly recommended that efforts be made to avoid disturbance to these areas from the construction of Kwinana Quay.
- The interannual variation in the fish communities in Cockburn Sound was not investigated in this study. Notably, the distribution and abundances of snapper may be significantly different between years considering the 2007 year class sampled in this study represented a strong recruitment year, which typically occurs infrequently for this species in Cockburn Sound. Thus, further sampling using BRUVs is recommended.

Objective 4*. Describe the movement patterns of adult (mature) snapper relating to their spawning aggregations in the nearshore areas of Cockburn Sound, Owen Anchorage and Warnbro Sound.*

- The technology involved in this type of research is relatively expensive and could not be reduced and still achieve the desired outcomes. Thus this objective was not undertaken.
- This research would be important if there were thought to be any negative interactions between spawning aggregations of snapper and the Kwinana Quay development or associated increased shipping traffic in the area.
- The connectivity between snapper from these spawning aggregations and the contribution of recruitment from these embayments to the larger west coast would be supported by genetic or age-related otolith microchemistry analysis.

Objective 5*. Investigation of the potential impacts of the Kwinana Quay development on juvenile blue swimmer crab stocks in Cockburn Sound*

- The actual physical area encompassed by the Footprint of Options 1 and 4 provides a very low proportion of crab recruitment (3% and 4% respectively) and therefore the permanent loss of this area due to reclamation is likely to have minimal long-term impact on crab recruitment in Cockburn Sound.

- The Footprint and Channels combined of Option 1 represents a greater proportion of crab recruitment (9%), but Option 4 is even higher (11%) due to high catch rates at the site representing the Land-back component of the Footprint.
- The area surrounding the site of the proposed Kwinana Quay development, *i.e.* the Vicinity, represented 67% of the area considered to be important for blue swimmer crab recruitment in Cockburn Sound. The catch rates of juvenile blue swimmer crabs in this area was significantly high, with an average of 0.012 m^{-2} compared to 0.07 m^{-2} for the rest of the Recruitment Areas. Thus, the proportion of juvenile crabs that occurred in the Vicinity of the proposed site of Kwinana Quay development represented 83% of recruits for Cockburn Sound in 2008.
- It is important to note that assessment of potential impacts of the development on juvenile recruitment (and adult stocks) will be difficult until short-term and long-term environmental changes associated with each option have been determined.
- Despite blue swimmer crabs being a short-lived species with highly variability recruitment, the rebuilding of the recently depleted stocks is taking longer than expected. Thus, a cautious approach to potential impacts to recruitment needs to be adopted and it is anticipated that further sampling relative to different construction phases would be required.

Objective 6. *Investigation of the potential impacts of the Kwinana Quay development on adult blue swimmer crab stocks in Cockburn Sound.*

- It is likely that the risk of long-term impact on adult blue swimmer crab stocks in Cockburn Sound from the proposed Footprint and Channels associated with the development will be low, as these areas only support approximately 3% of the adult population.
- It should be noted that approximately one third of the relative abundances of adult crabs in 2008 were recorded within the Vicinity of this development.
- This assessment of adult crabs is based on the assumption of recruitment entering these areas from the juvenile nursery habitat. If these nursery areas are significantly affected by the Kwinana Quay development (see Section 6.0), this will have a flow-on effect to the adult population.
- The Department of Fisheries is dedicated to rebuilding the biomass of the currently depleted adult stocks of blue swimmer crabs. Therefore future assessments of the potential impacts on these adults from the proposed Kwinana Quay development should more accurately be based on historic abundances (*i.e.* pre-2006).
- Future monitoring in the vicinity of the Kwinana Quay development should be considered in the event of any impact to recruitment at the larval or juvenile stage to assess any flow-on effects.

***Objective** 7. Using genetic analysis, identify the relationship between blue swimmer crabs from Cockburn Sound, Warnbro Sound and the Swan River.*

- A genetic assessment of the relationships among the assemblages of the blue swimmer crab *Portunus pelagicus* in Cockburn Sound, the adjacent Swan River Estuary and near-by Warnbro Sound in south-western Australia was undertaken by Chaplin and Sezmiş (2008, Appendix 1).
- The assessment was based upon the patterns of variation at four polymorphic microsatellite loci in samples of *P. pelagicus* collected from Cockburn Sound, the Swan River Estuary and Warnbro Sound in 2007 and 2008.

- Results indicate that the genetic compositions of the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound were homogeneous at the time of sampling (2007/2008) and thus that *P. pelagicus* is represented by either a single biological stock, or a series of overlapping stocks, in these water bodies. However, the amount of gene exchange between the assemblages in Cockburn Sound, Swan River and Warnbro Sound is temporally variable and generally insufficient to have major impact on the abundances between these water bodies. On this basis the blue swimmer crab population in Cockburn Sound is managed as a single stock with limited recruitment from elsewhere.

It needs to be considered that for many of the studies included in this report there may be high levels of interannual variation that cannot be accounted for from this one-year investigation. The aspects of this report that require further annual monitoring to determine the significance of any interannual variation and thus provide a more comprehensive investigation of the potential impacts of the proposed Kwinana Quay development include objectives three, five and possibly six. In addition, this investigation by the Department of Fisheries represents key commercially and recreationally important fish and crab species and the impacts of this development may have broader biodiversity implications that are not addressed in this report.

KEYWORDS: Kwinana Quay, Port Development, Cockburn Sound, Owen Anchorage, Warnbro Sound, Shipping, Snapper, Blue Swimmer Crabs, Marine Embayment.

1.0 Introduction

C. Wakefield and D. Johnston

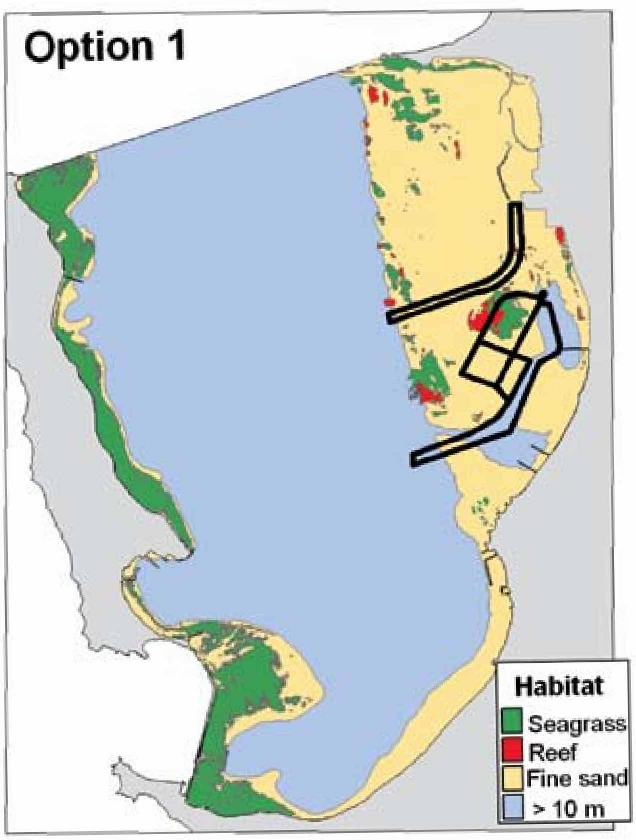
1.1 Background

It is expected that by 2015 the port of Fremantle will reach maximum working capacity. To alleviate the port of Fremantle from the increase in shipping operations, the Fremantle Ports have proposed the construction of an additional port in the protected waters of Cockburn Sound, *ca* 20 km south of Fremantle, with construction expected to commence in 2012. This outer harbour facility, named Kwinana Quay, is to be located on the eastern margin of Cockburn Sound, north of James Point (Fig. 1.1). The design of Kwinana Quay has been narrowed down from four to two different configurations, *i.e.* Option 1, island component only and Option 4, island and land-backed components (Fig. 1.1).

There are many aspects of the development of Kwinana Quay that will potentially impact on the marine fauna in Cockburn Sound. These include environmental disturbance during the construction process (*e.g.* increased turbidity, toxicity or physical alteration), impacts associated with the island and/or land-backed reclamation (*e.g.* removal and/or modification of natural habitat and/or redistribution of existing marine fauna and potential alterations to hydrodynamics), increased boat/shipping traffic (*e.g.* high risk for introduced species) and the introduction of restricted areas for commercial and recreational fishing.

It has been recognised that the two commercially and recreationally important species, blue swimmer crab and snapper, utilise Cockburn Sound as an integral part of their life cycle. Currently, the stocks of these two species are at depleted levels most likely resulting from high fishing pressure and below average recruitment for both species in recent years (Johnston *et al.* 2007; Wise *et al.* 2007). Thus, it is of importance to the Department of Fisheries that these stocks be managed to achieve a prompt recovery to acceptable stock levels. Therefore, any potentially detrimental influence that may affect the rebuilding of these stocks needs to be avoided, reduced or mitigated/managed. Notwithstanding this, there are numerous other commercially, recreationally and ecologically important marine species that also reside in Cockburn Sound, for which their biology and trophic interactions are poorly understood. Thus, both the physical and biological impacts of this development on the marine species may have broader biodiversity implications in this area, which also needs to be considered. The Department of Fisheries has recently completed a study describing the structure of the faunal community from trawling in an attempt to provide sound quantitative data from which changes to the broader ecosystem may be detected (Johnston *et al.* 2008).

The research outlined in this study that was funded by the Fremantle Ports, aims to establish methods and provide one year of data on important biological aspects of key species in Cockburn Sound. Many of the objectives in this study will require further investigation to determine any interannual variation. Thus, allowing a comprehensive evaluation of the potential impacts of this development on the marine fauna and associated user groups by the Department of Fisheries.



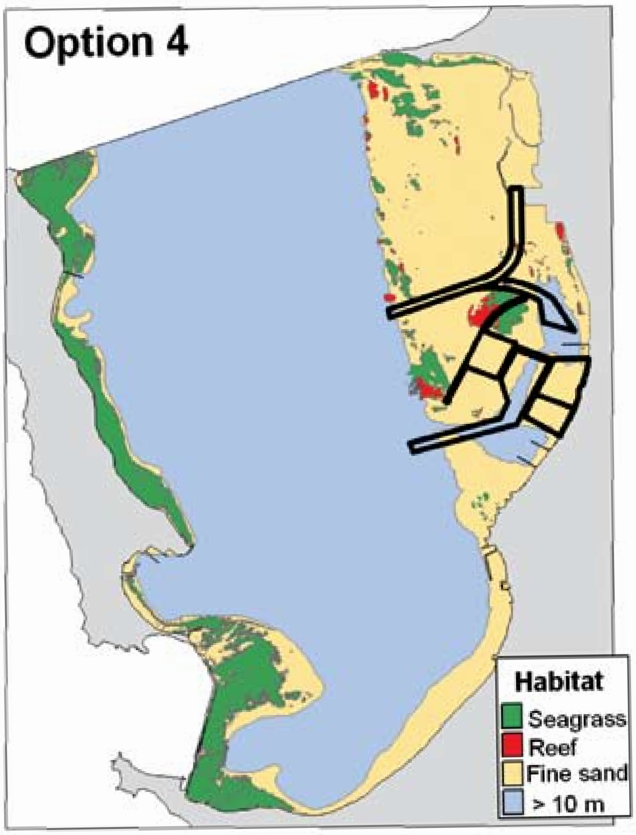


Figure 1. The two configurations of the Kwinana Quay development being considered and broad habitat types in depths < 10 m in Cockburn Sound (courtesy of the environmental consulting company *Oceanica*).

1.1.1 Cockburn Sound

The geomorphology that comprises the embayment of Cockburn Sound is typically a depressed land contour between the Spearwood and Garden Island ridges that lie along the eastern and western margins, respectively. To the north, two shallow submerged sand ridges, *i.e.* Success and Parmelia Banks, represent the respective northern and southern boundaries of Cockburn Sound and Owen Anchorage (Fig. 1). The southern entrance of Cockburn Sound has been almost closed through the construction of a rock-filled causeway, built in 1971-73 to provide vehicle access to Garden Island. This construction effectively reduced water flow into Cockburn Sound by 40 % and wave energy by 75 % (D.A. Lord & Associates Pty Ltd 2001). These boundaries provide a sheltered marine embayment *ca* 16 by 9 km in size, with a sea surface area of *ca* 100.5 km^2 and a maximum depth of 23 m. Large and relatively deep marine embayments, such as this, are rare on the south-western coast of Australia, with the closest areas with similar geomorphic attributes being Shark Bay to the north (*ca* 700 nm) and King George Sound on the south coast (Seddon 1972).

The main habitat types found in Cockburn Sound include small patches of limestone reef, extensive soft sediment areas (typically silt) and seagrass meadows. These diverse habitats support a wide variety of marine fauna including numerous species of fishes, crustaceans, molluscs, marine mammals and seabirds (see Section 1.1.2). For a majority of these species Cockburn Sound has been found to constitute an important area during certain stages of their life history, *i.e.* spawning and/or nursery. These species include invertebrates, *e.g.* blue swimmer crabs (Potter *et al.* 2001) and western king prawns (Penn 1975; Penn 1976); fish, *e.g.* snapper (Lenanton 1974; Wakefield 2006), white bait (Gaughan *et al.* 1996) and king george whiting (Hyndes *et al.* 1998); marine mammals, *e.g.* bottlenose dolphins (Finn 2005) and Australian sea-lions (Simpson *et al.* 1993) and seabirds, *e.g.* fairy penguins (Simpson *et al.* 1993). Thus, any changes to environmental conditions or habitat within the Sound will potentially impact on marine species from all trophic levels.

Cockburn Sound's sheltered waters and close proximity to the capital city of Western Australia, Perth (*ca* 20 km to the north), made it an ideal location for numerous industrial, shipping, naval, aquaculture, and commercial and recreational fishing activities. Industrial development commenced in this area in 1955 and over an extended period of time has resulted in the accumulation of pollutants and nutrient enrichment in Cockburn Sound. Physical alteration of the benthos in this area also occurred with the mining of shell sand and dredging of shipping channels. The combination of these anthropogenic inputs has been detrimental to the ecosystem and this is evident through the extensive depletion of seagrass meadows, with estimates of less than 20 % of their original coverage remaining (Kendrick *et al.* 2002). Intensive management over the past two decades has seen water quality in Cockburn Sound improve. However, there has been no evidence of seagrass recovery in Cockburn Sound despite signs of recovery occurring at Success and Parmelia Banks in the northern adjacent embayment of Owen Anchorage (Kendrick *et al.* 2000; Kendrick *et al.* 2002).

1.1.2 Snapper

Snapper, *Pagrus auratus*, is a widely distributed sparid found predominantly in the temperate waters of the Indo-Pacific region, from New Zealand and Australia to China and Japan (Paulin 1990). This species is highly valued by commercial and recreational anglers throughout its distribution, which in Western Australia includes marine waters from Exmouth Gulf (*ca* 18°S) southwards along the entire west and south coasts. Within this extensive distribution the species occurs in habitats ranging from shallow coastal lagoons and nearshore embayments to depths exceeding 200 m on the continental slope.

This species is highly vulnerable to overexploitation given its predictable reproductive strategy of forming large spawning aggregations in protected nearshore areas at the same time and location each year. It is believed that high levels of fishing pressure targeting spawning aggregations of this species contributed to the serious depletion of stocks in the eastern gulf of Shark Bay (Stephenson & Jackson 2005) and in the oceanic waters off the coast of Carnarvon in Western Australia (Moran *et al.* 2004). The hydrodynamics of a large majority of these nearshore areas, which are utilised by spawning aggregations of snapper, result in the retention of progeny as eggs and pre-settled larvae (Nahas *et al.* 2003; Doak 2004). As a consequence, these areas are important nursery or recruitment locations for this species. Some examples of these nearshore embayments include Gulf St Vincent and Spencer Gulf in South Australia (Fowler *et al.* 2005), Port Phillip Bay in Victoria (Hamer *et al.* 2005) and Hauraki Gulf in New Zealand (Crossland 1980; Francis 1995).

Although this species occurs along a large area of the Western Australian coast, recent studies on the biology of this species have identified very few spawning and nursery/recruitment areas (Wakefield 2006; Jackson 2007; St John *et al.* in press). The locations identified for recruitment of snapper from these three studies include three self-replenishing areas within the inner gulfs of Shark Bay; Koks, Bernier and Dorre Islands and Turtle Bay for the oceanic stocks off Carnarvon; Cockburn and Warnbro Sounds in the Perth metropolitan area; the area surrounding and including the Blackwood River on the lower west coast; and Wilsons Inlet and King George Sound along the south coast.

Cockburn Sound was first identified as a nursery area for snapper in 1971 from monthly trawl surveys conducted by the Department of Fisheries, from which juvenile snapper were found to remain in the area for at least the first 14 months of their life (Lenanton 1974). In addition, Cockburn Sound was recognised as an important location for annually occurring spawning aggregations of snapper as a result of catches of large mature fish taken during the spawning period by commercial fishers since 1979 (from compulsory catch statistics provided by commercial fishers to the Department of Fisheries Western Australia). To reduce fishing mortality on the spawning aggregations of this species, a seasonal closure (currently 1 October to 31 January) prohibiting the fishing of snapper by commercial and recreational anglers in Cockburn Sound during their vulnerable spawning period, was first introduced in 2000. Recent studies have suggested that this marine embayment may represent an important area for spawning and recruitment for a significant portion of the west coast managed bioregion, which extends from *ca* 27°00'S (slightly north of Kalbarri) to *ca* 115°30'E (slightly south of Augusta) (Wakefield 2006; St John *et al.* in press).

Given this demonstrated importance of Cockburn Sound for snapper, an improved understanding of the faunal composition of this marine embayment would provide key indicators of the areas environmental health and ultimately benefit the future conservation and sustainable management of this important area for snapper.

1.1.3 Blue swimmer crab

Distribution and biology

The blue swimmer or blue manna crab, *Portunus pelagicus,* occurs in nearshore, marine embayment and estuarine systems throughout the Indo-West Pacific region (Stephenson 1962).

They live in a wide range of inshore and continental shelf habitats, including sandy, muddy or algal and seagrass habitats, from the intertidal zone to at least 50 m depth (Williams 1982; Edgar 1990). Blue swimmer crabs have been recorded in all States of Australia, except Tasmania (Stephenson 1962). In Western Australia their distribution extends from Cape Naturaliste in the southwest, north along the coast to the Northern Territory. They are a highly valued species to commercial and recreational fishers, with the Shark Bay fishery the largest commercial blue swimmer crab fishery in Australia. Blue swimmer crabs are also the most important recreationally fished species in Western Australia in terms of community participation rate.

The reproductive cycle of blue swimmer crabs is influenced strongly by water temperature. In Cockburn Sound mating occurs in late summer – autumn (January to April), when females have finished spawning and recently matured recruits are soft-shelled (Kangas 2000). These females store the sperm for a number of months over winter, after which eggs are extruded and fertilised, with females becoming ovigerous and spawning between October and January (Penn 1977; Smith 1982). Incubation takes 10 to 18 days, depending upon water temperature, with each female releasing up to one million eggs during this period (Kangas 2000). The larval phase, *i.e.* egg, zoea, megalopa, extends for up to six weeks in coastal waters, with larvae drifting as far as 60 km out to sea in some locations, before settling in inshore waters (Kangas 2000). Rapid growth occurs over summer during the juvenile phase with recruits entering the fishery between March and June after which they move into deeper water. The size at which maturity occurs can vary with latitude or location and between individuals at any location. In Cockburn Sound, most (50 %) are mature in less than 12 months at a carapace width (CW) of between 86 and 96 mm. Blue swimmer crabs in estuaries and embayments in southwestern Australia typically start to attain minimum legal size (130 mm CW commercial and 127 mm CW recreational) in late summer, when they are approximately 12-16 months of age. Most animals in exploited crab stocks have died either through natural or fishing mortality by the time they are 20 months old (Potter *et al.* 2001), but without fishing pressure, blue swimmer crabs can live for three to four years.

Genetic studies have indicated that the population of blue swimmer crabs in Cockburn Sound is generally independent of other stocks in the State, such as the Peel-Harvey Estuary (Chaplin *et al*. 2001). This implies that it is unlikely there would be pronounced recruitment of blue swimmer crabs from outside Cockburn Sound into this embayment. Hence, adverse changes in environmental conditions or high levels of fishing pressure in the embayment could have highly detrimental and long-term effects on crab stocks in Cockburn Sound (Chaplin *et al*. 2001).

Status

Historically, commercial blue swimmer crab catches in Cockburn Sound have shown large fluctuations, *e.g.* 92 t in 2001/02 *vs.* 362 t in 1996/97. These fluctuations have previously been attributed to changes both in commercial fishing practices and normal variations in recruitment strength. In recent years, commercial catches have declined significantly from 231 tonnes in 2002/03 to 42 tonnes in 2005/06. Recruitment surveys in 2006 revealed the abundance of 0+ crabs was the lowest on record, with numbers in 2007 only marginally higher. It was concluded that high levels of fishing pressure, coupled with three years of reduced recruitment due to unfavourable environmental conditions, namely lower than average water temperatures, resulted in significantly reduced levels of relative egg production in 2004/05. Recruitment data has been used to generate an index from which catch prediction for the following year can be made. Based on these indices the predicted catch for the 2006/07 and 2007/08 seasons were

59 and 80 tonnes, respectively. On this basis the fishery has been closed to commercial and recreational fishing for the 2006/07 and 2007/08 seasons to allow levels of spawning stock and subsequent recruitment to recover. Current assessments during 2008 have indicated that recovery is slower than expected, despite warmer water temperatures, and that blue swimmer crabs have perhaps been more vulnerable than previously thought. Past reliance on minimum size limits (130 mm CW commercial and 127 mm CW recreational), set well above the size at sexual maturity (98 mm CW), clearly do not provide adequate protection to breeding stock if there are a number of years of adverse environmental conditions. Future management arrangements will focus on protecting the spawning stock under all environmental conditions to ensure recruitment is at an acceptable level.

1.2 Objectives

1. Determine the spatial extent of spawning of snapper, during their peak spawning period, in Cockburn Sound and surrounding areas and compare these findings with data collected during the spawning periods from 2001 to 2004 (Wakefield 2006).
2. Identify the distributions of fish larvae in Cockburn Sound to ascertain which species use this embayment as a spawning area.
3. Determine the distribution and abundance of demersal fish species, focussing on juvenile snapper, in Cockburn Sound and surrounding areas and identify any associations of fish assemblages with benthic habitat, topography and/or artificial structures.
4. Describe the movement patterns of adult (mature) snapper relating to their spawning aggregations in the nearshore areas of Cockburn Sound, Owen Anchorage and Warnbro Sound.
5. Assessment of potential impacts of Kwinana Quay development on juvenile blue swimmer crab stocks in Cockburn Sound
6. Assessment of potential impacts of Kwinana Quay development on adult blue swimmer crab stocks in Cockburn Sound.
7. Using genetic analysis, identify the relationship between blue swimmer crabs from Cockburn Sound, Warnbro Sound and the Swan River.

2.0 Objective 1

C. Wakefield, P. Lewis and M. Mackie

Objective 1. Determine the spatial extent of spawning of snapper, during their peak spawning period, in Cockburn Sound and surrounding areas and compare these findings with data collected during the spawning periods from 2001 to 2004 (Wakefield 2006).

2.1 Introduction

Spawning aggregations of many tropical reef fish species have been found to occur at the same time and locations each year (Colin 1992; Domeier & Colin 1997; Sadovy de Mitcheson *et al.* 2008). This is also the case for *Pagrus auratus*, where large spawning aggregations are known to form at the same time each year in protected nearshore marine embayments throughout its geographic distribution, *e.g.* Shark Bay (Jackson 2007), Cockburn Sound (Wakefield 2006) and King George Sound in Western Australia (Wakefield 2006), northern Spencer Gulf and Gulf St Vincent in South Australia (Fowler & Jennings 2003; Fowler *et al.* 2004), Port Phillip Bay in Victoria (Coutin *et al.* 2003) and Hauraki Gulf in New Zealand (Crossland 1980). The hydrodynamics associated with these marine embayments have been shown to retain eggs and larvae (Gersbach 1993; Nahas *et al.* 2003; Doak 2004). It has also been demonstrated that these marine embayments act as nursery areas for *P. auratus* for up to the first 2 years of their life (Lenanton 1974; Hamer *et al.* 2006; Wakefield *et al.* 2007). Studies of the age-related elemental concentrations of otoliths of *P. auratus* from South Australia (Fowler *et al.* 2005) and Victoria (Hamer *et al.* 2006) have clearly outlined the importance of these marine embayments through the considerable contributions of recruits to their respective adult populations. In South Australian, all *P. auratus* from a single, highly abundant age cohort, collected at nine years of age from over 2000 km of coastline, were found to originate from only one or two points of origin. Thus, given the relative paucity of nearshore marine embayments on the west coast of Western Australia, the embayments of Cockburn Sound, Warnbro Sound and Owen Anchorage most likely play an integral part in the early life history stages of *P. auratus* and may contribute a high proportion of recruits to the adult population over a large part of this coast.

The aims of this part of the research were to determine the temporal and spatial distributions of *P. auratus* eggs in and surrounding Cockburn Sound in 2007 to elucidate cycles in reproduction and examine the relationship between these cycles and environmental parameters. These findings were compared with data collected during the spawning periods from 2001 to 2004 (Wakefield 2006). The relationship between reproductive cycles and environmental parameters will provide information on the mechanisms influencing spawning in this embayment and provide useful information on the potential impacts on spawning from their alterations.

2.2 Methods

Ichthyoplankton was sampled during daylight in Cockburn Sound on the new moons in November and December in 2007. Sampling was also undertaken in the waters surrounding Cockburn Sound, *i.e.* Owen Anchorage, Five Fathom Bank and Warnbro Sound, on each day either side of these new moons. Sampling was confined around the new moon because the spawning fraction of *P. auratus* is highest during this period (Wakefield 2006). A total of 95 stations were sampled, 30 of which were the same stations sampled in Cockburn Sound in 2001 to 2004 (Fig. 2.1, Wakefield 2006). The stations were arranged in a grid formation with the distances between stations being 1' of latitude and longitude in Cockburn and Warnbro Sounds and 1.5' in Owen Anchorage and Five Fathom Bank.

Sampling involved the use of a double bongo net, each with a 60 cm diameter opening and 500µm mesh. The nets were towed obliquely for 2 minutes at approximately 2 knots. The warp (length of rope) used for each tow was approximately 2.5 times the depth at each station, allowing the net to sample the neutrally-buoyant eggs from just above the substrate to the surface of the water column. The volume of water filtered was measured using a 'General Oceanics' flowmeter fitted in the centre of one of the net openings. The contents of the two cod ends from each tow were sieved into a 500 ml jar containing 5% buffered formaldehyde.

Data collected previously using the same sampling gear and methods on, 1) four new moons during the 2001, 2002, 2003 and 2004 spawning seasons at the same 30 stations in Cockburn Sound, and 2) over 3 days around the new moons in October and November in 2003 from Owen Anchorage to Warnbro Sound were used to compare with the data collected in 2007 (Wakefield 2002, unpub. honours thesis; Wakefield 2006).

To identify the relationship between reproduction and water temperature (sea surface temperature) and salinities, measurements were taken at each station at the time of sampling using a conductivity meter with a built-in temperature probe (WTW 315i conductivity meter with a WTW tetracon®325 conductivity cell). Measurements were taken approximately 1 m below the sea surface.

Snapper eggs were identified in the samples on the basis of the following unique combination of characteristics, *i.e.* a chorion diameter ranging from 0.8 to 1.0 mm and a particularly prominent oil globule with a diameter ranging from 0.15 to 0.25 mm (Wakefield 2002, unpub. honours thesis; Wakefield 2006). Each snapper egg was then allocated to 1 of 19 developmental stages using the criteria of Norriss and Jackson (2002). The concentration of eggs at each station on each sampling occasion were calculated by dividing the number of eggs in the tow by the volume of water filtered by the bongo nets and expressed as eggs 100 m^{-3}.

As the samples of eggs contained two cohorts with respect to developmental stages, the analyses were restricted to the eggs in the first and younger of those cohorts. The number of *P. auratus* eggs present at each sampling station was estimated as:

$$N_i = (C_i / 100) \,.\, SA_i \,.\mathrm{D_i},$$

where N = total number of snapper eggs, i = sample station (i = 1 to 30 for Cockburn Sound), C = concentration of snapper eggs (eggs 100 m^{-3}), SA = surface area represented by the sample (m^2) and D = depth (m).

The relative abundance of *P. auratus* eggs in Cockburn Sound on each new moon in each year was calculated as ΣN_i. The resulting trends in the relative abundances of *P. auratus* eggs within and among years were compared with those exhibited by sea surface temperatures, which, for each new moon, was calculated as the mean of the temperatures recorded at each of the 30 stations.

The times of day when *P. auratus* spawns were estimated by back-calculating the estimated age of their eggs (derived from Wakefield 2006) from the times the eggs were caught. The relationship between the times of day when *P. auratus* spawns and the daily tidal fluctuations were then examined. The tidal data were obtained from measurements taken at the Fremantle Boating Harbour *ca* 20 km north of Cockburn Sound, by the Department of Planning and Infrastructure.

The spatial distribution of spawning of *P. auratus* was analysed from maps fitted with contours calculated using kriging and generated using 'Surfer 8.0' (Golden Software, Inc.).

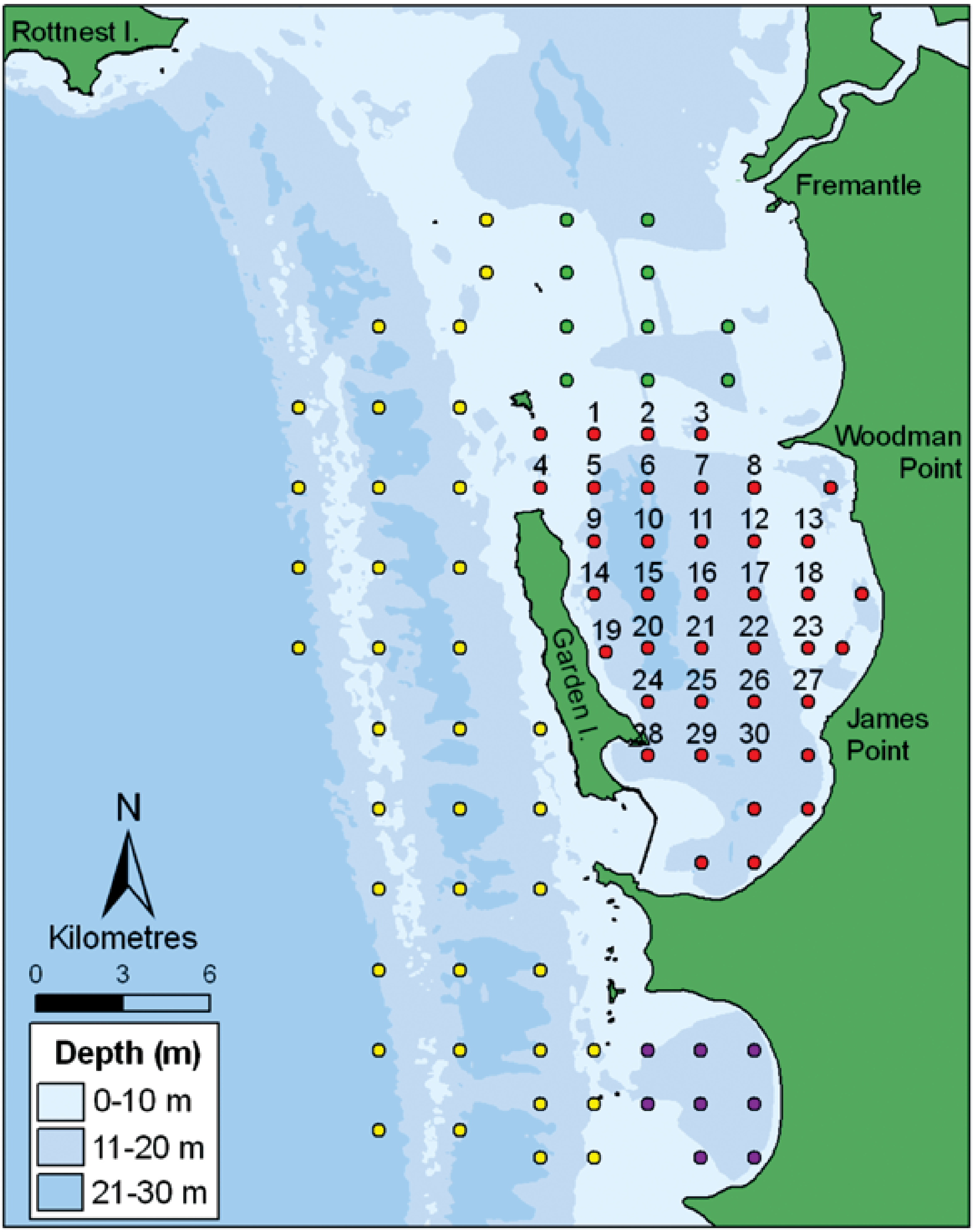


Figure 2.1. Map showing the locations of sampling sites in November and December 2007 in Cockburn Sound (red circles), Owen Anchorage (green circles), Five Fathom Bank (yellow circles) and Warnbro Sound (purple circles). Note that stations 1 to 30 are located inside Cockburn Sound and were the same locations sampled previously in 2001 to 2004 (Wakefield 2006).

2.3 Results

2.3.1 Temporal distribution of snapper eggs

The abundances of *P. auratus* eggs from sampling in 2007 were higher in November than December, at water temperatures of 19.4 and 20.5°C, respectively (Fig. 2.2). As was the case in 2007, previous sampling has shown that egg abundances are highest in those months when water temperatures lay between 19 and 20°C (Fig. 2.2, Wakefield 2006). This situation typically occurs in November each year with the exception of 2002, where water temperatures remained within this range over the new moons in November and December and egg production was equally as high (Fig. 2.2).

The relative abundances of eggs collected in Cockburn Sound in November 2007 were much higher than had been collected previously (Fig. 2.3). Likewise, the abundances of eggs collected in Warnbro Sound in November were much higher than that collected in the only other year in which Warnbro Sound had been sampled, *i.e.* 2003 (Fig. 2.3). In contrast, the abundances of eggs collected in Owen Anchorage in November were very similar between 2003 and 2007 (Fig. 2.3). The abundances of *P. auratus* eggs collected in all three regions in December in 2007 were very similar to those collected in previous years in December (Fig. 2.3).

The back-calculated times of spawning of *P. auratus* showed that, despite the fact that the time of the nightly high tide varied among sampling occasions, the mean time of spawning occurred on or up to three hours following the time of the nightly high tide (Fig. 2.4).

2.3.2 Spatial distribution of snapper eggs

In November 2007, the spatial distribution of *P. auratus* eggs formed three distinct groups, with a single group occurring in Owen Anchorage, Cockburn Sound and Warnbro Sound (Fig. 2.5). The concentrations of eggs in these groups were higher in Cockburn and Warnbro Sounds than in Owen Anchorage (Fig. 2.5). The spatial distribution of eggs in December 2007 also displayed three distinct groups, however, the eggs in Owen Anchorage were situated closer to Carnac Island and had higher concentrations than those in Cockburn Sound (Fig. 2.5). In comparison, although the abundances of *P. auratus* eggs were lower in 2003 their distributions also formed distinct groups in Owen Anchorage, Cockburn Sound and Warnbro Sound, which was particularly evident in November during the peak period of egg production (Fig. 2.6).

Previously, sampling on successive new moons throughout the spawning periods in each year from 2001 to 2004 has shown that the distributions of *P. auratus* eggs display a similar pattern. *Pagrus auratus* eggs first appear in significant concentrations at the north to north-east end of Cockburn Sound in October. One month later, in November, and during the overall peak in egg abundance the distribution of eggs occurs in the middle of Cockburn Sound, after which time on the subsequent new moon egg concentrations are substantially less and typically situated towards the north to north-western areas of Cockburn Sound (Figs 2.6 & 2.7).

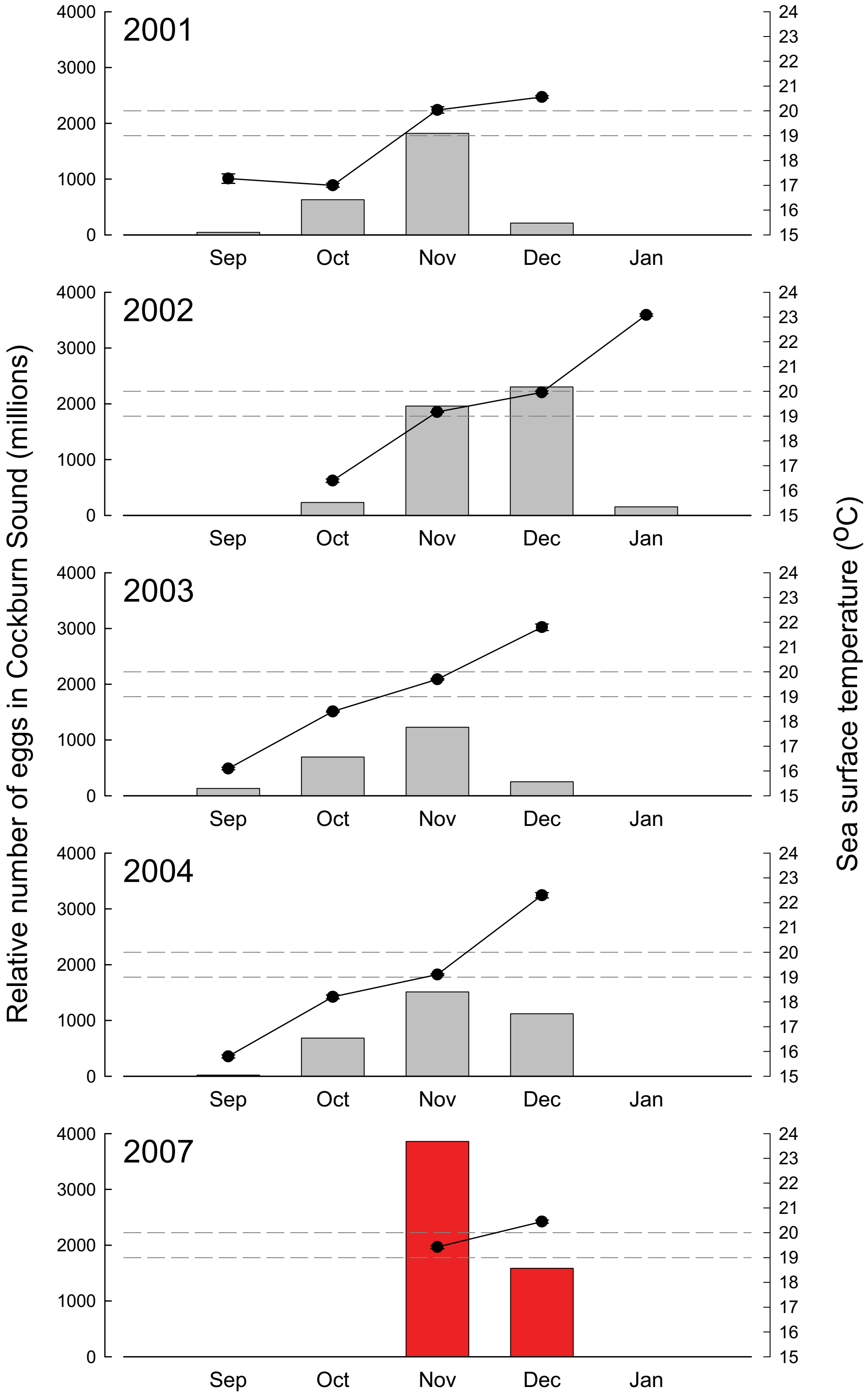


Figure 2.2. Relative number of *Pagrus auratus* eggs in Cockburn Sound on each of four new moons in each spawning season in each year between 2001 and 2004 (grey bars, from Wakefield 2006) and two new moons in 2007 (red bars); with the mean sea surface temperatures (black circles) on each of those occasions. Dashed lines represent temperatures of 19 and 20°C.

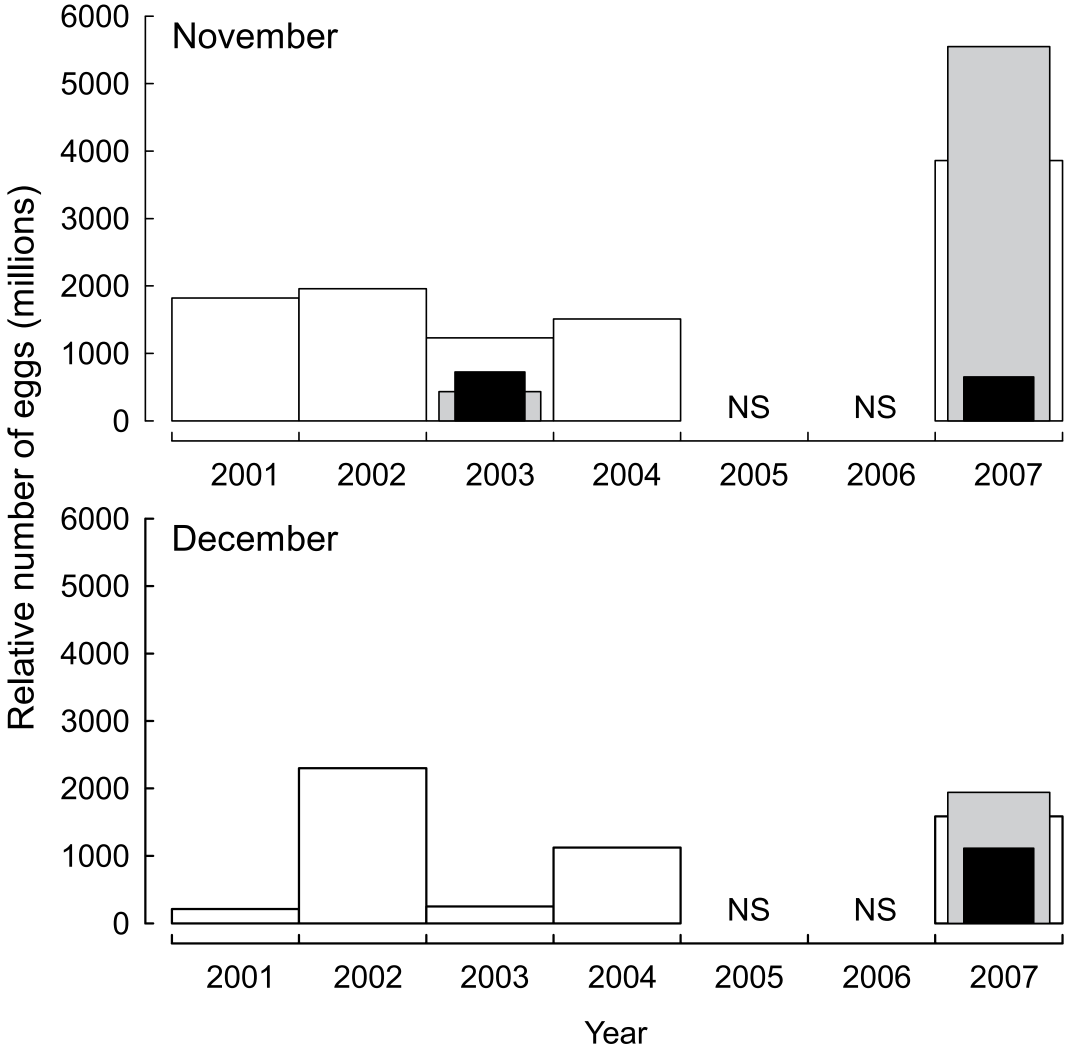


Figure 2.3. Relative number of *Pagrus auratus* eggs in Owen Anchorage (black bars), Cockburn Sound (white bars) and Warnbro Sound (grey bars) on the new moon from 2001 to 2007 (modified from Wakefield 2006). NS, not sampled.

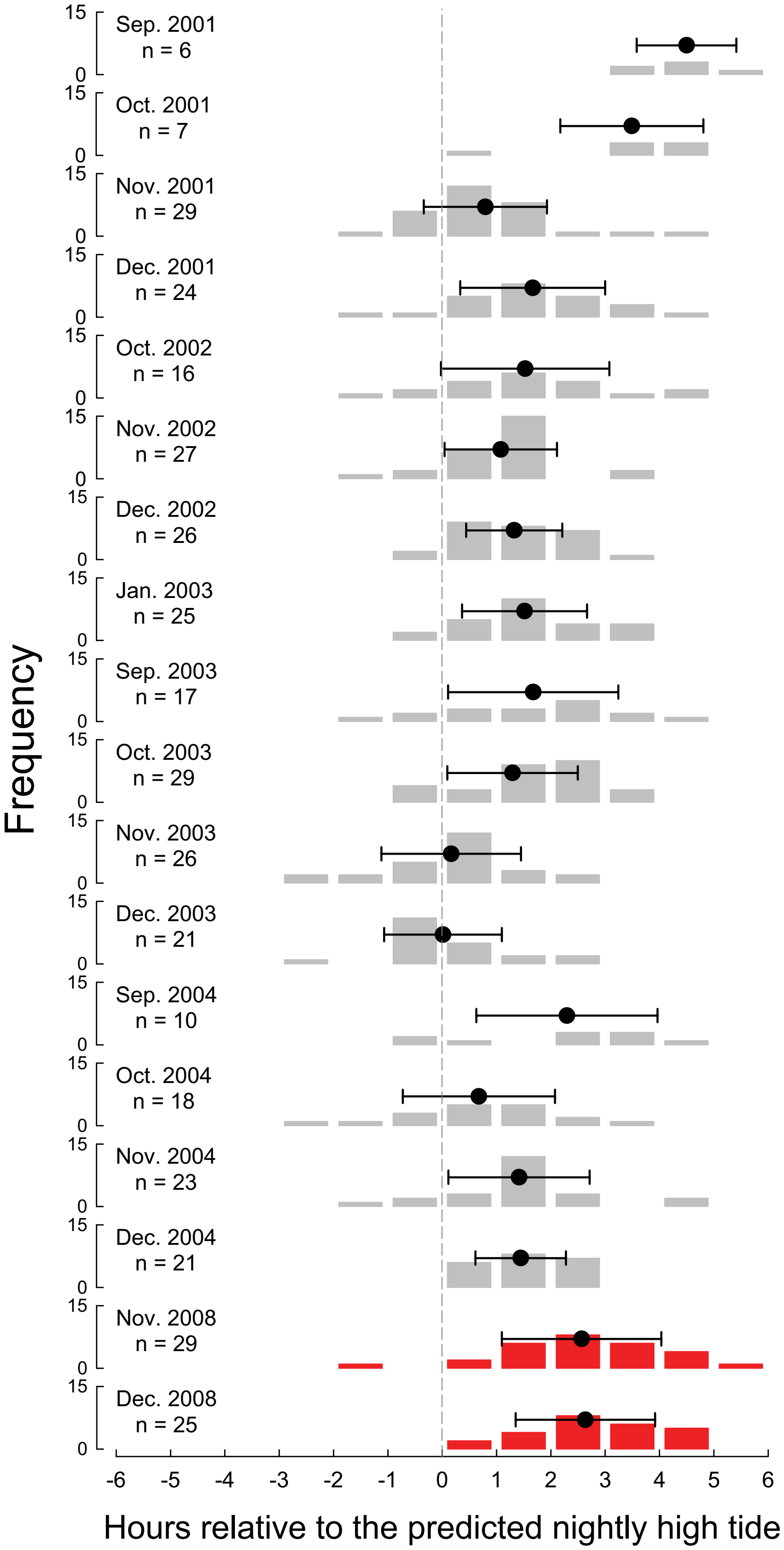


Figure 2.4. Distributions of back-calculated spawning times (bars) relative to the time of the nightly high tide (dashed line) for each of the 16 surveys between 2001 to 2004 from a previous study (grey bars, Wakefield 2006) and 2 surveys in 2007 from this study (red bars) on the new moons in Cockburn Sound. Circles and error bars (± 1 SD) represent mean spawning times.

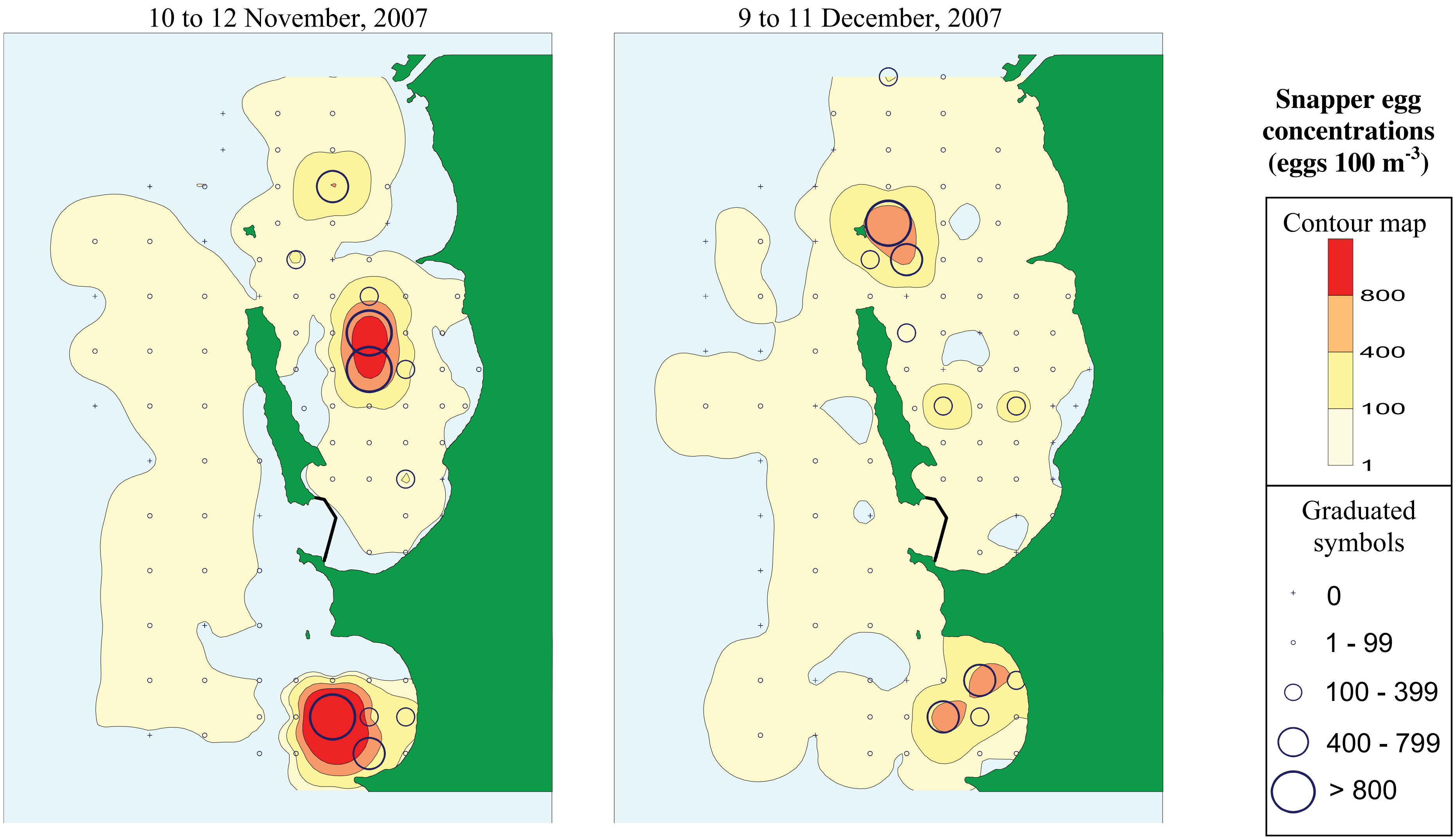


Figure 2.5. Distribution of *Pagrus auratus* eggs in the nearshore area from the Swan River to Warnbro Sound on the new moon in November (left) and December (right) 2007. Graduated circles (blue) represent egg concentrations at each site, which have been fitted with contours. Note, that the same scales for graduated symbols and contour maps are used in Figs 2.6, 2.7 & 2.8.

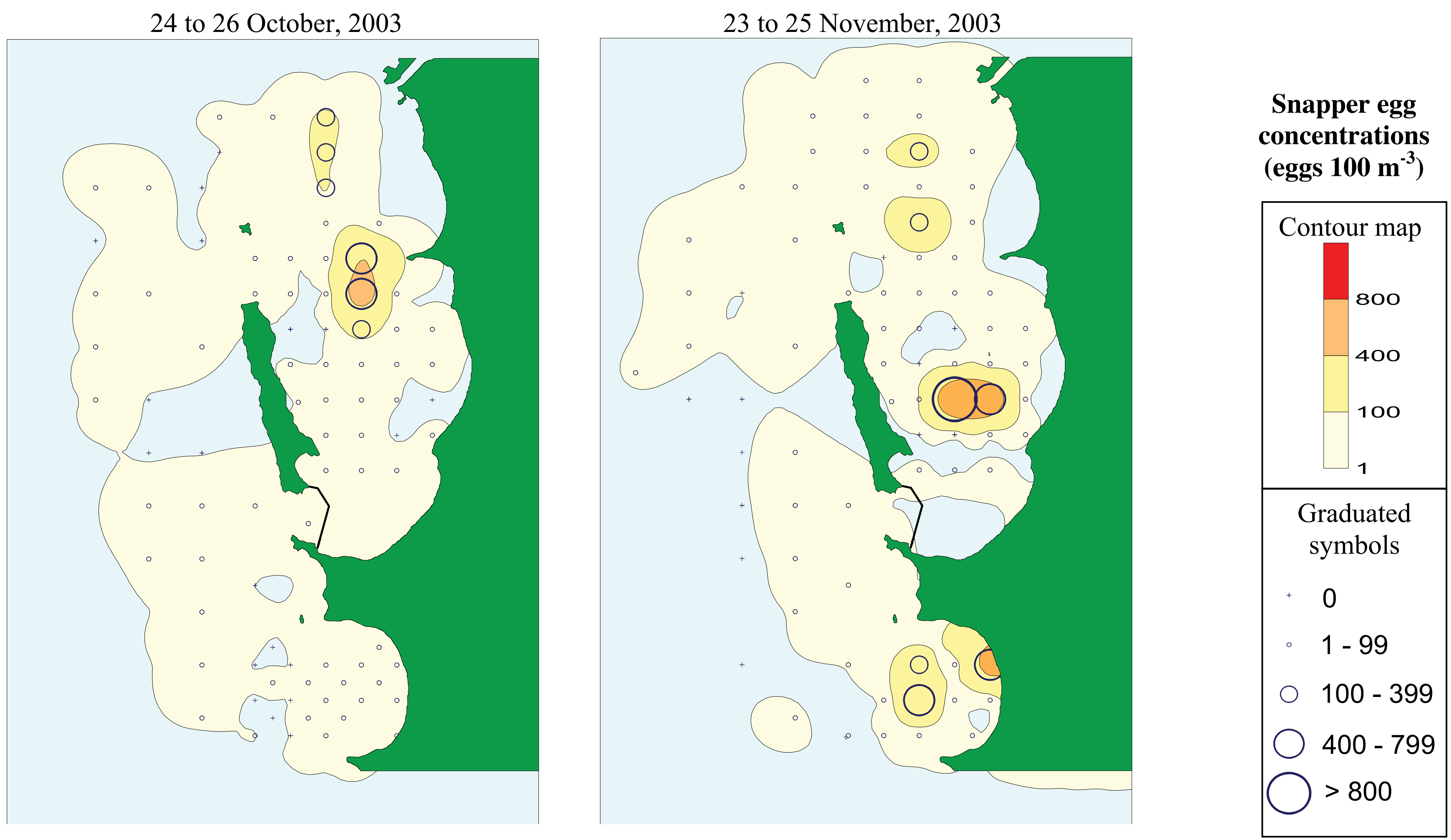


Figure 2.6. Distribution of *Pagrus auratus* eggs in the nearshore area from the Swan River to Warnbro Sound on the new moon in October (left) and November (right) 2003. Graduated circles (blue) represent egg concentrations at each site, which have been fitted with contours (from Wakefield 2006).

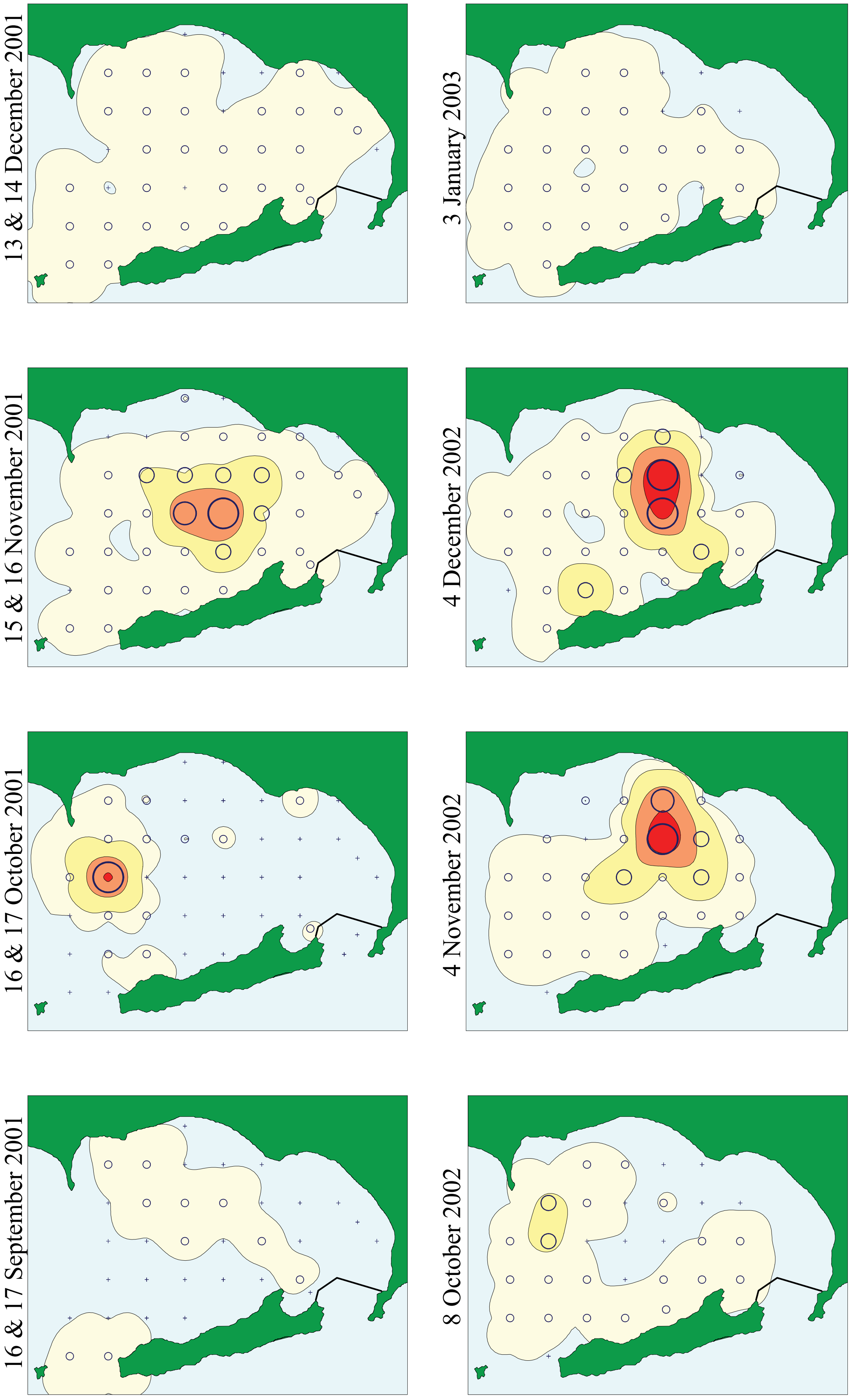


Figure 2.7. Distribution of *Pagrus auratus* eggs in Cockburn Sound on four new moons during the spawning seasons of 2001 and 2002 (from Wakefield 2006).

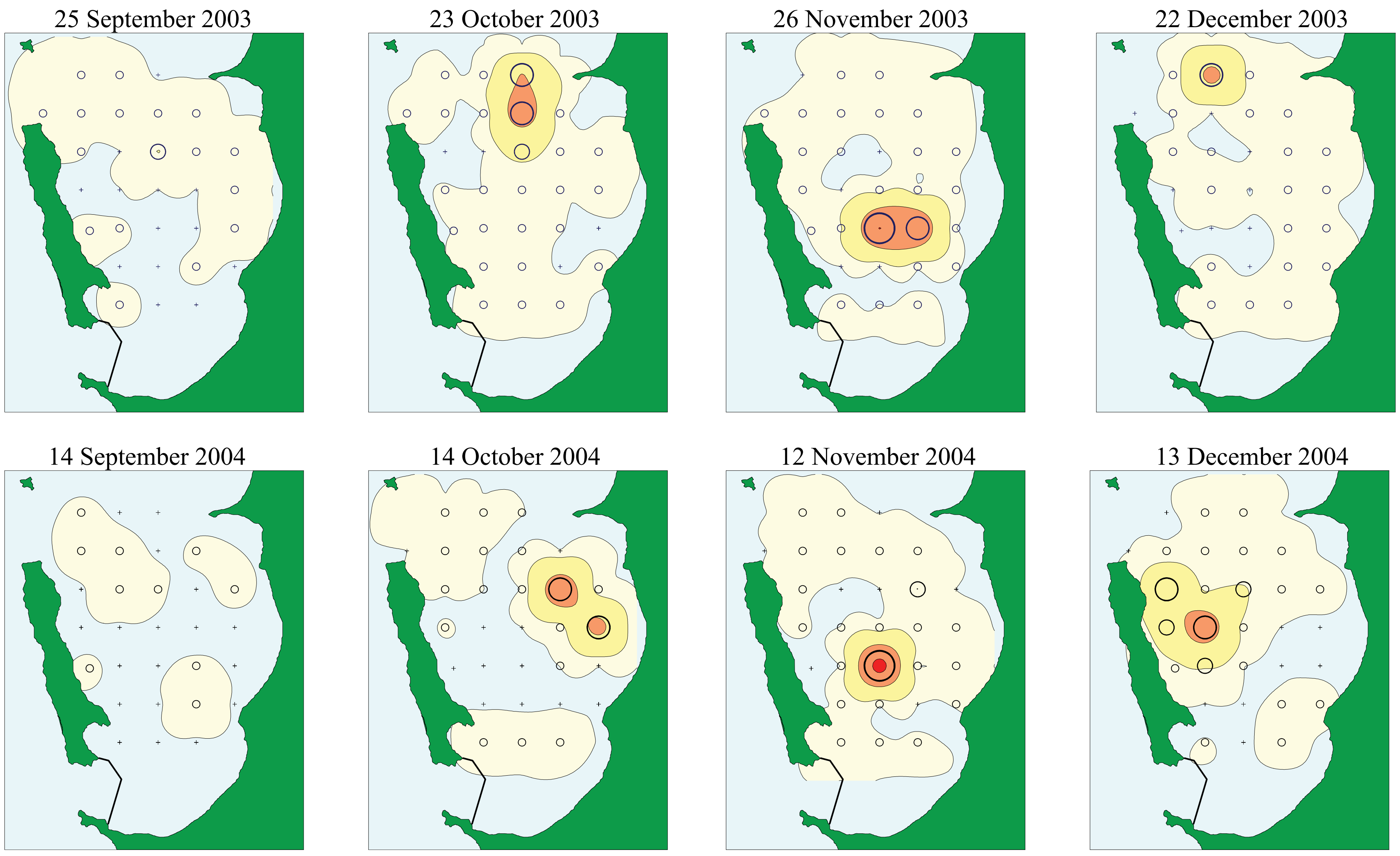


Figure 2.8. Distribution of *Pagrus auratus* eggs in Cockburn Sound on four new moons during the spawning seasons of 2003 and 2004 (from Wakefield 2006).

2.4 Discussion

The proposed site of the Kwinana Quay development, on the eastern margin of Cockburn Sound north of James Point, is not located in an area where high abundances of *P. auratus* eggs are typically sampled. However, it is considered that any potential impacts on the spawning success of *P. auratus* from this development would be through the disruption or alteration of physical environmental conditions in this embayment during their spawning period, considering the strong relationship between environmental parameters and the reproductive cycles of this species.

The relative abundance and distribution of early-stage *Pagrus auratus* eggs in ichthyoplankton samples collected from Cockburn Sound in 2001 to 2004 and 2007 have enabled the period when spawning peaked and the locations where it occurred to be clearly defined. The results demonstrated that the vast majority of spawning took place between October and December, with the peak occurring in November in all years except 2002 where egg abundances were equally as high in November and December. The fact that the mean monthly sea surface temperatures in those months when egg abundances peaked lay within the narrow range of 19 to 20°C provided substantial evidence that the spawning of *P. auratus* is strongly correlated to water temperatures.

Ichthyoplankton sampling of *P. auratus* eggs was conducted on the new moon in November and December in 2007 as the spawning fraction of females of this species in Cockburn Sound is highest during this period (Wakefield 2006). The back-calculated ages of the eggs of *P. auratus* collected in Cockburn Sound at different times of the day and during the 18 new moon surveys demonstrated that spawning occurred at night and predominantly during the three hours following the high tide.

The sampling of *P. auratus* eggs in the areas surrounding Cockburn Sound in 2003 and 2007 have indicated that, while spawning did not occur in the waters west of Cockburn Sound, *i.e.* Five Fathom Bank, it did take place just to the north in Owen Anchorage and to the south in Warnbro Sound. The patterns of distribution of the eggs within Cockburn Sound throughout the spawning season in four consecutive years were consistent. They indicated, that, in each year, the main locations of spawning moved in a clockwise direction from the north to north-east area of the embayment in September/October, to the middle of the embayment in November and then finally to its north-western region in December. Modelling of water body movements in Cockburn Sound has demonstrated that there is a prominent wind-driven eddy during the spawning period of *P. auratus* (Apai 2001; Doak 2004). A similar eddy also forms during this period in Warnbro Sound (Gersbach 1993). These water movement characteristics would help facilitate the retention of eggs and larvae of *P. auratus* in these marine embayments and thereby ensure that the juveniles have the potential to utilise the waters of this system as a nursery area (see Lenanton 1974; Johnston *et al.* 2008).

The proposed site of the Kwinana Quay development, on the eastern margin of Cockburn Sound north of James Point, is not located in an area where high abundances of *P. auratus* eggs are typically sampled. The largest perceived risk to the reduction of spawning success for snapper from the Kwinana Quay development would most likely result from alterations to water circulation that would disrupt the retention of progeny in Cockburn Sound. Three recent studies on the biology of *P. auratus* throughout its distribution in Western Australia (Wakefield 2006; Jackson 2007; Lenanton *et al.* 2008) identified the adjacent embayments of Owen Anchorage, Cockburn Sound and Warnbro Sound, as the main and possibly only locations of annually occurring spawning aggregations and subsequent recruitment areas of this species on the lower west coast of Western Australia. Thus, any detrimental alteration to the physical environmental during their spawning period in Cockburn Sound could have much broader ramifications.

3.0 Objective 2

C. Wakefield

Objective 2. Identify the distributions of fish larvae in Cockburn Sound to ascertain which species use this embayment as a spawning area.

The fish larvae collected during ichthyoplankton sampling from Objective 1 (see Section 2.0) were to be identified to determine their distribution and abundance in Cockburn Sound. This will provide information on other fish species that utilise this nearshore marine embayment during spring/summer to reproduce. These fish larvae have been preserved in 70% ethanol and are being stored at the Western Australian Fisheries and Marine Research Laboratories (WAFMRL) for identification in 2009, from funding provided by the Fremantle Ports, Murdoch University and the Department of Fisheries. There are concerns over dredge plume induced mortality of fish larvae through gill fouling. Information on the distribution and abundance of fish larvae in Cockburn Sound are to be used in a model to predict the potential risk associated with suspended sediment from dredging during the construction of Kwinana Quay, based on lethal concentrations established by Partridge and Michael (2008).

4.0 Objective 3

C. Wakefield and P. Lewis

Objective 3. Determine the distribution and abundance of demersal fish species, focussing on juvenile snapper, in Cockburn Sound and surrounding areas and identify any associations of fish assemblages with benthic habitat, topography and/or artificial structures.

4.1 Introduction

Studies of the fish communities in Cockburn Sound are limited and have principally focussed on associations with seagrass (Dybdahl 1979; Scott *et al.* 1986; Vanderklift 1996; Vanderklift & Jacoby 2003). Recently, the fish communities were described from suitable trawl grounds in the central basin and eastern shelf areas of Cockburn Sound (Johnston *et al.* 2008). However, the fish communities in Cockburn Sound that are associated with habitats other than seagrass or extensive soft sediment (typically silt or fine sand) have not been determined and given the selectivity's associated with different sampling techniques, the fish communities described in these previous studies are difficult to compare.

An important aspect for determining the potential impacts of the Kwinana Quay development on the fish communities in Cockburn Sound is to have an understanding of the species that occur and their distributions and abundances associated with the different naturally occurring habitats in this embayment. In addition, given the vast expanses of relatively featureless soft sediment in Cockburn Sound, large-scale topographic features may also be important in defining the distributions and abundances of these species. There will also be some artificial structures created by the construction of the Kwinana Quay facility that may be utilised by some fish species that will ultimately need to be assessed.

This study compared two methods, *i.e.* baited remote underwater videos (BRUVs) and traps, to determine the most appropriate technique to describe the fish communities in Cockburn Sound with respect to habitat, topography and artificial structures. It is important that this method be easily repeated as further assessment will be required to investigate interannual variations and any changes to the fish community structures during and for some time after construction.

4.2 Methods

4.2.1 Pilot study

Initial assessments of traps and BRUVs were conducted in May 2008, which involved trialling four different trap configurations including two Opera-house and two rectangle traps of different colours and slightly different openings, *i.e.* internal cone or flat (Fig. 4.1), at locations where juvenile *P. auratus* had been caught previously (Wakefield 2006; Johnston *et al.* 2008). All four traps used identical bait canisters that were circular with a diameter of 13.5 cm and a height of 4 cm.

In conjunction, multiple trials with BRUVs were used to determine a preferable distance at which the camera could be situated from the substrate and the bait from the camera to attain the widest field of view possible, while still maintaining sufficient visibility. The BRUVs consisted of a single Canon HV20 high definition video camera in an underwater housing mounted in a galvanised steel frame with a bait canister (same dimensions as those used in the traps) positioned in the field of view (Fig. 4.1).

Fish were observed escaping from both rectangle traps during retrieval and the green Opera-house trap caught consistently higher numbers of fish than the black Opera-house trap. Thus, the green Opera-house trap was used to compare with the BRUVs in this study. The optimal dimensions of the BRUVs included the camera to be situated 75 cm from the substrate and the bait canister to be 100 cm from the camera (Fig. 4.1). During this pilot study high variations in the numbers of fish caught in traps and the maximum number of fish of each species in a single frame of video footage, *i.e.* Max N, were observed at the same location and time. It was therefore apparent that replicate sampling was required at each site during this study.

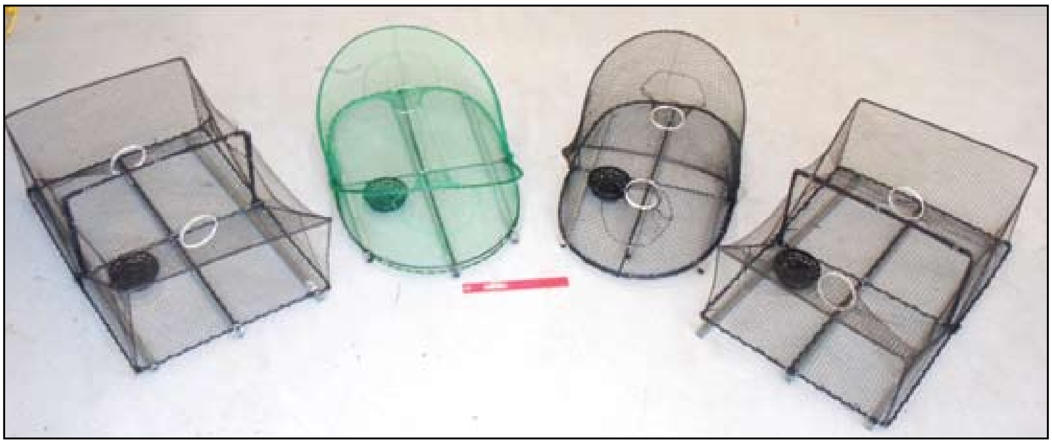


Figure 4.1. Pictures of the four types of traps used during the pilot study (above, scale is 30 cm) and the green Opera-house trap (above) and BRUV setup (below, note camera housing to ground is 75 cm and camera housing to bait canister is 100 cm) used during the study.

4.2.2 Sampling

The survey was designed to incorporate the main types of habitat, topography and anthropogenic structures in Cockburn Sound. Thus, the categories considered included (see Fig. 4.2):

- Seagrass – habitat consisting predominantly of *Posidonia* and *Amphibolus* species.
- Reef - naturally occurring high relief limestone reef.
- Silt Basin - incorporates a large majority of the central area of Cockburn Sound and consists of flat, soft sediment (typically silt) in depths > 15 m.
- Lower Slope – located in the Silt Basin area but is situated at the base of distinguishing topographic features, *i.e.* the ridge that is orientated from north to south and separates the Silt Basin from the Eastern Plateau.
- Upper Slope – located in the shallower areas of a distinguishing topographic feature immediately adjacent to the lower slope sites. Note on the upper slope of this north to south ridge there are small, interspersed outcrops of naturally occurring limestone.
- Sand Plateau – is located along the eastern margin of Cockburn Sound and consists of extensive flat areas of soft sediment (typically fine sand).
- Rockwall – an anthropogenic structure used as a barrier or bridge and is constructed of large limestone blocks/boulders.
- Dredged Channel – anthropogenically altered areas where substrate has been removed to increase depth to facilitate access for large vessels. Periodicity of dredging has not been considered.

At least three sites were sampled at each benthic category to allow for sufficient statistical power (see Table 4.2). There were a total of 51 sites with 3 replicates at each site that were sampled at the same locations (using GPS) by BRUVs and Opera-house traps (Fig. 4.2). The replicates were orientated in a triangle pattern *ca* 100 to 150 m apart at each site unless the site warranted their orientation to follow a particular feature, *i.e.* rockwall. The bait canisters used for BRUVs and Opera-house traps were identical and the bait was replaced for each replicate. The bait consisted of four Australian pilchards (*Sardinops neopilchardus*) weighing *ca* 150 g. As sampling with BRUVs is much less invasive than traps, they were used first. Sampling for both methods required four days each and took place in late June to early July 2008.

There were six BRUV setups used such that two sites were sampled at the same time. The BRUVs were left to record for at least 35 minutes based on the methods of Morrison and Carbines (2006). The recorded footage was later analysed in the laboratory (see Section 4.2.3). There were 30 Opera-house traps used such that ten sites were sampled at the same time. The Opera-house traps were left for at least 90 minutes, as catch rates are thought to asymptote after this period (Ferrell & Sumpton 1996). All fish caught were identified and measured the nearest 1 mm. Scientific and common names of fish species were validated with those of the Codes for Australian Aquatic Biota (CAAB, CSIRO 2008).

4.2.3 Data analysis

The footage recorded from each BRUV was analysed using the BRUVS 2.1 database (Cappo and Ericson *pers. comm.*, Australian Institute of Marine Science) to obtain the following parameters:

- Time of first appearance of each species.

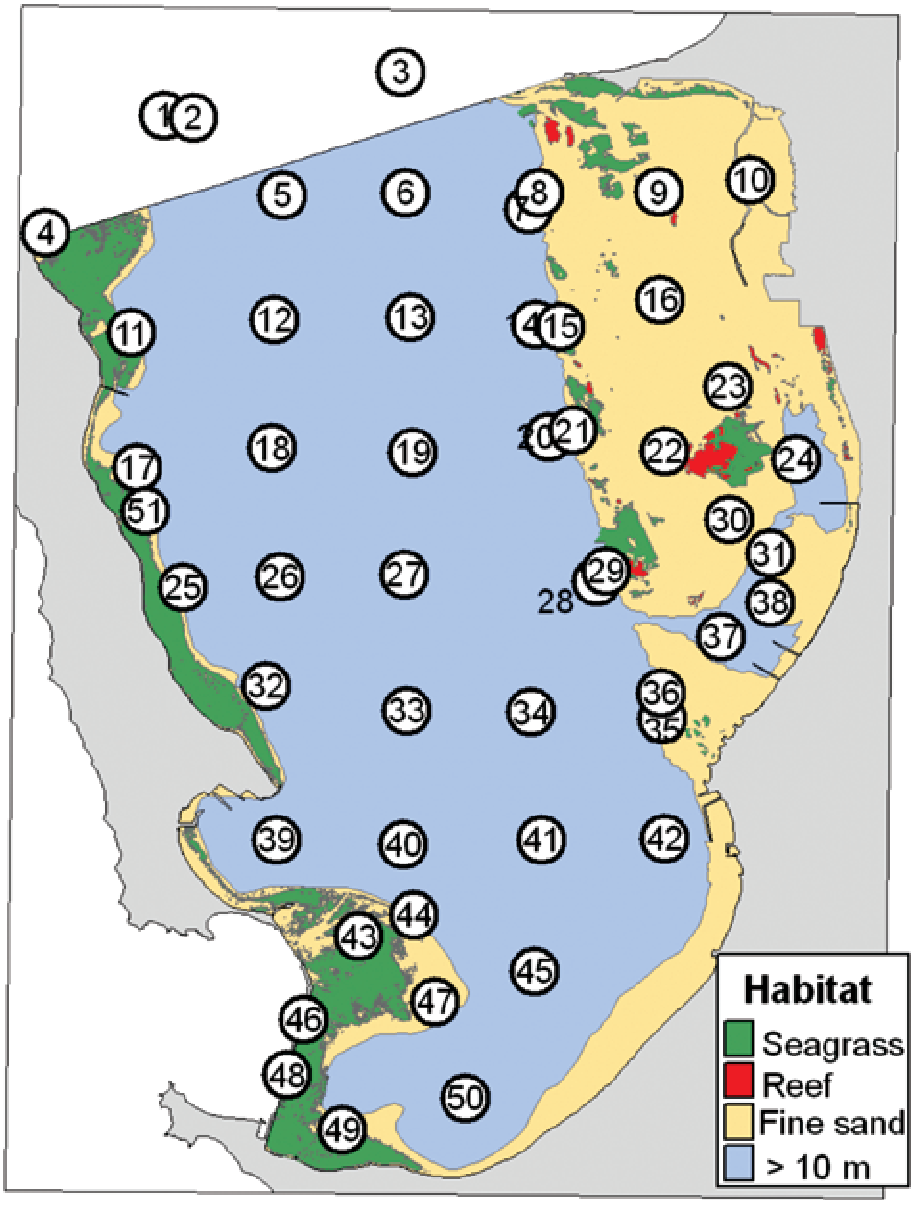
- Relative abundance, *i.e.* MaxN (maximum number of fish of each species visible in a single frame).
- Time at which MaxN occurred for each species.
- Activity of each species (*e.g.* passing, feeding)
- Time of first feed by each species.
- Confirmation of habitat characteristics (*e.g.* reef, seagrass).

Note 0+ and 1+ age cohorts of *Pagrus auratus* were easily distinguished visually by their overall size.

The species and their abundances (MaxN) were compared between BRUVs and Opera-house traps, from which it was apparent that Opera-house traps inadequately sampled demersal fish (see Results, Section 4.3). Thus, the subsequent analyses was only performed on the data collected from the BRUVs. Multivariate analyses were conducted on the abundances of demersal fish species, *i.e.* excluding pelagic or 'baitfish' species (*e.g.* Clupeidae species), to identify correlations with benthic habitats. Data were transformed prior to analyses to meet the assumptions of normality and homogeneity of variances based on the gradient of the lineal relationship between the logarithms of standard deviation and mean according to Clarke and Warwick (2001). Consequently, the abundances of species were fourth root transformed. Similarities between the abundances of each species were tested using the Bray-Curtis similarity measure, as this preserved the abundance structure of the data set (Clarke & Warwick 2001). Non-metric multidimensional scaling (nMDS) and cluster analyses were used to explore how the fish community grouped with the significance of the groupings assessed using the similarity profile test (SIMPROF, Clarke & Gorley 2006).

Significant differences in the fish assemblages were then tested between the sites and benthic categories (which were validated from the video footage) using permutational multivariate analysis of variance (PERMANOVA) and canonical analysis of principal coordinates (CAP). The species that contributed to these differences among sites and benthic categories were assessed using a Pearson's correlation coefficient > 0.43.

Differences between the mean numbers of these contributing species were tested between benthic categories using analysis of variance (ANOVA). The significance of these benthic categories were assessed using multiple comparison tests, *i.e.* Tukey's and Bonferroni, the former of which is thought to be more conservative (Zar 1999).



Benthic category	n	Site numbers
Seagrass	5	3, 43, 47, 49, 51
Reef	3	4, 11, 17
Silt Basin	16	5, 6, 12, 13, 18, 19, 26, 27, 33, 34, 39, 40, 41, 42, 45, 50
Lower Slope	9	2, 7, 14, 20, 25, 28, 32, 35, 44
Upper Slope	6	1, 8, 15, 21, 29, 36
Sand Plateau	5	9, 16, 22, 23, 30
Rockwall	3	10, 46, 48
Dredged Channel	4	24, 31, 37, 38

Figure 4.2. Locations and sites sampled for the eight categories of benthic habitat or structure in Cockburn Sound. Note there were 3 replicates at each site. The environmental consulting company *Oceanica* supplied the map of the broad habitat types in Cockburn Sound.

4.3 Results

4.3.1 Comparison of BRUVs and Opera-house traps

The BRUVs sampled 44 species of fish compared to 27 from Opera-house traps. Only two species sampled by the Opera-house traps were not sampled by the BRUVs, *i.e.* longspine flathead (*Platycephalus longispinis*) and soldier fish (*Gymnapistes marmoratus*, Table 4.1). For those species that were sampled by both methods, in all cases the numbers of fish were higher or similar for BRUVs than Opera-house traps, with the exception of western butterfish (*Pentapodus vitta*, Table 4.1, Fig. 4.3). Thus, the Opera-house traps did not sample species at some sites despite evidence of their occurrence at these sites from the BRUVs. This was particularly evident for snapper (*Pagrus auratus*, Fig. 4.3). Therefore, the Opera-house traps were considered an inadequate method for sampling demersal fish species in Cockburn Sound and subsequent analyses were only performed on data collected from BRUVs.

4.3.2 Fish assemblages and habitat associations

The unconstrained ordination of the abundances of each species resulted in nine groups that were significant at 5% Bray-Curtis similarity (Fig. 4.4). Using this method all seagrass sites were contained in a single group, whereas all other benthic categories were distributed amongst all groups.

The constrained ordination (CAP) separated all sites based on the abundances of each species and demonstrated that the seagrass sites were markedly different from the other seven benthic categories (Fig. 4.5). Note site 4 was categorised as reef but was surrounded by seagrass and the composition of fish species at this site was consistent with that of seagrass (Fig. 4.5). The species that contributed towards the distinction of seagrass, *i.e.* Pearson's correlation coefficient > 0.43, included Australian herring (*Arripis georgianus*), weeping toadfish (*Torquigener pleurogramma*), western striped grunter (*Pelates octolineatus*), sixspine leatherjacket (*Meuschenia freycineti*) and snook (*Sphyraena novaehollandiae*, Fig. 4.5). The spatial distributions of these key species were almost exclusively constrained to seagrass areas in Cockburn Sound (Figs 4.6 & 4.7). The seagrass sites had the second highest number of species and third highest mean number of fish per replicate (Table 4.2).

There appeared to be two other broad groups, which were more apparent when the constrained ordination (CAP) was performed on sites excluding seagrass (Fig. 4.5). The second group included sites with benthic categories that comprised extensive sand or silt areas, *i.e.* the lower slope, silt basin and sand plateau (Fig. 4.5). The fish species that contributed the most toward the separation of this group (Pearson's correlation coefficient > 0.43) were all species of rays (Fig. 4.5). This was consistent with the distribution of southern eagle rays (*Myliobatus australis*) in Cockburn Sound (Fig. 4.6). The numbers of species and mean number of fish observed in each replicate at the sites that contributed to this second group, *i.e.* the lower slope, silt basin and sand plateau, were markedly lower than all other benthic categories (Table 4.2).

The third group included those sites with benthic categories that consisted of some form limestone structure, *i.e.* the upper slope, reef and rockwall. The fish species that contributed the most toward the separation of this group were (Pearson's correlation coefficient > 0.43) snapper (*Pagrus auratus*), western butterfish (*Pentapodus vitta*) and trevally species (*Pseudocaranx sp.*, Fig. 4.5). The spatial distributions of these species and numerous other commercially and recreationally important species were situated in areas comprising some form of limestone structure, most notably along the upper slope of the eastern margin of the Basin area (categorised as Upper Slope) that comprised small, interspersed outcrops of naturally occurring limestone reef (Fig. 4.6). The numbers of species and the mean number of fish per replicate at the Reef and Upper Slope sites were higher than all other benthic categories. In contrast the Rockwall sites contained similar numbers of species but much lower mean numbers of fish per replicate. Thus, although the species compositions were similar between Reef, Upper Slope and Rockwall sites the carrying capacity of these species at the Rockwall sites appeared lower. The mean numbers of fish observed at each of the benthic categories and the statistical significance of these categories for six commercially and recreationally important species are displayed in Figure 4.8.

The highest numbers of snapper were found in the dredged areas, with Upper Slope, Reef and Rockwall sites having slightly less numbers but still providing significant habitat (Fig. 4.8). Conversely, snapper were observed at some of the Lower Slope and Silt Basin sites however they were not in significant numbers, and snapper were not observed at any of the Sand Plateau or Seagrass sites (Fig. 4.8).

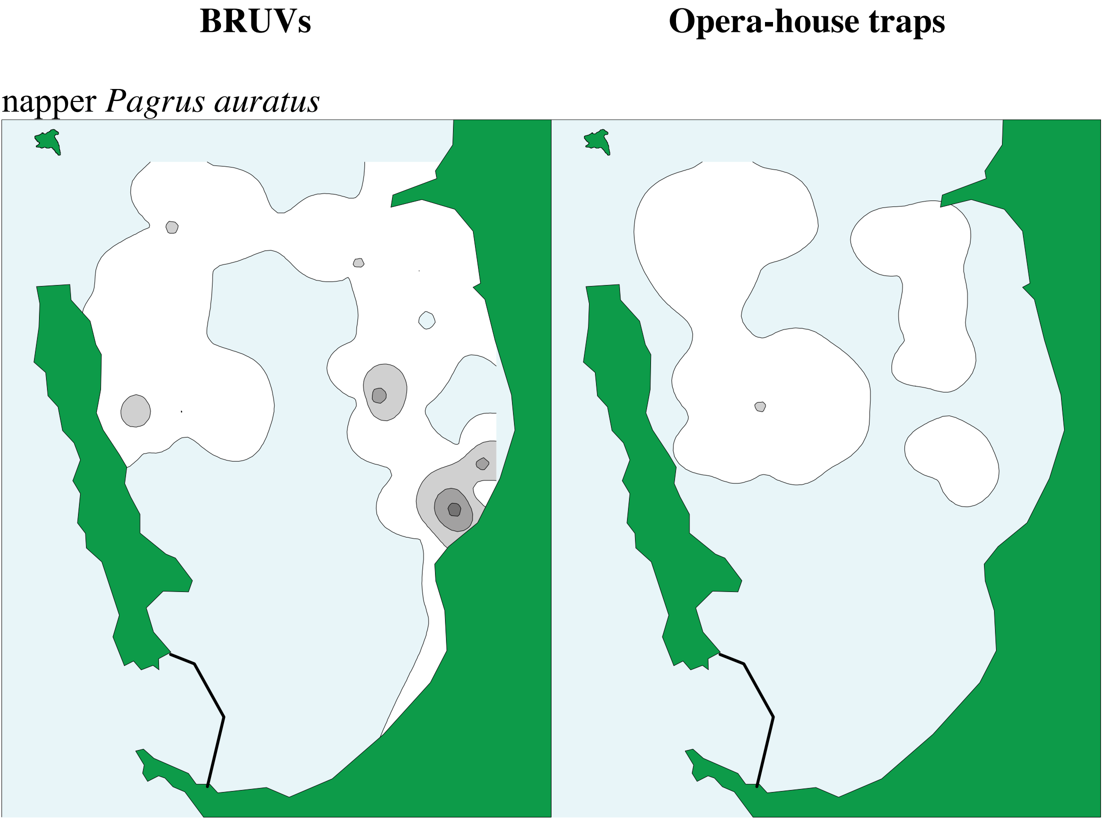
The dredged Channel sites could not be grouped with other benthic categories as they displayed highly varying species compositions among sites. This may be due to the periodicity and time since each of these sites were dredged. Overall, the dredged sites comprised very low numbers of species, similar to that of the group that comprised extensive areas of sand or silt (Table 4.2). However, the species that did occur at the dredged sites were highly abundant (Table 4.2). This was particularly evident for snapper with their numbers highest at these sites (Fig. 4.8).

Table 4.1. List of all species sampled, highest number recorded by a single set (MaxN) and total number sampled (Sum MaxN) by the BRUVs and Opera-house traps and their retention by either commercial or recreational fishers is noted.

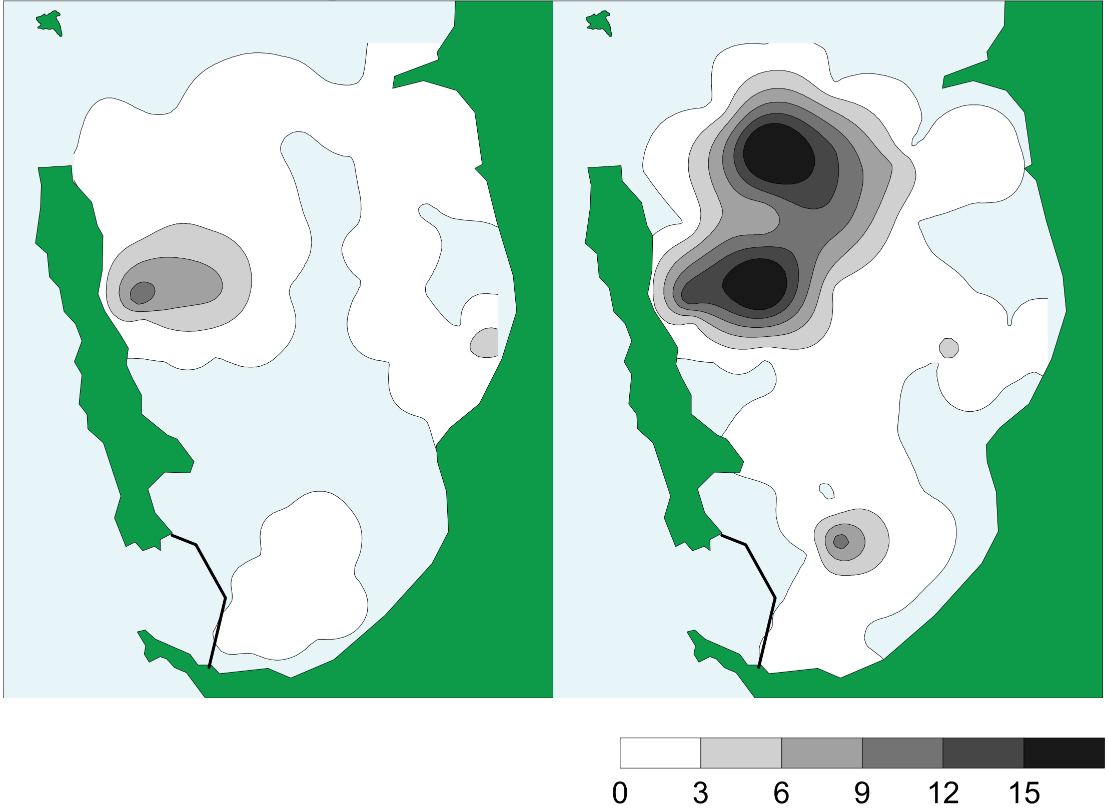
Taxa		Common Name	BRUVs		Traps		Commercial or Recreational sig.
			MaxN	Sum MaxN	MaxN	Sum MaxN	
Pisces							
Apogonidae							
Apogon	*rueppellii*	Western gobbleguts	150	226	25	116	
Arripidae							
Arripis	*georgianus*	Australian herring	2	19	1	1	•
Carangidae							
Pseudocaranx	*wrightii*	Skipjack trevally			6	38	•
Pseudocaranx	*georgianus*	Silver trevally			43	84	•
Pseudocaranx	indeterminate	Trevally sp.	150	1599			•
Seriola	*dumerili*	Amberjack	1	1			•
Seriola	*hippos*	Samson fish	1	2			•
Trachurus	*novaezelandiae*	Yellowtail scad	200	413	1	1	•
Chaetodontidae							
Chelmonops	*curiosus*	Western talma	2	2			
Cheilodactylidae							
Cheilodactylus	*gibbosus*	Crested morwong	2	6			
Dactylophora	*nigricans*	Dusky morwong	1	2			
Clupeidae							
indeterminate		Baitfish	2000	5240			•
Dasyatidae							
Dasyatis	*brevicaudata*	Smooth stingray	1	9			
Gerreidae							
Parequula	*melbournensis*	Silverbelly	14	30	11	48	
Hemiramphidae							
Hyporhampus	*melanochir*	Southern garfish	34	46			•
Heterodontidae							
Heterodontus	*portusjacksoni*	Port Jackson shark	1	2	1	6	
Kyphosidae							
Kyphosus	*sydneyanus*	Silver drummer	7	8			
Labridae							
Coris	*auricularis*	Western king wrasse	4	21	1	2	
Notolabrus	*parilus*	Brownspotted wrasse	10	31	11	35	•
indeterminate		Wrasse sp.	1	1			
Monacanthidae							
Acanthaluteres	*spilomelanurus*	Bridled leatherjacket	50	120	1	2	
Acanthaluteres	*vittiger*	Toothbrush leatherjacket	30	36	1	1	•
Meuschenia	*freycineti*	Sixspine leatherjacket	8	46	6	26	
Monacanthus	*chinensis*	Fanbelly leatherjacket	1	1	1	1	
Scobinichthys	*granulatus*	Rough leatherjacket	1	4	2	6	•
Mugiloidae							
Parapercis	*haackei*	Wavy grubfish	1	2	2	8	
Mullidae							
Upeneichthys	*vlamingii*	Bluespotted goatfish	2	9	2	3	•
Upeneus	indeterminate	Goatfish	1	1			

Table 4.1. Continued.

Taxa		Common Name	BRUVs		Traps		Commercial or Recreational sig.
			MaxN	Sum MaxN	MaxN	Sum MaxN	
Pisces continued							
Myliobatidae							
Myliobatus	*australis*	Southern eagle ray	1	13			•
Nemipteridae							
Pentapodus	*vitta*	Western butterfish	21	139	32	395	•
Odacidae							
Haletta	*semifasciata*	Blue weed whiting	26	29	4	14	
Neoodax	*balteatus*	Little weed whiting	2	3	1	1	
Ostraciidae							
Anoplocapros	*amygdaloides*	Western smooth boxfish	2	25			
Platycephalidae							
Platycephalus	*speculator*	Southern bluespotted flathead	1	1	2	3	•
Platycephalus	*longispinis*	Longspine flathead			4	9	•
Rhinobatidae							
Trygonorhina	*fasciata*	Southern fiddler ray	1	4			
Sillaginidae							
Sillaginodes	*punctata*	King george whiting	2	8	1	1	•
Sillago	*bassensis*	Southern school whiting	12	22	6	23	•
Sillago	indeterminate	Whiting sp.	1	2			•
Sparidae							
Pagrus	*auratus*	Snapper	24	134	9	29	•
Rhabdosargus	*sarba*	Tarwhine	6	9			•
Sphyraenidae							
Sphyraena	*novaehollandiae*	Snook	2	6			
Sphyraena	*spp.*	Striped seapike	200	300			
Tetrarogidae							
Gymnapistes	*marmoratus*	Soldier			1	1	
Terapontidae							
Pelates	*octolineatus*	Western striped grunter	50	299	31	69	•
Tetraodontidae							
Torquigener	*pleurogramma*	Weeping toadfish	40	298	32	127	
Urolophidae							
Trygonoptera	*ovalis*	Striped stingaree	1	1			
Syngnathidae							
Hippocampus	*subelongatus*	West Australian seahorse			1	1	
Unknown			10	14			
Crustacea							
Portunidae							
Portunus	*pelagicus*	Blue swimmer crab	1	6	3	56	•
Thalamita	*sima*	Four-lobed swimmer crab			7	11	
Cephalopoda							
Octopodidae							
Octopus	*sp.*	Octopus			1	2	•
Aves							
Phalacrocoracidae							
Phalacrocorax	*varius*	Pied cormorant	1	3			
Mammalia							
Delphinidae							
Tursiops	*sp.*	Bottlenose dolphin	1	1			
Otariidae							
Neophoca	*cinerea*	Australian sea-lion	1	1			



Snapper *Pagrus auratus*



Western butterfish *Pentapodus vitta*

Figure 4.3. Comparison of distribution of snapper *Pagrus auratus* (above) and western butterfish *Pentapodus vitta* (below) in Cockburn Sound estimated from BRUVs (left) and Opera-house traps (right). Scale for contour plots refers to mean number of fish at each site (n = 3 replicates at each site).

Table 4.2. Summary of the number of sites, replicates, fish species and mean number of fish per replicate for each category of benthic habitat or structure. Note analysis excludes baitfish, *e.g.* Clupeidae species.

Benthic characteristics	Number of			Mean no. fish
	Sites	Replicates	Fish species	per replicate
Reef	3	9	23	75.11
Upper slope	6	18	19	71.50
Seagrass	5	15	22	50.53
Dredged channel	4	12	10	33.58
Rockwall	3	9	18	22.11
Lower slope	9	27	11	7.93
Silt basin (> 15 m)	16	48	10	6.88
Sand Plateau (< 15 m)	5	15	8	4.33

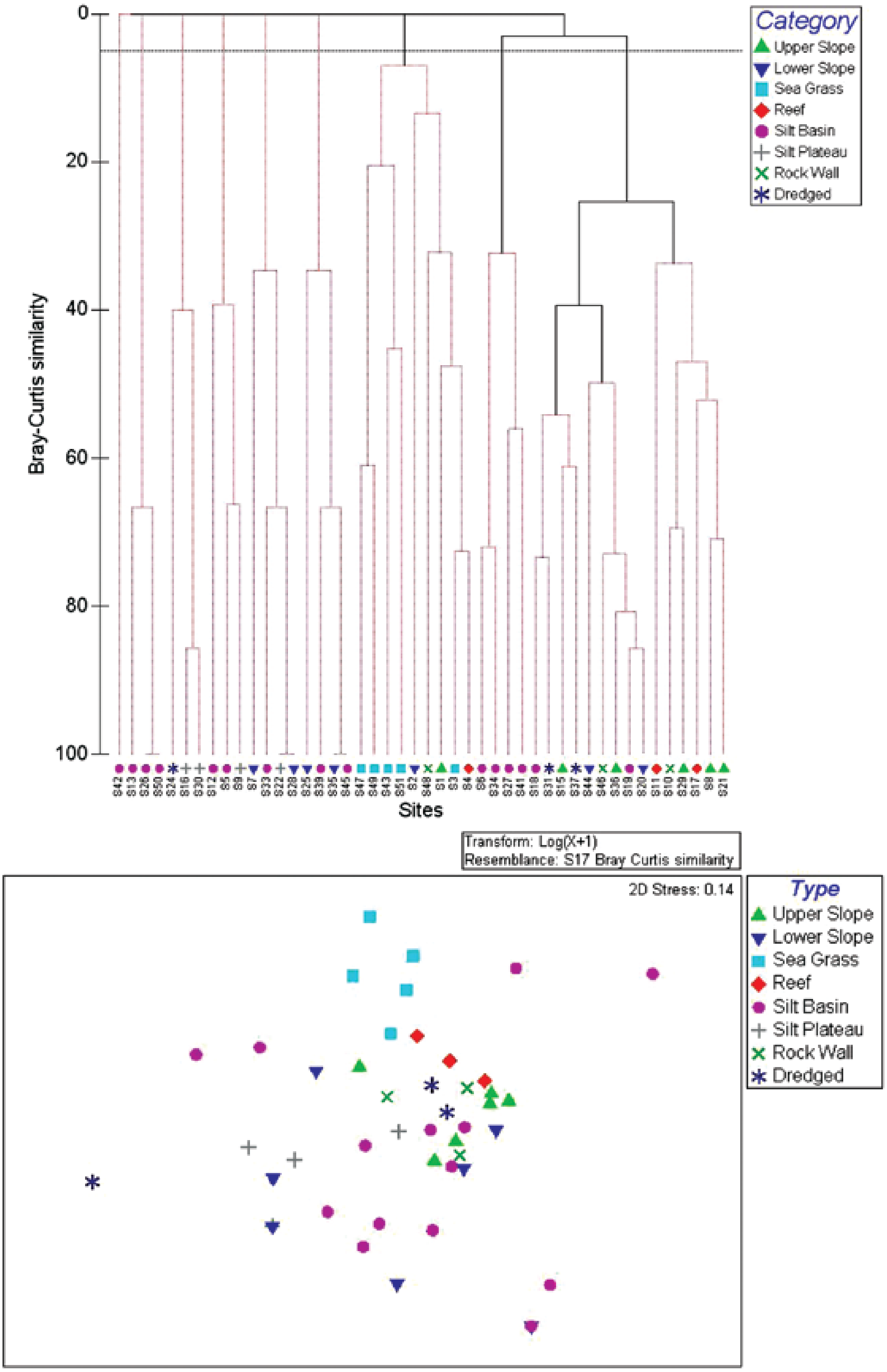


Figure 4.4. Dendrogram (above, groupings at 5% Bray-Curtis similarity) and two-dimensional ordination (below) of mean numbers of fish for each species at each site (n = 3 replicates at each site) and category of benthic habitat or structure from BRUVs. Note analysis excludes baitfish, *e.g.* Clupeidae species.

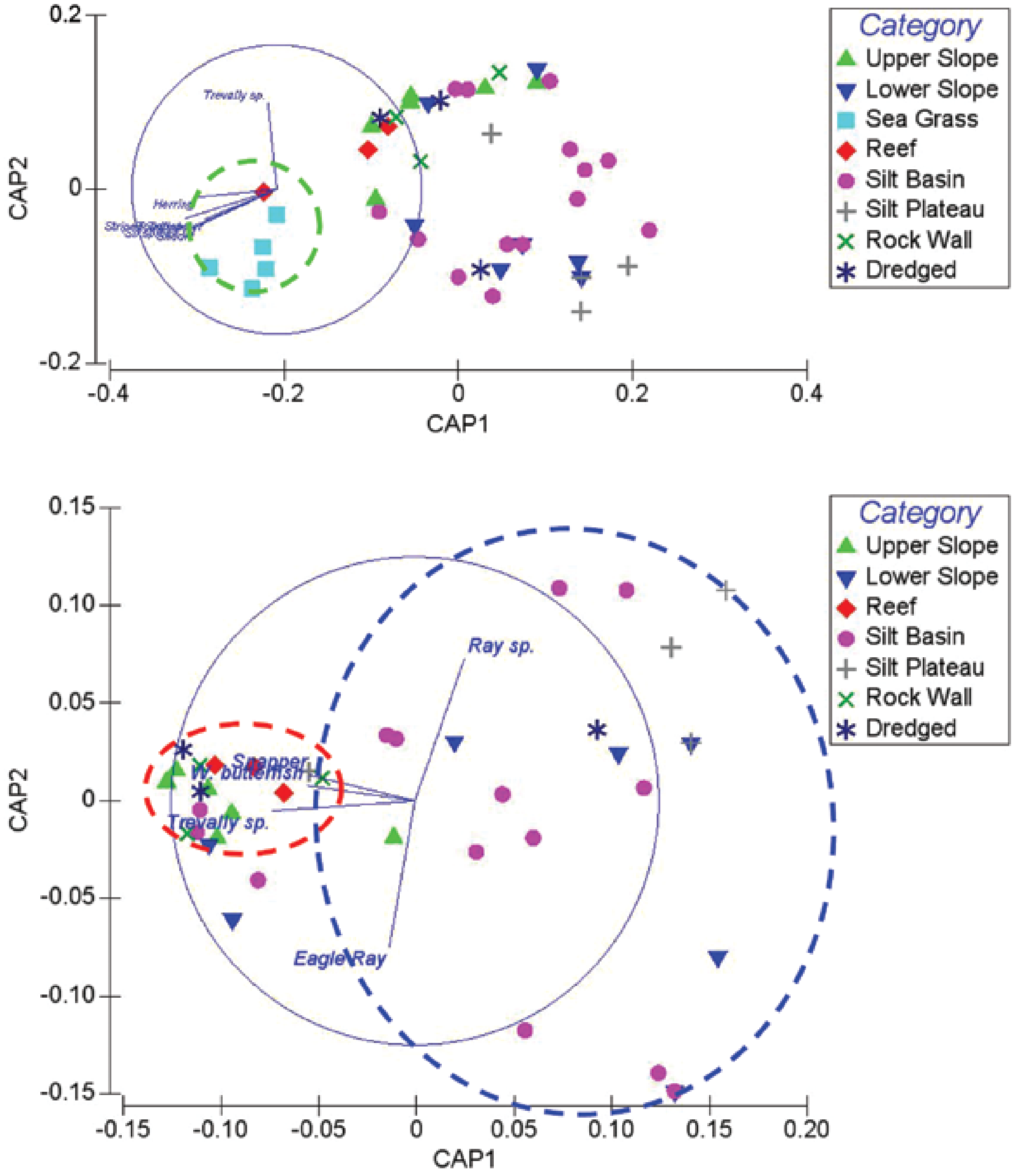
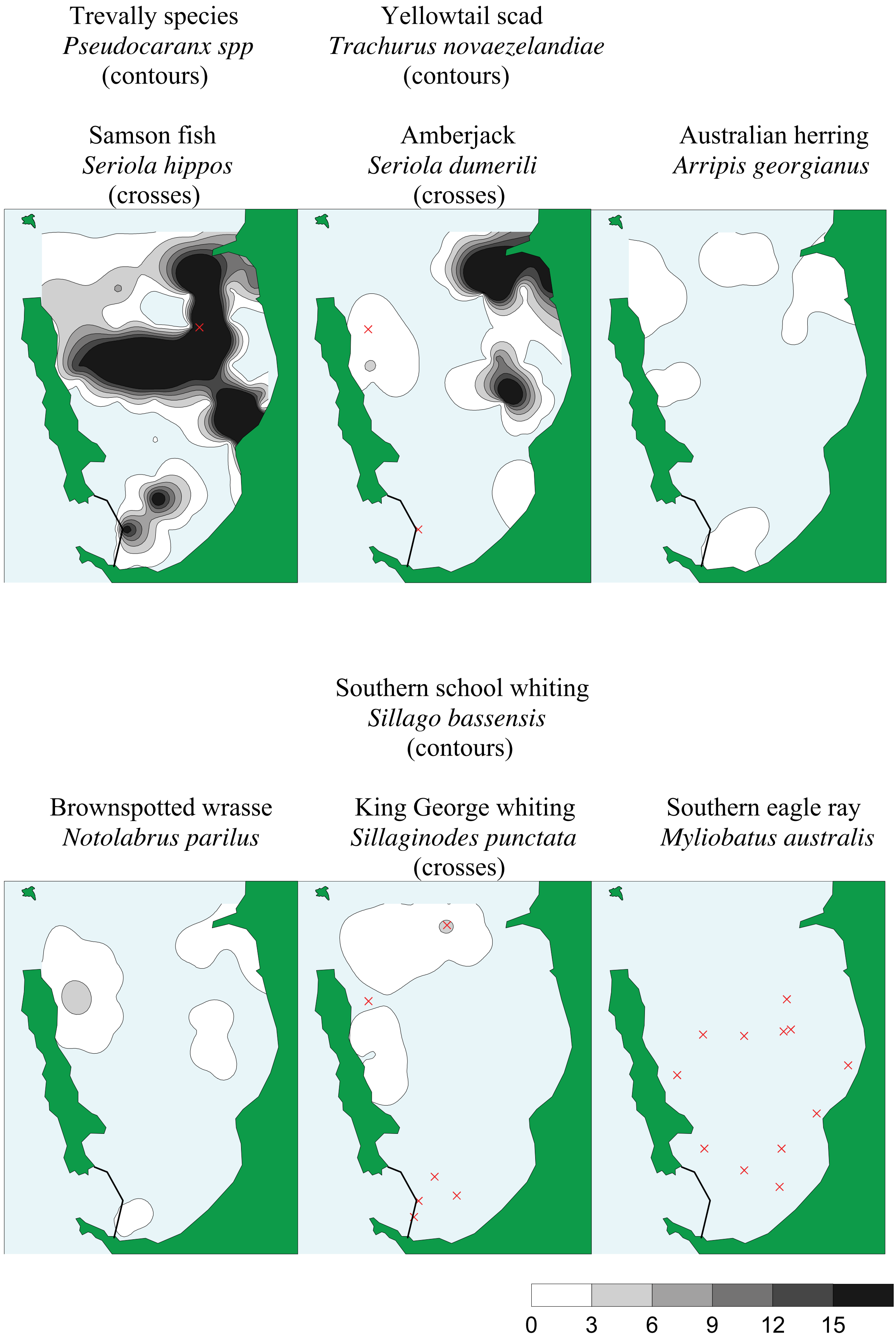


Figure 4.5. Canonical analysis of principal coordinates (CAP) of mean numbers of fish for each species at each site (n = 3 replicates at each site) and all categories of benthic habitat or structure (above) and all categories excluding seagrass (below) from BRUVs. Species with a Pearson's correlation coefficient > 0.43 are plotted and the length of the lines and their direction for each of these species represents the strength of the correlation. Note analysis excludes baitfish, *e.g.* Clupeidae species. Green circle (dashed line, above) denotes seagrass sites, red circle (dashed line, below) denotes categories of benthos that contain some form of hard structure, *i.e.* upper slope, reef and rockwall, and blue circle (dashed line, below) denotes categories of benthos that contain extensive sand areas, *i.e.* lower slope, silt basin, silt plateau. Note the dredged category is split between the two latter groups.


Trevally species
Pseudocaranx spp
(contours)

Yellowtail scad
Trachurus novaezelandiae
(contours)

Samson fish
Seriola hippos
(crosses)

Amberjack
Seriola dumerili
(crosses)

Australian herring
Arripis georgianus

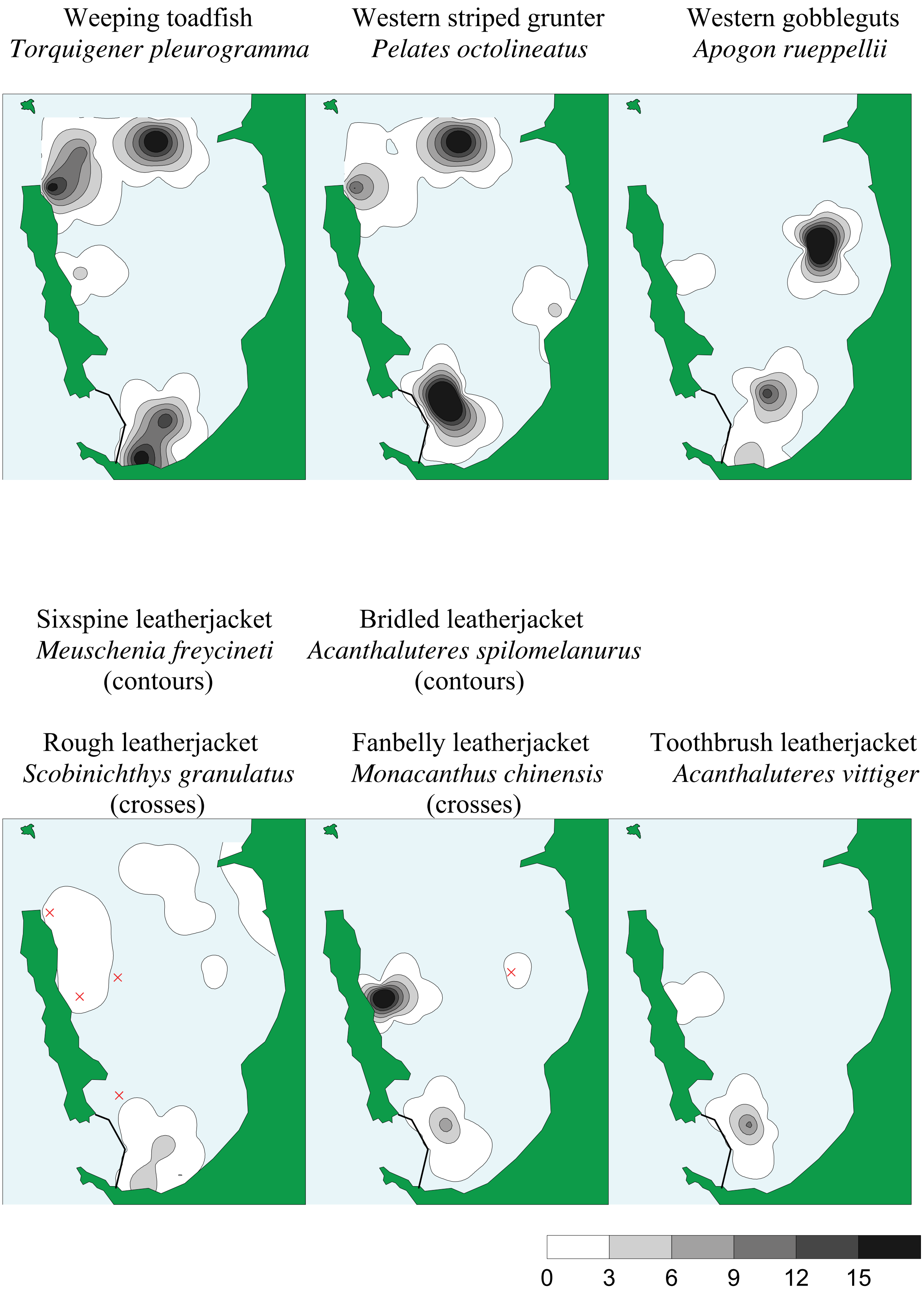
Southern school whiting
Sillago bassensis
(contours)

Brownspotted wrasse
Notolabrus parilus

King George whiting
Sillaginodes punctata
(crosses)

Southern eagle ray
Myliobatus australis

Figure 4.6. Distribution of commercially or recreationally important fish species estimated from BRUVs. Scale for contour plots refers to mean number of fish at each site (n = 3 replicates at each site).



Weeping toadfish
Torquigener pleurogramma

Western striped grunter
Pelates octolineatus

Western gobbleguts
Apogon rueppellii

Sixspine leatherjacket
Meuschenia freycineti
(contours)

Bridled leatherjacket
Acanthaluteres spilomelanurus
(contours)

Rough leatherjacket
Scobinichthys granulatus
(crosses)

Fanbelly leatherjacket
Monacanthus chinensis
(crosses)

Toothbrush leatherjacket
Acanthaluteres vittiger

Figure 4.7. Distribution of fish species that were predominantly associated with seagrass estimated from BRUVs. Scale for contour plots refers to mean number of fish at each site (n = 3 replicates at each site).

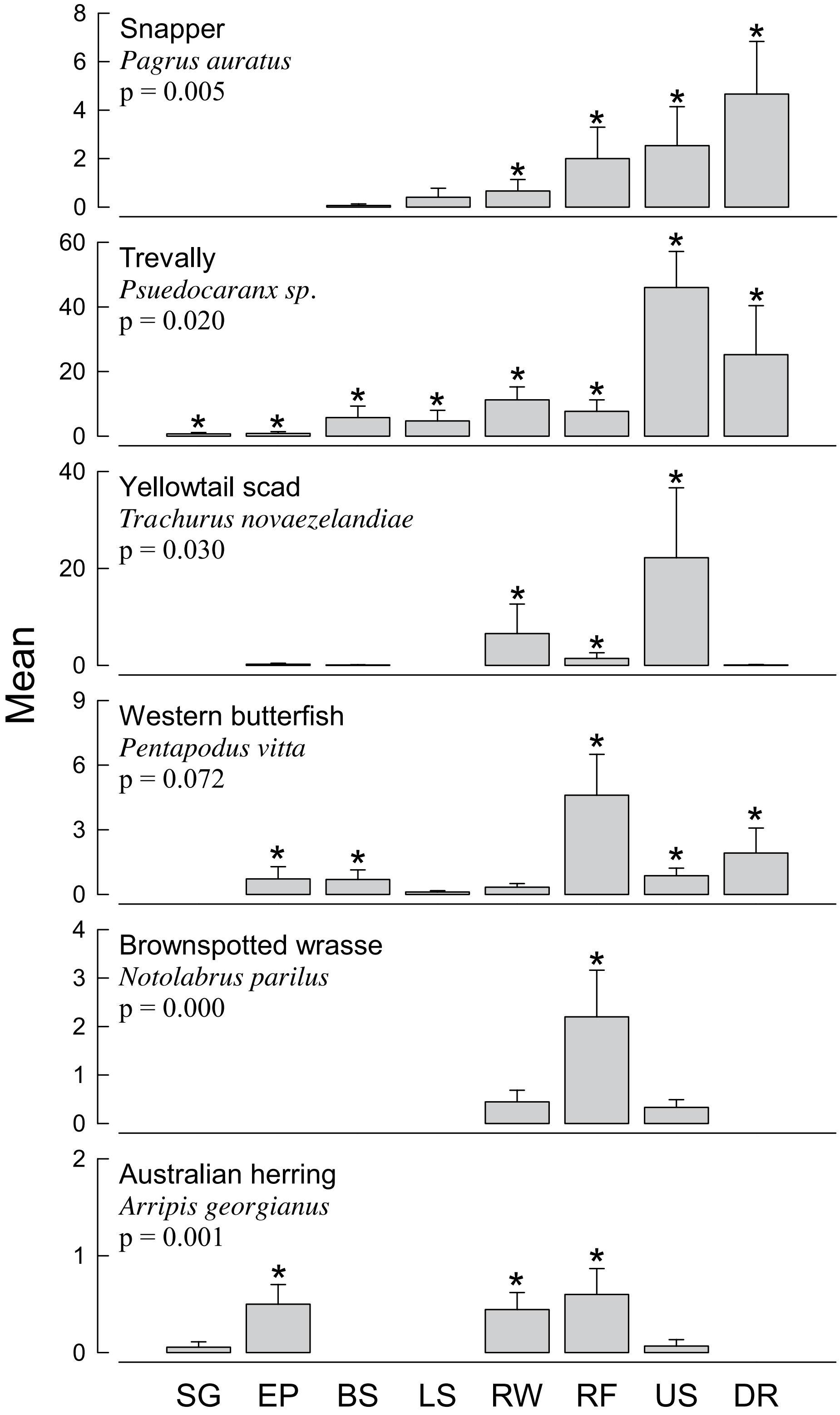


Figure 4.8. Mean (± 1 SE) number of fish for six key commercially or recreationally important species at each category of benthic habitat or structure with the significant categories for each species denoted from BRUVs (*, p values displayed). SG, seagrass; EP, eastern plateau; BS, silt basin; LS, lower slope; RW, rockwall; RF, reef; US, upperslope; DR, dredged channel. Note analysis excludes baitfish, *e.g.* Clupeidae species**.**

4.4 Discussion

The baited remote underwater videos (BRUVs) provided a better description of the fish assemblages in Cockburn Sound than the Opera-house traps. The differences in the number of species and their abundances between the two methods at identical locations provided circumstantial evidence that, in a lot of cases the Opera-house traps falsely provided an absence of many species. This was most likely due to predator-prey interactions in the confined space of the traps between both fish and non-fish species, for example, blue swimmer crabs and octopus were frequently caught in the traps. Conversely, the BRUVs were able to sample species that were attracted by the bait (evident through their feeding behaviour) and those that swam passed. The use of BRUVs has been demonstrated to increase the numbers of predatory or scavenging species without decreasing the abundances of herbivorous or omnivorous species (Harvey *et al.* 2007).

There were broadly three groups of fish assemblages identified from the BRUVs, they included those associated with 1) seagrass, 2) extensive areas of sand or silt and 3) areas comprising some form of limestone structure. A large majority of Cockburn Sound is typically flat and relatively featureless with sand or silt substrate. However, a large majority of demersal fish species were found to occur in the seagrass and limestone reef areas. These habitats have also been demonstrated to be important areas for fish communities in the nearshore waters of WA, outside of this embayment (Howard 1989). A similar number of species were sampled at the rockwall sites compared to those with naturally occurring limestone reef, *i.e.* reef and upper slope. However, the abundances of these species at the rockwall sites were markedly lower, which suggests this artificial structure has a relatively lower carrying capacity for these species than naturally occurring habitats. The numbers of species in the dredged areas were low and similar to those found in the relatively featureless soft sediment areas, *i.e.* silt basin, lower slope and sand plateau. However, the highest numbers of 0+ aged snapper were found in the dredged areas.

Naturally occurring habitats such as areas with small and interspersed outcrops of limestone reef (*e.g.* upper slope), high relief limestone reef (not including rockwall) and seagrass were associated with significantly higher numbers and abundances of fish species. Given the importance of these habitats to the fish communities and the small area they occupy in Cockburn Sound, it is highly recommended that efforts be made to avoid disturbance to these areas from the construction of Kwinana Quay.

This is the first time BRUVs have been used to describe the demersal fish assemblages and their habitat associations in Cockburn Sound. Therefore, the interannual variation in these assemblages needs further investigation. This is particularly true for snapper considering the 2007 year class sampled in this study, was highly abundant, which typically occurs infrequently for this species in Cockburn Sound (Lenanton 1974; Johnston *et al.* 2008). To improve our understanding of the distribution and abundance of snapper, future sampling using BRUVs should also include Warnbro Sound and Owen Anchorage, as annually occurring spawning aggregations of snapper also occur in these areas and given their similar hydrodynamics to Cockburn Sound, it is highly likely that they are also nursery areas for snapper. In addition, given the relative paucity of marine embayments on the lower west coast of WA it is highly likely these embayments contribute a high proportion of recruits to the adult populations along a large part of this coast. Furthermore, as with the situation in Cockburn Sound, the embayments of Warnbro Sound and Owen Anchorage most likely play an integral role in the life history strategies of many other demersal fish species and inclusion during sampling would provide an improved interpretation of the findings in Cockburn Sound.

5.0 Objective 4

C. Wakefield

Objective 4. Describe the movement patterns of adult (mature) snapper relating to their spawning aggregations in the nearshore areas of Cockburn Sound, Owen Anchorage and Warnbro Sound.

The aim of this objective was to use acoustic tags to determine the movement patterns of adult snapper within and between the annually occurring spawning aggregations in Cockburn Sound, Owen Anchorage and Warnbro Sound. The technology involved in this type of research is relatively expensive and could not be reduced to still achieve the desired outcomes. Thus, this research will need to be postponed. This research should be considered if there were thought to be any negative interactions between spawning aggregations of snapper and the Kwinana Quay development or associated increased shipping traffic in the area. These nearshore areas are currently the only known spawning aggregation sites for the depleted snapper stocks along the lower west coast of Western Australia (Wakefield 2006; Lenanton *et al.* 2008). The connectivity between snapper from these spawning aggregations and the contribution of recruitment from these embayments to the larger west coast would be supported by genetic or age-related otolith microchemistry analysis.

6.0 Objective 5

D. Johnston and D. Harris

Objective 5. Investigation of the potential impacts of the Kwinana Quay development on juvenile blue swimmer crab stocks in Cockburn Sound.

6.1 Introduction

The blue swimmer crab fishery in Cockburn Sound started in the 1970s and traditionally used gill nets to supply the domestic Perth market. In 1994/95 the fishery was converted from gill nets to purpose-designed traps to reduce the impact on non-target species. Historically, commercial catches in Cockburn Sound fluctuated dramatically, and were attributed to changes in commercial fishing practices, and normal variations in recruitment strength. However, since 2002/03 commercial catches have declined significantly, with the low stock abundance resulting in closure of the fishery in December 2006. High levels of fishing pressure, particularly on pre-spawn females in winter months, coupled with three years of reduced recruitment due to unfavourable environmental conditions resulted in a significant reduction in the levels of relative egg production and ultimately a decline in stocks. The fishery has remained closed with predicted catches, based on juvenile recruitment indices, below historic levels.

Monitoring of juvenile blue swimmer crab abundance by the Western Australia Department of Fisheries has been conducted annually during peak recruitment months (April-August) since 2002. This data has been used to assess the strength of blue swimmer crab recruitment in the fishery annually and a model developed from which a recruitment index can be used to predict commercial catch in the following season. The current Fremantle Ports project has used the existing juvenile blue swimmer crab recruitment program and added trawl sites in and around the proposed Kwinana Quay development during 2008, to assess short-term and long-term impacts of the various components of Options 1 and 4 on crab juvenile recruitment. Blue swimmer crab nursery areas are characteristically inshore shallow coastal and estuarine waters that provide necessary habitat such as seagrass beds and have high abundances of potential prey items including benthic infauna. Examples of these recruitment areas within Cockburn Sound include Mangles Bay and the Eastern Shelf (depths < 10 m) located north of James Point.

6.2 Methods

6.2.1 Sampling design

Research trawling to collect data on juvenile blue swimmer crab abundance in Cockburn Sound has been conducted annually by the Department of Fisheries since 2002, as part of a long-term annual assessment of the strength of crab recruitment. During 2008, three replicate 750 m trawls were undertaken at six sites monthly between February and August as part of the long-term monitoring program. A further nine sites were sampled in each of these months in and around the proposed site of the Kwinana Quay development in Jervoise Bay (Fig. 6.1).

Trawling was conducted over three consecutive nights each month aboard a 7.3 m DoF research vessel at a speed of 2.8 kts, commencing 30 minutes after sunset. The trawl net employed had a headrope of 4.5 m, with 2 inch (*ca* 5.1 cm) mesh in the panels and 9 mm roschell mesh in the codend. The effective spread of the net on the seabed was estimated as 0.6 x net headrope length (m), giving the net an effective opening of ~3 m wide by 0.4 m high. The net was fitted with 8mm ground chain and 6mm drops, with the ground chain set two links ahead of the footrope. The wooden otter boards measured 615, 320 and 50 mm. A warp length to depth ratio of 5:1 was observed.

Biological data collected from each trawl included crab carapace width (the distance between the tips of the two lateral spines of the carapace) measured to the nearest millimetre, sex, moult stage (soft, hard) and female breeding condition.

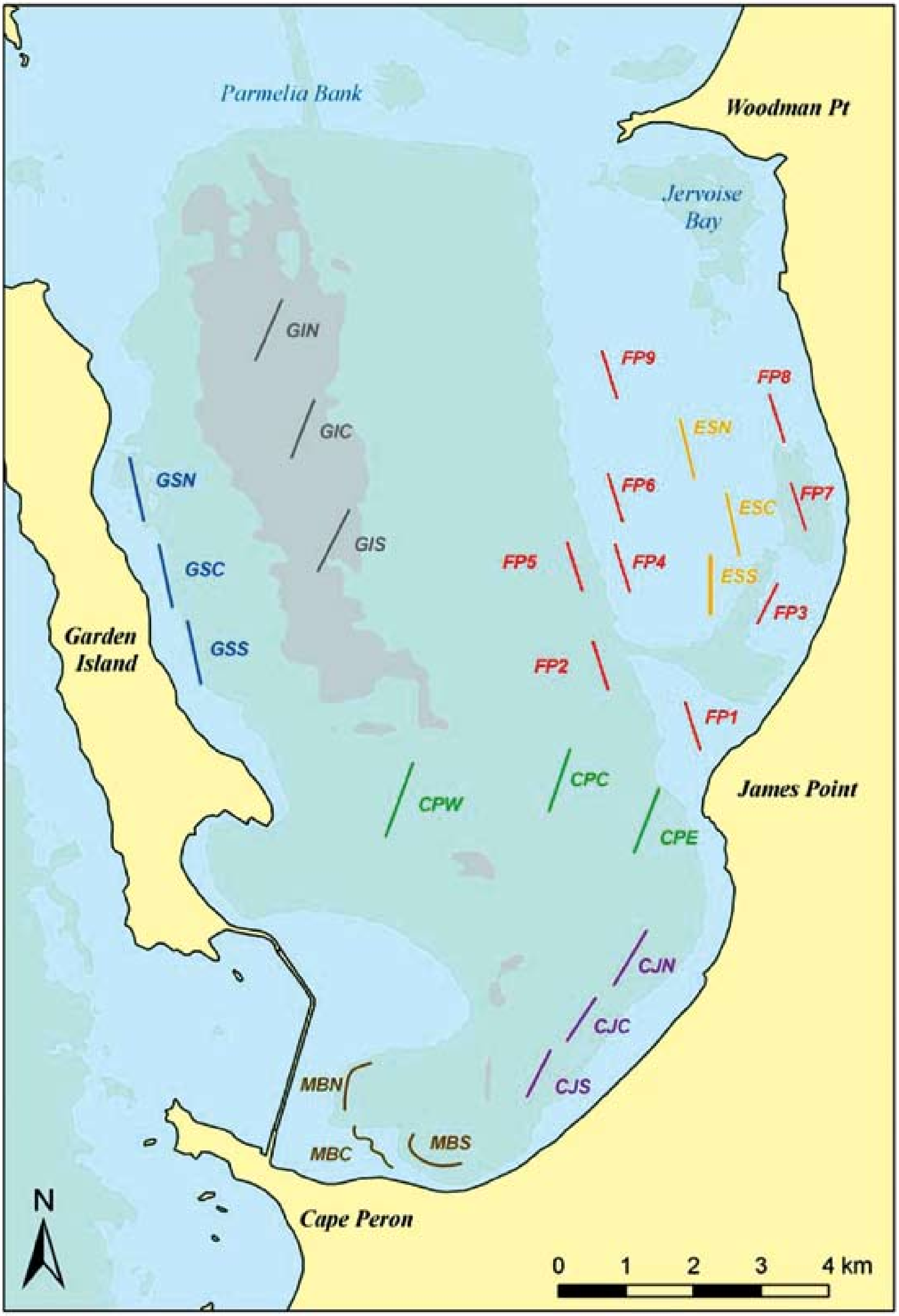


Figure 6.1. Juvenile blue swimmer crab (recruitment) sites trawled monthly between February and August 2008. (MBS – Mangles Bay south; MBC – Mangles Bay centre; MBN – Mangles Bay north; CJS – CBH Jetty south; CJC – CBH Jetty centre; CJN – CBH Jetty north; CPE – Colpoy's Point east; CPC – Colpoy's Point centre; CPW – Colpoy's Point west; GSS – Garden Island Shore south; GSC – Garden Island Shore centre; GSN – Garden Island Shore north; GIS – Garden Island Deep south; GIC – Garden Island Deep centre; GIN – Garden Island Deep north; ESS – Eastern Shelf south; ESC – Eastern Shelf centre; ESN – Eastern Shelf north; FP1-FP9 – Fremantle Ports sites 1 – 9).

6.2.2 Data analysis

An assessment of the potential impact of both Option 1 (Island only) and Option 4 (Island and Land-back) of the Kwinana Quay development was determined for three different components (Fig. 6.2):

Component 1: Footprint.	Juvenile crab habitat that will be permanently lost following reclamation of the physical area for the development.
Component 2: Footprint & Channels.	The area of juvenile crab habitat to be lost as part of the Footprint or modified through initial and maintenance dredging for the shipping Channels. Also represents the area lost or restricted to fishing.
Component 3: Vicinity.	The area of Cockburn Sound that may be affected during the construction of the development, also incorporates the Footprint and Channel areas. In the absence of specific data defining the area of potential impact from dredging and construction, an arbitrary boundary encompassing sampling sites around the Kwinana Quay development was used to define the Vicinity. This area can be refined after impacts from construction and dredging are determined.

The area covered by each of these components was calculated using shape-file geometry in ArcGIS (Version 9.3) and presented as a proportion of the total nursery area within Cockburn Sound (Figs 6.2 & 6.3, Table 6.1). These nursery or recruitment areas have been previously determined during the Department of Fisheries long term monitoring program of juvenile recruitment in Cockburn Sound.

The numbers of juvenile (0+) crabs in each trawl was determined by fitting a length frequency distribution of the catch to a probability model that takes into account mean lengths and standard deviations in length for various age-classes of crabs to assign an individual to an age cohort (Schnute & Fournier 1980). The model assumes age cohorts are normally distributed, and a chi-squared statistic was used to choose between competing solutions to the model. The catch from each trawl at a given site was grouped into 5 mm size classes, and the resulting length frequency distributions fitted to the model to differentiate the juvenile cohort (spawned the previous year) from the residual (1+) stock. The model provided an abundance of juveniles for each 5 mm size class, which were summed to provide the total number of juvenile crabs in that trawl. Distributions of male and female juvenile crabs were found to be homogenous so sexes were pooled for each sample. The size of recruits differed slightly between sites depending on the structure of the crab population trawled at that site, but also differed throughout the year as the cohort grew with recruits ranging in size from as little as 30mm CW in February to 120 mm CW in August.

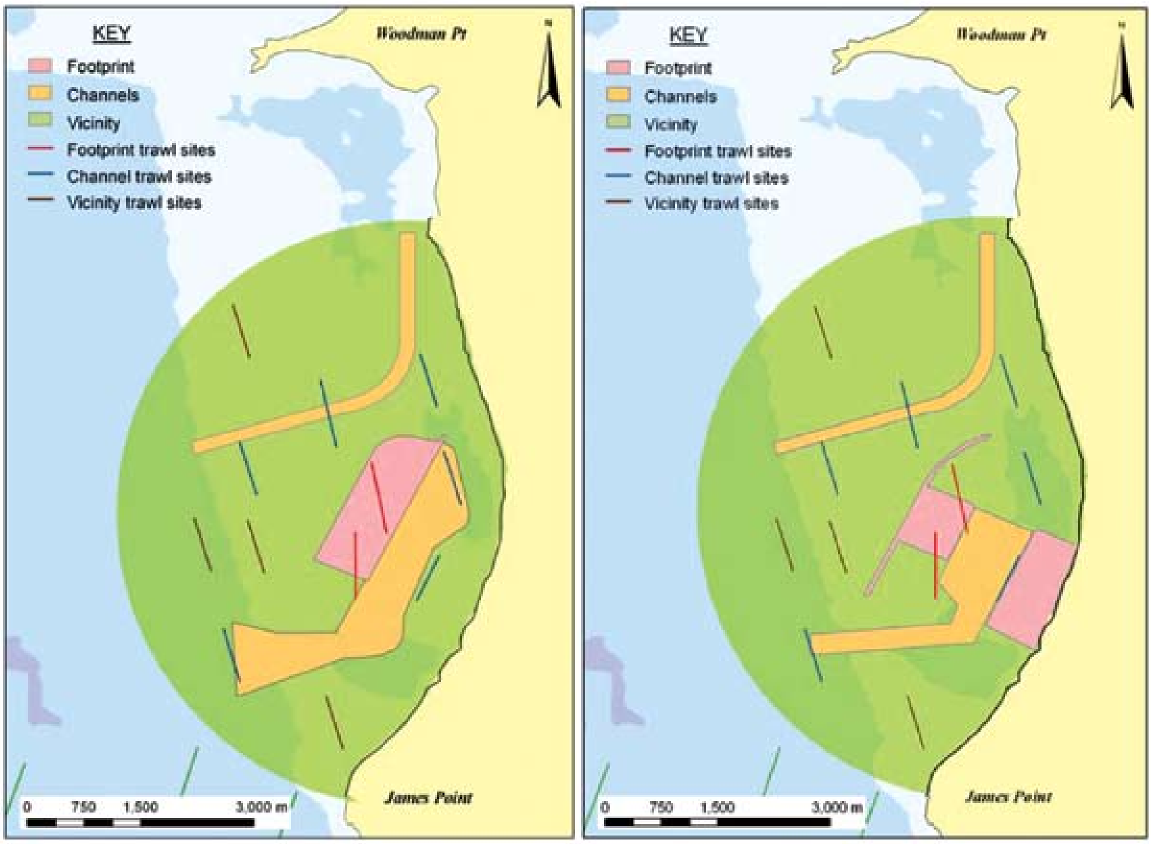


Figure 6.2. Juvenile trawl sites used to provide representative catch rates of each component for Option 1 (island only on the left) and Option 4 (island and land-back on the right) of the proposed Kwinana Quay development.

Monthly juvenile catch rates (number of juvenile crabs caught per square metre of ground trawled) were determined for each trawl site, and then averaged across months to provide an overall catch rate for that site for 2008. Specific trawl sites were considered representative of each component (Fig. 6.2; Table 6.1), with the mean of the overall catch rates of these sites providing a representative catch rate for that component. This representative catch rate was assumed to be uniform across the area covered by the component. The contribution of each component to the 2008 recruitment was calculated by multiplying the representative catch rate of the component by its area (in square metres). Finally, this contribution was presented as a proportion of the total recruitment of juvenile blue swimmer crabs to the Cockburn Sound crab stock for 2008.

Table 6.1. Area of each component and the juvenile trawl sites used to calculate representative recruitment catch rates for each component for Option 1 and Option 4 of the proposed Kwinana Quay development. Asterisks indicate that all the sites from that location were used in the analysis of that component (*e.g.* ES* means ESS, ESC and ESN were incorporated).

COMPONENT		**OPTION 1**		**OPTION 4**	
		Area (ha)	**Sites**	**Area (ha)**	**Sites**
Footprint	Island	126	ESS, ESC	143	ESS, ESC
	Land-back		–		FP3
Footprint & Channels		350	ES*, FP2,3,6,7,8	327	ES*, FP2,3,6,8
Vicinity (inc. Footprint & Channels)		3507	ES*, FP*	3507	ES*, FP*
Rest of Recruitment Area	James Pt	462	CPE, CPC	462	CPE, CPC
	Kwinana	498	CJ*	498	CJ*
	Mangles Bay	484	MB*	484	MB*
	Garden Island	289	GS*	289	GS*
TOTAL RECRUITMENT AREA		5240		5240	

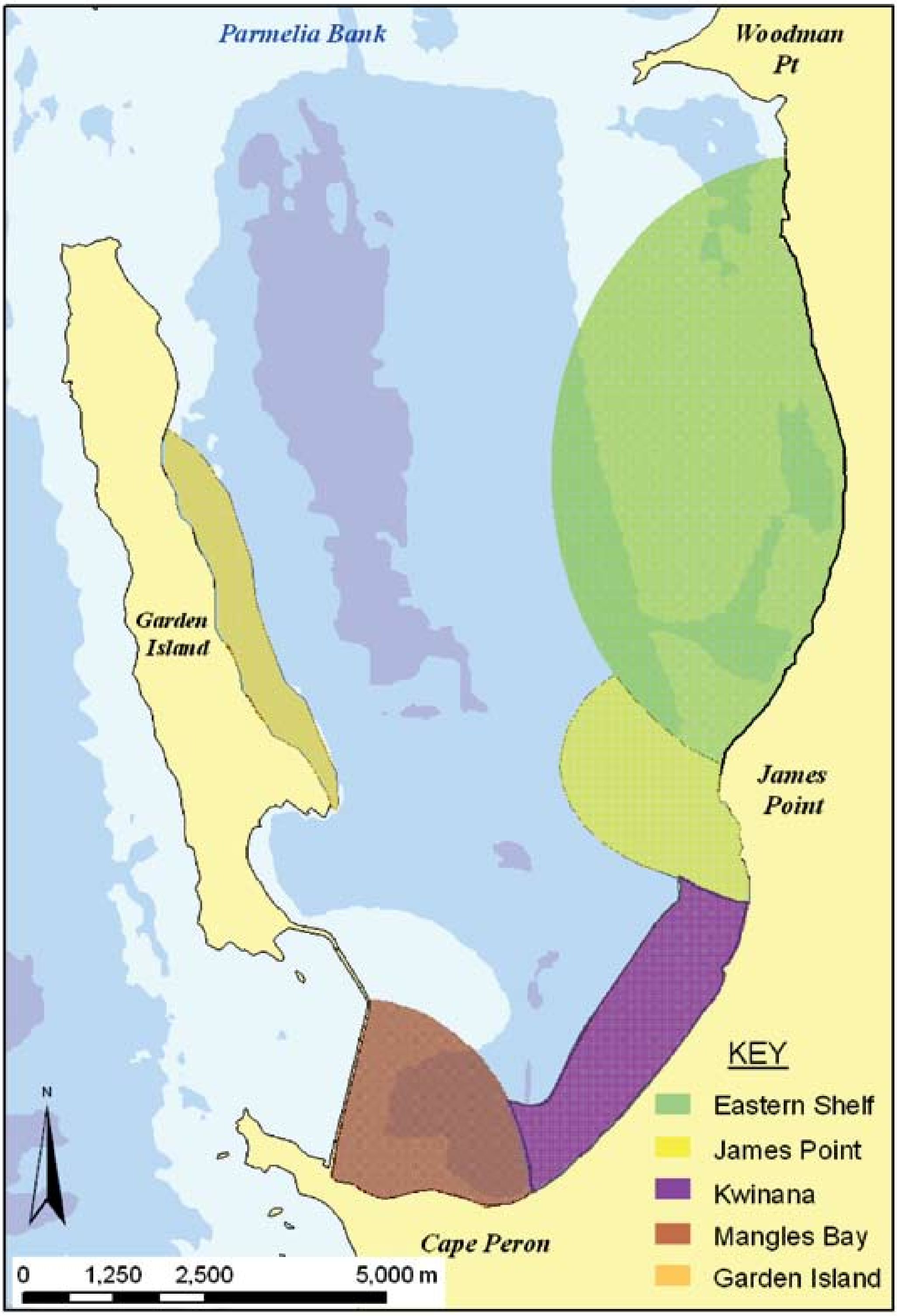


Figure 6.3. Juvenile blue swimmer crab nursery areas in Cockburn Sound used to calculate the likely impact on recruitment of the Kwinana Quay development north of James Point. These areas have been determined as important nursery areas for blue swimmer crabs based on the Department of Fisheries long-term juvenile recruitment monitoring program.

6.3 Results

A total of 189 trawls were undertaken between February and August 2008 in Cockburn Sound to collect data on juvenile blue swimmer crab abundance.

The physical area covered by the Footprint of Option 1 represents two percent of the total blue swimmer crab nursery area in Cockburn Sound. The mean catch rate within the Footprint of 0.011 juveniles.m^{-2} (which equates to approximately 14000 juveniles) represents 3% of the total recruitment to blue swimmer crab stocks in Cockburn Sound for 2008 (Table 6.2). The physical area of the Footprint for Option 4 (Island and Land-back) also covers three percent of the crab nursery grounds in the Sound but contributed 4% of the 2008 recruitment (Table 6.3).

The Footprint & Channels component of Option 1 covers 7% of Cockburn Sound's crab nursery area. The eight trawl sites representative of this area had a mean catch rate of 0.015 juvenile crabs.m^{-2}, equating to approximately 45000 juveniles or 9% of the 2008 recruitment (Table 6.2). In comparison, the area covered by the Footprint & Channels of Option 4 accounts for 6% of the juvenile grounds and contributed 11% of the 2008 recruitment (Table 6.3).

The Vicinity (for both Option 1 and Option 4) accounts for 67% of the physical area of Cockburn Sound's nursery grounds. However, the mean catch rate of 0.012 juveniles.m^{-2} across the 12 sites that were considered representative of this area equated to 421000 juvenile crabs, or 83% of the blue swimmer crab recruitment to the Sound for 2008 (Tables 6.2 & 6.3).

Finally, the remainder of the nursery areas in Cockburn Sound contributed just over 17% of the 2008 crab recruitment, despite covering 33% of the physical nursery grounds (Tables 6.2 & 6.3).

Table 6.2. Area, catch rate and proportion of juvenile crabs represented within each component for Option 1 (Island only) of the proposed Kwinana Quay development.

COMPONENT	AREA		JUVENILE CRABS		
	ha	%	crabs.m^{-2}	Total	%
Footprint	126	2	0.011	14000	3
Footprint & Channels	350	7	0.015	45000	9
Vicinity	3507	67	0.012	421000	83
Rest of Recruitment Area	1733	33	0.007	90000	17

Table 6.3. Area, catch rate and proportion of juvenile crabs represented within each component for Option 4 (island and land-back) of the proposed Kwinana Quay development.

COMPONENT	AREA		JUVENILE CRABS		
	ha	%	crabs.m^{-2}	Total	%
Footprint	143	3	0.013	22000	4
Footprint & Channels	327	6	0.016	57000	11
Vicinity	3507	67	0.012	421000	83
Rest of Recruitment Area	1733	33	0.007	90000	17

6.4 Discussion

This project represents a preliminary assessment of the impacts of the proposed Kwinana Quay development on juvenile blue swimmer crabs and is based solely on the 2008 data collected. Consequently, discussion of the data is limited to crab stocks sampled during 2008. Future additional monitoring will be needed prior to, during and after the dredging and construction phases to accurately assess the short term and long term impacts of the proposed Kwinana Quay development on crab stocks. This discussion is also limited to blue swimmer crabs and does not comment on broader biodiversity implications of the development.

The mean catch rate and proportion of juvenile crabs represented within the Footprint of Options 1 and 4 of the proposed Kwinana Quay development were relatively low (3% and 4%, respectively) indicating that the actual physical area encompassed by the footprint is likely to have minimal long-term impact on crab recruitment in Cockburn Sound.

The Footprint and Channels of Option 1 represent an increased proportion of juvenile crabs (9%). The Footprint and Channels of Option 4 represent a similar area to Option 1 but a greater proportion of juvenile crabs (11%), despite the actual dredged area being less than Option 1. This is due to high catch rates of juvenile crabs at the site representing the land-backed component of the Footprint for Option 4 (FP3). Although dredged areas do not represent a direct loss of habitat to blue swimmer crabs, as they are frequently caught in these areas. Changes in their abundances in dredged areas are uncertain and may fluctuate depending on the frequency of maintenance dredging.

The area surrounding the site of the proposed Kwinana Quay development, *i.e.* the Vicinity, represented 67% of the area considered to be important for blue swimmer crab recruitment in Cockburn Sound. The catch rates of juvenile blue swimmer crabs in this area on the Eastern Shelf was also significantly higher, with an average of 0.012 m^{-2} compared to 0.07 m^{-2} for the rest of the Recruitment Areas. Thus, the proportion of juvenile crabs that occurred in the Vicinity of the proposed site of Kwinana Quay development represented 83% of recruits for Cockburn Sound in 2008. Therefore, any detrimental influence from the construction of Kwinana Quay could potentially have a large impact on recruitment of blue swimmer crabs in Cockburn Sound. It is recognised that an assessment of the potential impacts of the Kwinana Quay development on juvenile crab recruitment will be difficult until short-term and long-term environmental changes associated with each option have been determined.

Considering blue swimmer crabs are short lived and recruitment is almost exclusively from within Cockburn Sound (deemed to be essentially an independent stock, see Chaplin & Sezmis 2008, Appendix 1), one or two years of induced low recruitment could compromise the stocks viability for a much longer period. This is evident from the slower than expected rebuilding of crab stocks in Cockburn Sound following a significant decline in biomass between 2002 and 2006. The cause of this decline most likely resulted from a combination of high fishing pressure on pre-spawning females in winter and consecutive years of low recruitment due to cooler than average water temperatures. Currently, the risk of fishing to the blue swimmer crab stocks in Cockburn Sound is non-existent, as a closure to commercial and recreational fishing for crabs has been in place since December 2006. Following the eventual reopening of the crab fishery in Cockburn Sound, the risk to stocks from fishing will be kept low by imposing regulations to limit catch and reduce effort to ensure their sustainability. It is difficult to assign a level of risk to naturally occurring environmental cycles that influence recruitment. Future management regimes for this fishery will account for any environmental influences that may adversely affect recruitment. Thus, an important risk to blue swimmer crab stocks in Cockburn Sound will be the potential impact to recruitment from the Kwinana Quay development, given its proximity to the major recruitment area.

7.0 Objective 6

D. Johnston and D. Harris

Objective 6. Investigation of the potential impacts of the Kwinana Quay development on adult blue swimmer crab stocks in Cockburn Sound.

7.1 Introduction

Adult stocks of blue swimmer crab in Cockburn Sound form the basis of what was, until recently, a highly productive commercial and recreational crab fishery. The Western Australia Department of Fisheries has conducted a commercial monitoring program of catch and effort data since 1999, in addition to compulsory catch and effort returns, to provide a good understanding of adult stocks in the area. Following the closure of this fishery in December 2006, a commercial vessel has been contracted to continue the commercial monitoring program and provide information pertinent to the recovery of stocks. The current Fremantle Ports project has used this vessel to provide additional data on the potential impacts of the proposed Kwinana Quay development on adult crab stocks in Cockburn Sound, by placement of additional potlines in and around the proposed site during 2008. Results from this potting program will be presented in this chapter. In addition, the data will be compared to our historical data on adult stocks to provide a preliminary assessment of the potential short-term and long-term impacts of the various components of Options 1 and 4 of the Kwinana Quay development.

7.2 Methods

7.2.1 Sampling design

Commercial monitoring of blue swimmer crab catch and effort data in Cockburn Sound has been conducted by the Department of Fisheries since 1999. Fisheries staff accompanied commercial fishers during normal daily fishing operations to quantify that day’s catch. Different fishers are surveyed each month during the commercial crabbing season (December through October of the following year). Each fisher services between 100-200 hourglass traps a day, set in lines of 10-20 traps. The carapace width (the distance between the tips of the two lateral spines of the carapace) of each crab in a line is measured to the nearest millimetre and it’s sex recorded, along with information on moult stage (soft, hard) and female breeding condition. The number of pots in that line is noted, along with the soak time (number of hours the pots have been in the water since they were last serviced) and the latitude, longitude and depth. Initially three days were monitored during each month of the fishing season, but this was refined to two days per month from 2005.

Following the closure of the fishery in December 2006, a commercial fisher has been contracted by the Department of Fisheries to replicate commercial fishing in Cockburn Sound. Accompanied by research staff, the fisher set 100 hourglass pots twice a month throughout the traditionally fished areas. To assess the potential impact of the Kwinana Quay development, the fisher has set an additional 20 pots (four lines of five pots) in and around the proposed site in Jervoise Bay during 2008 (Fig. 7.1). It is important to note that the fishing and monitoring procedures were identical to that of pre-2006 except that all crabs were returned to the water at their place of capture during sampling from 2006 onwards.

The traps used in all years sampled were collapsible, with a metal base ring and a pneumatic upper ring to set the trap. They measured 1.2 m in diameter and 0.4 m in height, with an internal volume of *ca* 0.2 m^3. The lower half of the trap was fitted with 2" (stretched) mesh and the upper half with 3.5" (stretched) mesh. The soak time for all traps was 24 hours.

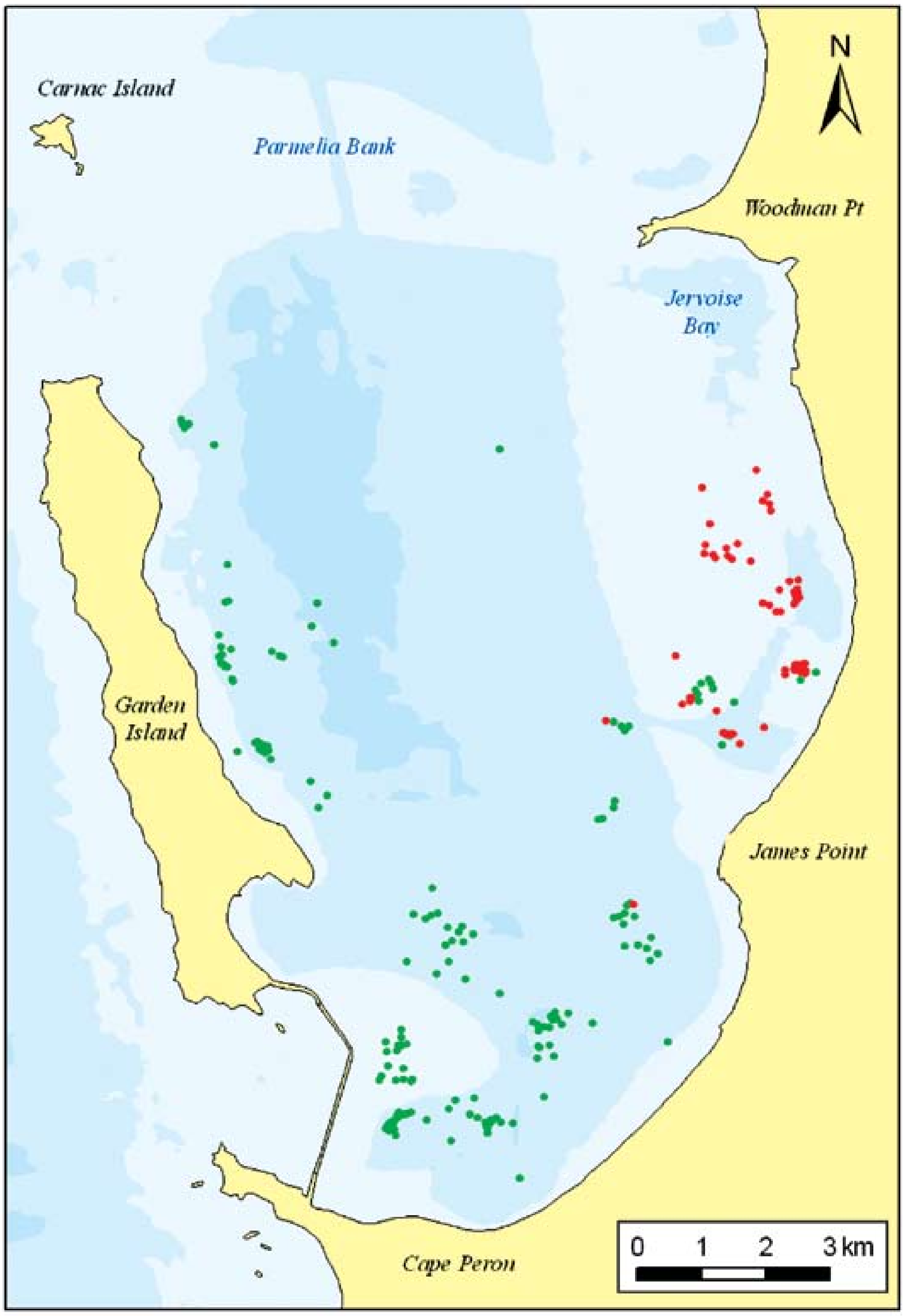


Figure 7.1. Location of potlines for the long-term commercial monitoring program (●) and Fremantle Port (●) sites in 2008.

7.2.2 Data analysis

An assessment of the potential impact of both options of the proposed Kwinana Quay development on the adult blue swimmer crab stocks in Cockburn Sound was undertaken for three different components (Fig. 7.2):

Component 1: Footprint.	Juvenile crab habitat that will be permanently lost following reclamation of the physical area for the development.
Component 2: Footprint & Channels.	The area of juvenile crab habitat to be lost as part of the Footprint or modified through initial and maintenance dredging for the shipping Channels. Also represents the area lost or restricted to fishing.
Component 3: Vicinity.	The area of Cockburn Sound that may be affected during the construction of the development, also incorporates the Footprint and Channel areas. In the absence of specific data defining the area of potential impact from dredging and construction, an arbitrary boundary encompassing sampling sites around the Kwinana Quay development was used to define the Vicinity. This area can be refined after impacts from construction and dredging are determined.

The area covered by each of these components was calculated using shapefile geometry in ArcGIS (Version 9.3) and presented as a proportion of the total area of Cockburn Sound (Fig. 7.2, Table 7.1).

Table 7.1. Area of each component for Option 1 and 4 of the proposed Kwinana Quay development.

COMPONENT	AREA (ha)	
	Option1	Option 4
Footprint	126	143
Footprint & Channels	350	327
Vicinity (inc. Footprint & Channels)	3507	3507
Rest of Cockburn Sound	6493	6493

2008 data

Samples collected during monthly catch monitoring surveys in 2008 were pooled across the year to create a robust sample size for each component. Catch rates (number of crabs per potlift) were calculated for each individual potline. A representative catch rate for each component was then determined by averaging the catch rates of the potlines in the immediate vicinity of that component (Fig. 7.2).

For components that were composed of several smaller areas, such as the Footprint of Option 4 and both the Footprint and Channels of both options, a weighted catch rate was calculated. A weighted catch rate for the Footprint and Channels in Option 1 was calculated using the formula:

Option 1

$$\text{Footprint \& Channels catch rate} = \left(\frac{x_1}{x}\right)CR_1 + \left(\frac{x_2}{x}\right)CR_2 + \left(\frac{x_3}{x}\right)CR_3$$

where x = the total area of the Footprint and Channels in Option 1, x_1 = area of the Footprint, x_2 = area of the northern channel, x_3 = area of the southern channel, CR_1 = the mean catch rate of potlines in the immediate vicinity of the Footprint, CR_2 = the mean catch rate of potlines in the immediate vicinity of the northern channel and CR_3 = the mean catch rate of potlines in the immediate vicinity of the southern channel.

A weighted catch rate for the Footprint in Option 4 was calculated using the formula:

Option 4

$$\text{Footprint catch rate} = \left(\frac{x_1}{x}\right)CR_1 + \left(\frac{x_2}{x}\right)CR_2$$

where x = the total area of the Footprint in Option 4, x_1 = area of the island component of the Footprint, x_2 = area of the land-back component of the Footprint, CR_1 = the mean catch rate for the potlines in the immediate vicinity of the island component of the Footprint, CR_2 = the mean catch rate for the potlines in the immediate vicinity of the land-back component of the Footprint.

A weighted catch rate for the Footprint and Channels in Option 4 was calculated using the formula:

Option 4

$$\text{Footprint \& Channels catch rate} = \left(\frac{x_1}{x}\right)CR_1 + \left(\frac{x_2}{x}\right)CR_2 + \left(\frac{x_3}{x}\right)CR_3 + \left(\frac{x_4}{x}\right)CR_4$$

where x = the total area of the Footprint and Channels in Option 4, x_1 = area of the island component of the Footprint, x_2 = area of land-back component of the Footprint, x_3 = area of the northern channel, x_4 = area of the southern channel, CR_1 = the mean catch rate for the potlines in the immediate vicinity of the island component of the Footprint, CR_2 = the mean catch rate for the potlines in the immediate vicinity of the land-back component of the Footprint, CR_3 = the mean catch rate for the potlines in the immediate vicinity of the northern channel and CR_4 = the mean catch rate for the potlines in the immediate vicinity of the southern channel.

This mean catch rate was then multiplied by the area (in hectares) of that component (Table 7.1) to provide an index of relative abundance for each area. This index allows for the comparison of that component's adult blue swimmer crab population with the rest of Cockburn Sound.

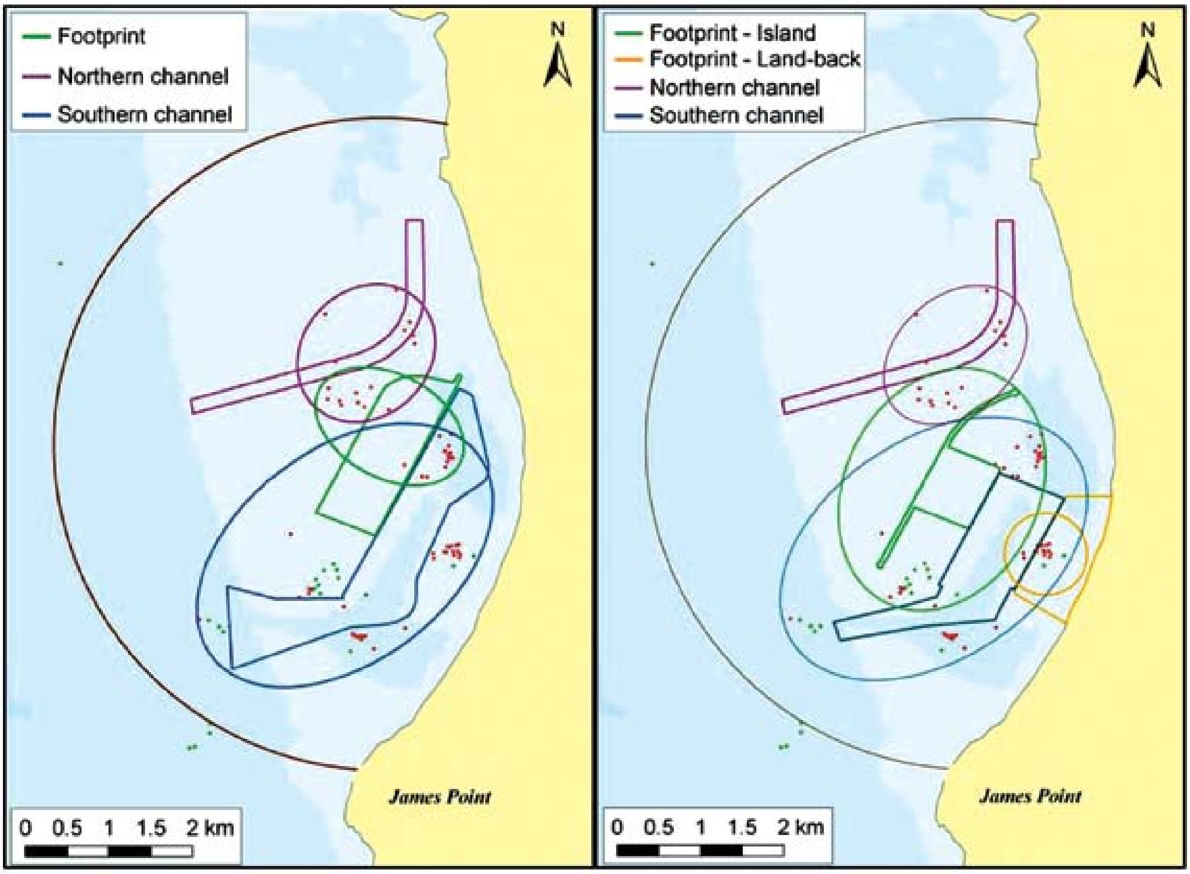


Figure 7.2. Locations of 2008 potlines used to provide representative catch rates of each component for Option 1 (Island only on the left) and Option 4 (Island and land-back on the right) of the proposed Kwinana Quay development.

Historic data (1999-2006)

A catch rate (number of crabs per potlift) was calculated for each individual potline sampled during monthly catch monitoring surveys between 1999 and 2006 (Fig. 7.4). These individual catch rates were then pooled across years to provide a more robust sample size for each component. A representative catch rate for each component was then determined by averaging the catch rates of the potlines in the immediate vicinity of that component (Fig. 7.3).

For components that were composed of several smaller areas, such as the footprint of Option 4 and the area of the Footprint and Channels for both options, a weighted catch rate was calculated.

A weighted catch rate for the Footprint and Channels in Option 1 was calculated using the formula:

Option 1

$$\text{Footprint \& Channels catch rate} = \left(\frac{x_1}{x}\right)CR_1 + \left(\frac{x_2}{x}\right)CR_2 + \left(\frac{x_3}{x}\right)CR_3$$

where x = the total area of the Footprint and Channels in Option 1, x_1 = area of the Footprint, x_2 = area of the northern channel, x_3 = area of the southern channel, CR_1 = the mean catch rate of potlines in the immediate vicinity of the Footprint, CR_2 = the mean catch rate of potlines in the immediate vicinity of the northern channel and CR_3 = the mean catch rate of potlines in the immediate vicinity of the southern channel.

A weighted catch rate for the Footprint in Option 4 was calculated using the formula:

Option 4

$$\text{Footprint catch rate} = \left(\frac{x_1}{x}\right)CR_1 + \left(\frac{x_2}{x}\right)CR_2$$

where x = the total area of the Footprint in Option 4, x_1 = area of the island component of the Footprint, x_2 = area of the land-back component of the Footprint, CR_1 = the mean catch rate for the potlines in the immediate vicinity of the island component of the Footprint, CR_2 = the mean catch rate for the potlines in the immediate vicinity of the land-back component of the Footprint.

A weighted catch rate for the Footprint and Channels in Option 4 was calculated using the formula:

Option 4

$$\text{Footprint \& Channels catch rate} = \left(\frac{x_1}{x}\right)CR_1 + \left(\frac{x_2}{x}\right)CR_2 + \left(\frac{x_3}{x}\right)CR_3 + \left(\frac{x_4}{x}\right)CR_4$$

where x = the total area of the Footprint and Channels in Option 4, x_1 = area of the island component of the Footprint, x_2 = area of land-back component of the Footprint, x_3 = area of the northern channel, x_4 = area of the southern channel, CR_1 = the mean catch rate for the potlines in the immediate vicinity of the island component of the Footprint, CR_2 = the mean catch rate for the potlines in the immediate vicinity of the land-back component of the Footprint, CR_3 = the mean catch rate for the potlines in the immediate vicinity of the northern channel and CR_4 = the mean catch rate for the potlines in the immediate vicinity of the southern channel.

This mean catch rate was then multiplied by the area (in hectares) of that component (Table 7.1) to provide an index of relative abundance. This index allows for the comparison of that component's adult blue swimmer crab population with the rest of Cockburn Sound.

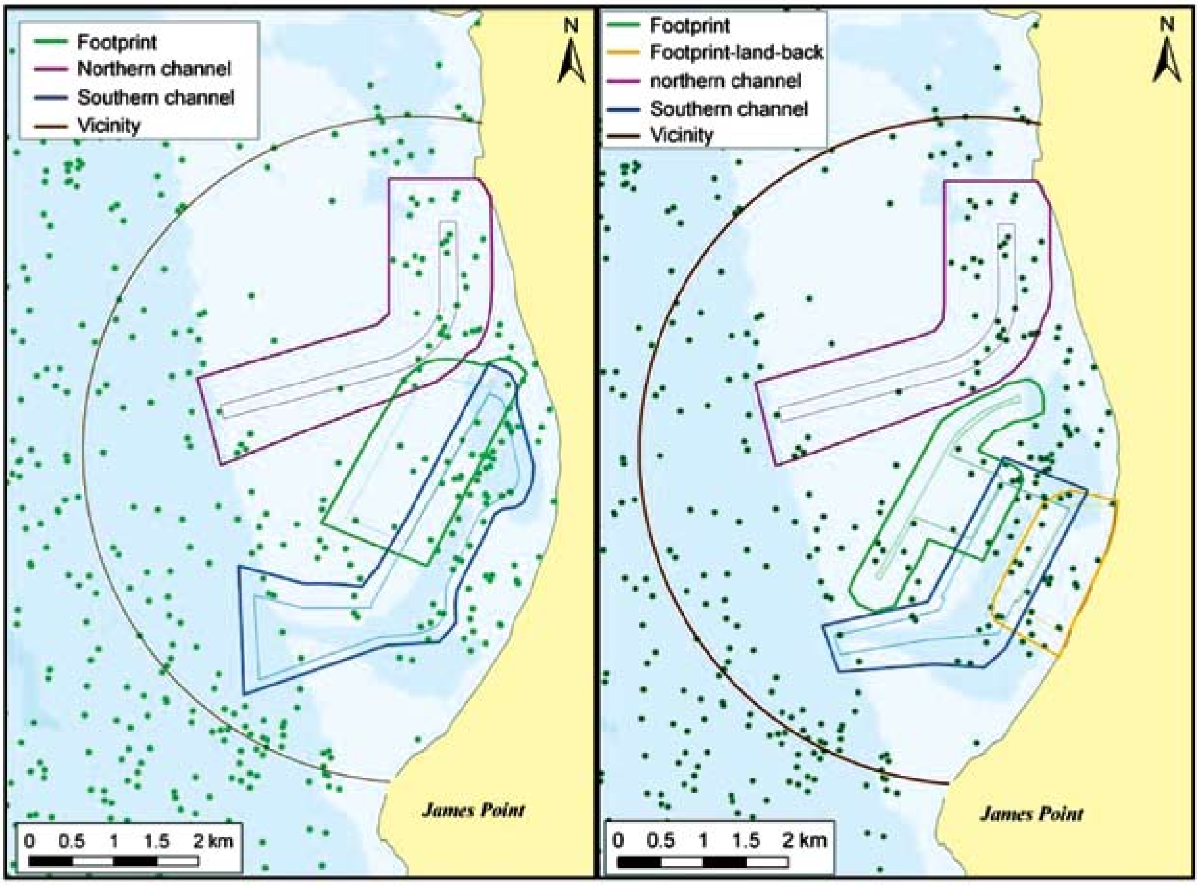


Figure 7.3. Locations of potlines (●) sampled between 1999 and 2006 used to provide representative catch rates of each component for Option 1 (Island only on the left) and Option 4 (Island and land-back on the right) of the proposed Kwinana Quay development. For each component, the thin coloured line represents the actual area of the component and the thick coloured line designates the potlines included in the calculation of a representative catch rate for that component.

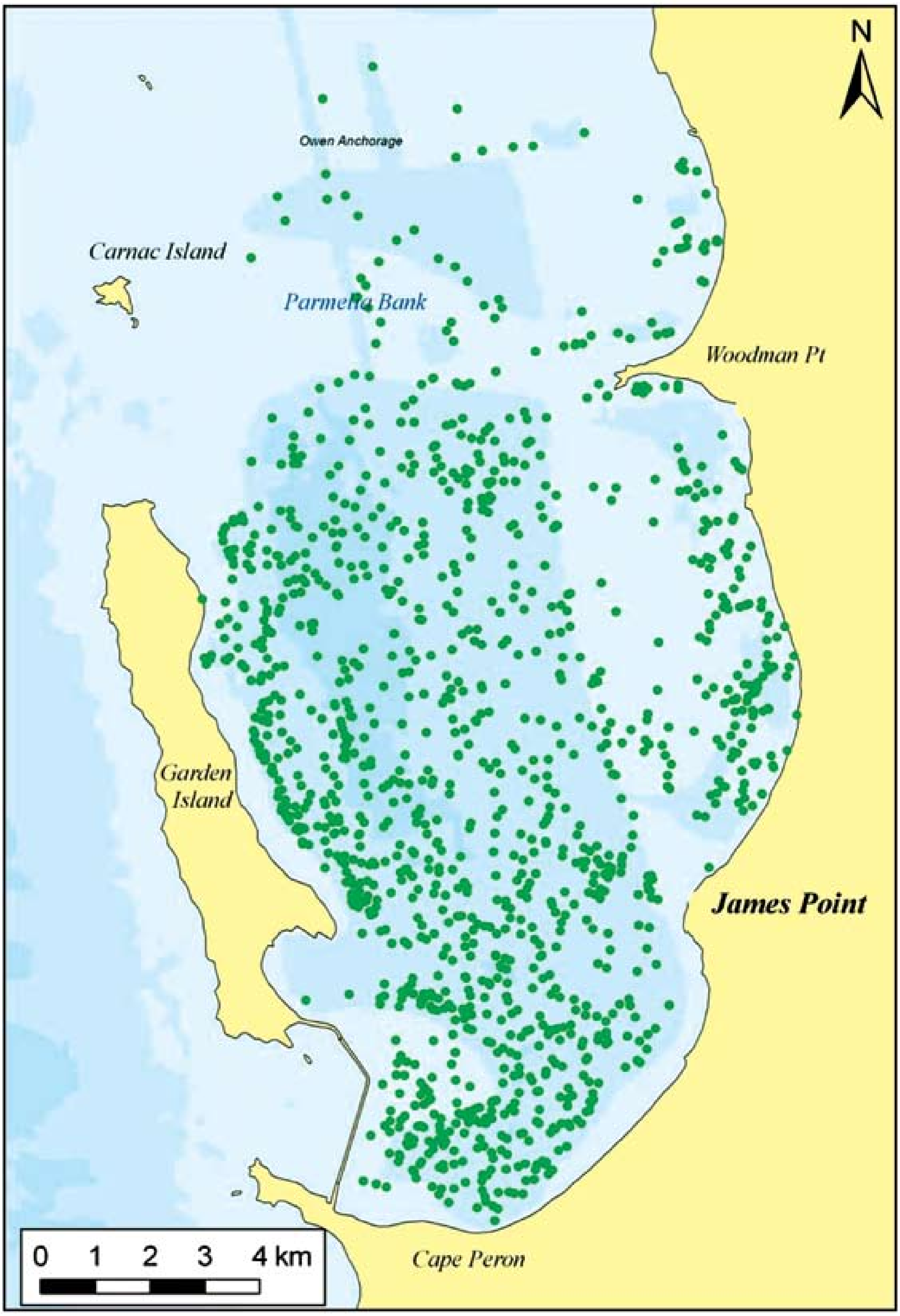


Figure 7.4. Location of potlines sampled during catch monitoring surveys aboard commercial crab vessels in Cockburn Sound between 1999 and 2006.

7.3 Results

7.3.1 2008 data

A total of 2,280 potlifts were completed during catch monitoring surveys in Cockburn Sound in 2008, with 190 standard lines of ten pots set throughout Cockburn Sound and an additional 76 lines of five pots set in and around the proposed site of the Kwinana Quay development in Jervoise Bay (Fig. 7.1).

The Footprint of Option 1 covers just over one percent of the physical habitat in Cockburn Sound that is considered important to resident adult blue swimmer crabs. With a comparatively low mean catch rate of 2.79 adult crabs/potlift, this area accounted for only 0.7% of the adult stock captured during 2008 (Table 7.2). The Footprint and Channels of Option 1 covers 4% of adult crab habitat and accounted for only 2% of the adult catch with a mean catch rate of 3.42 crabs/potlift. A mean catch rate of 4.13 crabs/potlift was recorded in the wider Vicinity area during 2008, which accounted for 27% of the 2008 adult crab catch, but represented 35% of the available adult crab habitat. The highest catch rate was recorded for the Rest of Cockburn Sound, with a mean catch rate of 6.08 adult crabs/potlift. Covering the remaining 65% of available habitat, this area produced 73% of the adult crabs caught during 2008 (Table 7.2).

As with Option 1, the habitat in and around the site of Option 4 of the proposed Kwinana Quay development accounted for proportionally less of the adult stock captured during 2008 than the area it covers (Table 7.3). The Footprint of Option 4 covers 1.4% of the physical habitat in Cockburn Sound considered important to resident adult blue swimmer crabs, but the comparatively low mean catch rate of 3.71 adult crabs/potlift of this area accounted for only 1% of the adult stock captured during 2008 (Table 7.3). The Footprint and Channels of Option 4 covers 3% of adult crab habitat and accounted for only 2% of the adult catch with a mean catch rate of 3.67 crabs/potlift. The relationships between area covered and contribution to adult crab stocks for the Vicinty and Rest of Cockburn Sound for Option 4 are the same as for Option 1 (Tables 7.1 & 7.3).

Table 7.2. Mean catch rate, relative abundance and proportion of adult crabs caught during the 2008 commercial monitoring program within each component for Option 1 (island only) of the proposed Kwinana Quay development.

COMPONENT		Area		Mean Catch Rate	Relative Abundance	
		ha	%	crabs/potlift	Index	%
Footprint		126	1.3	2.79	351	0.7
Footprint & Channels		350	4	3.42	1196	2
	footprint	104	1.04	1.01		
	southern channel	143	1.4	2.19		
	northern channel	40	0.4	0.30		
Vicinity		3507	35	4.13	14478	27
Rest of Cockburn Sound		6493	65	6.08	39461	73

Table 7.3. Mean catch rate, relative abundance and proportion of adult crabs caught during the 2008 commercial monitoring program within each component for Option 4 (island and land-back) of the proposed Kwinana Quay development.

COMPONENT		Area		Mean Catch Rate	Relative Abundance	
		ha	%	crabs/potlift	Index	%
Footprint		143	1.4	3.71	531	1
Footprint & Channels		327	3	3.67	1200	2
	footprint	104	1.04	1.01		
	southern channel	143	1.4	2.19		
	northern channel	40	0.4	0.30		
Vicinity		3507	35	4.13	14478	27
Rest of Cockburn Sound		6493	65	6.08	39461	73

7.3.2 Historic data (1999-2006)

A total of 19,129 potlifts were sampled aboard commercial crab vessels during catch monitoring surveys in Cockburn Sound between 1999 and 2006. Average catch rates for the various components of the proposed development were relatively constant, ranging from 5.7 crabs/potlift for the Footprint of Option 1 to 6.6 crabs/potlift for the Vicinity.

In general, catch rates for the respective components in and around the proposed Kwinana Quay development in Jervoise Bay were noticeably higher over the period from 1999-2006 than during 2008. Historically, the physical area covered by the Footprint for Option 1 produced a mean catch rate of 5.7 crabs/potlift, representing just over 1% of the adult crab catch (Table 7.4), compared to only 2.8 crabs/potlift or 0.7% of the adult crab catch during 2008 (Table 7.2). This trend was also repeated for the Footprint of Option 4 where the historical dataset generated a mean catch rate of 5.9 crabs/potlift or 1.4% of the adult catch for this component, compared to 3.8 crabs/potlift or 1.1% of the adult catch in 2008 (Tables 7.3 & 7.5).

This difference was also evident when comparing historic catch rates with those from 2008 in the Footprint and Channels. Catch rates of 6.1 and 6.2 crabs/potlift were calculated from the historic dataset for Options 1 and 4 respectively, representing 2.8% of the adult stock for Option 1 and 3.4% for Option 4 (Tables 7.4 & 7.5). By comparison, just 3.5 crabs/potlift (1.8% of adult catch) for Option 1 and 3.9 crabs/potlift (2.5% of adult stock) for Option 4 were recorded during 2008.

Historically, the Vicinity also proved more productive for adult blue swimmer crabs than during 2008. Sampling in this area during 2008 generated a catch rate of 4.1 crabs/potlift or 27% of the adult catch, compared to 6.6 crabs/potlift or 36% of adult crabs from the historic dataset (Tables 7.2 & 7.4). Consequently, the remainder of Cockburn Sound contributed 64% of the adult catch during this period compared to 73% in 2008 (Tables 7.2 & 7.4).

Table 7.4. Mean catch rate, relative abundance and proportion of adult crabs caught during catch monitoring surveys aboard commercial crab vessels between 1999 and 2006 attributed to each component for Option 1 (island only) of the proposed Kwinana Quay development.

COMPONENT		Area		Mean Catch Rate	Relative Abundance	
		ha	%	crabs/potlift	Index	%
Footprint		126	1.3	5.7	713	1
Footprint & Channels		350	4	6.2	2167	3
	footprint	104	1.04	1.01		
	southern channel	143	1.4	2.19		
	northern channel	40	0.4	0.30		
Vicinity		3507	35	6.6	23105	36
Rest of Cockburn Sound		6493	65	6.2	40216	64

Table 7.5. Mean catch rate, relative abundance and proportion of adult crabs caught during catch monitoring surveys aboard commercial crab vessels between 1999 and 2006 attributed to each component for Option 4 (island and land-back) of the proposed Kwinana Quay development.

COMPONENT		Area		Mean Catch Rate	Relative Abundance	
		ha	%	crabs/potlift	Index	%
Footprint		143	1.4	5.8	830	1.3
Footprint & Channels		327	3	6.2	2040	3
	footprint	104	1.04	1.01		
	southern channel	143	1.4	2.19		
	northern channel	40	0.4	0.30		
Vicinity		3507	35	6.6	23105	36
Rest of Cockburn Sound		6493	65	6.2	40216	64

7.4 Discussion

This project represents a preliminary investigation of the impacts of the proposed Kwinana Quay development on adult blue swimmer crabs and is based on the 2008 data collected. Comparisons with historical commercial monitoring data have also been made as adult stocks are currently depleted and a fishing closure has been effective since December 2006.

The impact of the two proposed development options (1 *vs*. 4) on the adult crab population appears to be similar, although Option 4 accounts for slightly greater relative abundance of crabs within the Footprint area (2008 data 0.7% *vs*. 1%; historical data 1% *vs*. 1.3%). However, it is important to note that direct comparisons of impacts between the two options are difficult until short-term and long-term environmental changes associated with each option have been determined. The mean catch rates and relative abundance of adult crabs caught within the various components of the proposed Kwinana Quay development during 2008 were generally lower than that for the historical dataset (1999 to 2006). This was expected considering the decline in adult biomass in 2006 and slower than expected recovery since. Given the Department of Fisheries is dedicated to rebuilding the biomass of adult blue swimmer crabs, it is recommended that future assessments of the potential impacts of the proposed Kwinana Quay development on these adults be based on historic abundances (*i.e.* pre-2006).

Comparisons of the relative abundance of crabs in various components of the Kwinana Quay development showed the Footprint and Channels areas supported only a small proportion of the adult blue swimmer crab population in Cockburn Sound. It is likely that the risk of long-term impact on adult blue swimmer crab stocks in this embayment from the Footprint and Channels will be low. However, approximately one third of the relative abundances of adult crabs in 2008 were recorded within the Vicinity of this development. This assessment of adult crabs is based on the assumption of recruitment entering these areas from the juvenile nursery areas as described in Section 6.0. Therefore, the largest perceived risk to blue swimmer crab stocks in Cockburn Sound would likely be through impacts to juvenile areas resulting in reduced recruitment, which is capable of resulting in longer term flow-on effects to adult stocks as witnessed in recent years. It is anticipated that additional monitoring in future years relative to different construction phases of the development may be required to accurately assess the short term and long term impacts of the proposed Kwinana Quay development on adult crab stocks.

8.0 Objective 7

J. Chaplin and E. Sezmiş

Objective 7. Using genetic analysis, identify the relationship between blue swimmer crabs from Cockburn Sound, Warnbro Sound and the Swan River.

See Appendix 1 for report from J. Chaplin and E. Sezmiş (Murdoch University).

9.0 Conclusions

This project represents a preliminary investigation of the potential impacts of the proposed Kwinana Quay development on important biological aspects of key fish species and blue swimmer crabs and is based on the 2008 data collected. Comparisons with historical data have been made where possible, *i.e.* objectives one and six. It is anticipated that for some components of this report additional monitoring will be needed in the future to accurately assess the short term and long term impacts of this proposed Kwinana Quay development. Thus, this discussion is limited to snapper, fish assemblages and juvenile and adult blue swimmer crabs and does not comment on broader biodiversity implications that may arise from the development. The key points from each of the studies in this report include:

Objective 1*. Determine the spatial extent of spawning of snapper, during their peak spawning period, in Cockburn Sound and surrounding areas and compare these findings with data collected during the spawning periods from 2001 to 2004 (Wakefield 2006).*

- There is a strong correlation between environmental parameters and reproductive cycles of snapper in Cockburn Sound.
- A strong year class has resulted from the 2007 spawning season.

The largest perceived risk to a reduction in spawning success for snapper from the Kwinana Quay development would most likely result from alterations to water circulation that would disrupt the retention of progeny in Cockburn Sound.

Objective 2*. Identify the species other than snapper that use Cockburn Sound as a spawning area.*

- Fish larvae from the ichyoplankton samples collected in Objective 1 have been preserved for identification at a later time.
- Given concerns over dredge plume induced mortality of fish larvae through gill fouling. The distribution and abundance of fish larvae in Cockburn Sound is to be used in a model to predict the potential risk associated with suspended sediment from dredging during the construction of Kwinana Quay, based on lethal concentrations established by Partridge and Michael (2008).

Objective 3*. Determine the distribution and abundance of demersal fish species, focussing on juvenile snapper, in Cockburn Sound and surrounding areas and identify any associations of fish assemblages with benthic habitat, topography and/or artificial structures.*

- BRUVs identified more species at higher abundances than traps and thus provided a better description of the fish communities in Cockburn Sound.
- There were three types of fish communities in Cockburn Sound, including those associated with seagrass, extensive areas of soft sediment (typically sand or silt) and areas comprising some form of limestone structure.
- Although a large part of Cockburn Sound comprises relatively featureless soft sediment habitat the majority of demersal fish species were found in seagrass or near naturally occurring limestone reef.
- A similar number of species were sampled at the rockwall sites compared to reef and upper slope sites (which consisted of interspersed small reef outcrops and were predominantly located on the upper slope of the topographic margin bordering the basin and eastern plateau). However, the abundances of these species at the rockwall sites were markedly

lower, which suggests this artificial structure has a lower carrying capacity for the species sampled using BRUVs.

- The numbers of species sampled in the dredged areas were low and similar to those found in the relatively featureless soft sediment areas. However, the highest numbers of 0+ aged snapper were found in these areas.
- The habitats that include high relief limestone reef (not including rockwall/groyne), small interspersed reef outcrops and seagrass were associated with significantly higher numbers and abundances of fish species. Given the importance of these habitats to the fish communities and the small area they occupy in Cockburn Sound, it is highly recommended that efforts be made to avoid disturbance to these areas from the construction of Kwinana Quay.
- The interannual variation in the fish communities in Cockburn Sound was not investigated in this study. Notably, the distribution and abundances of snapper may be significantly different between years considering the 2007 year class sampled in this study represented a strong recruitment year, which typically occurs infrequently for this species in Cockburn Sound. Thus, further sampling using BRUVs is recommended.

Objective 4. *Describe the movement patterns of adult (mature) snapper relating to their spawning aggregations in the nearshore areas of Cockburn Sound, Owen Anchorage and Warnbro Sound.*

- The technology involved in this type of research is relatively expensive and could not be reduced and still achieve the desired outcomes. Thus this objective was not undertaken.
- This research would be important if there were thought to be any negative interactions between spawning aggregations of snapper and the Kwinana Quay development or associated increased shipping traffic in the area.
- The connectivity between snapper from these spawning aggregations and the contribution of recruitment from these embayments to the larger west coast would be supported by genetic or age-related otolith microchemistry analysis.

Objective 5. *Investigation of the potential impacts of the Kwinana Quay development on juvenile blue swimmer crab stocks in Cockburn Sound*

- The actual physical area encompassed by the Footprint of Options 1 and 4 provides a very low proportion of crab recruitment (3% and 4% respectively) and therefore the permanent loss of this area due to reclamation is likely to have minimal long-term impact on crab recruitment in Cockburn Sound.
- The Footprint and Channels combined of Option 1 represents a greater proportion of crab recruitment (9%), but Option 4 is even higher (11%) due to high catch rates at the site representing the Land-back component of the Footprint.
- The area surrounding the site of the proposed Kwinana Quay development, *i.e.* the Vicinity, represented 67% of the area considered to be important for blue swimmer crab recruitment in Cockburn Sound. The catch rates of juvenile blue swimmer crabs in this area was significantly high, with an average of 0.012 m^{-2} compared to 0.07 m^{-2} for the rest of the Recruitment Areas. Thus, the proportion of juvenile crabs that occurred in the Vicinity of the proposed site of Kwinana Quay development represented 83% of recruits for Cockburn Sound in 2008.

- It is important to note that assessment of potential impacts of the development on juvenile recruitment (and adult stocks) will be difficult until short-term and long-term environmental changes associated with each option have been determined.
- Despite blue swimmer crabs being a short-lived species with highly variability recruitment, the rebuilding of the recently depleted stocks is taking longer than expected. Thus, a cautious approach to potential impacts to recruitment needs to be adopted and it is anticipated that further sampling relative to different construction phases would be required.

Objective 6*. Investigation of the potential impacts of the Kwinana Quay development on adult blue swimmer crab stocks in Cockburn Sound.*

- It is likely that the risk of long-term impact on adult blue swimmer crab stocks in Cockburn Sound from the proposed Footprint and Channels associated with the development will be low, as these areas only support approximately 3% of the adult population.
- It should be noted that approximately one third of the relative abundances of adult crabs in 2008 were recorded within the Vicinity of this development.
- This assessment of adult crabs is based on the assumption of recruitment entering these areas from the juvenile nursery habitat. If these nursery areas are significantly affected by the Kwinana Quay development (see Section 6.0), this will have a flow-on effect to the adult population.
- The Department of Fisheries is dedicated to rebuilding the biomass of the currently depleted adult stocks of blue swimmer crabs. Therefore future assessments of the potential impacts on these adults from the proposed Kwinana Quay development should more accurately be based on historic abundances (*i.e.* pre-2006).
- Future monitoring in the vicinity of the Kwinana Quay development should be considered in the event of any impact to recruitment at the larval or juvenile stage to assess any flow-on effects.

Objective 7*. Using genetic analysis, identify the relationship between blue swimmer crabs from Cockburn Sound, Warnbro Sound and the Swan River.*

- A genetic assessment of the relationships among the assemblages of the blue swimmer crab *Portunus pelagicus* in Cockburn Sound, the adjacent Swan River Estuary and near-by Warnbro Sound in south-western Australia was undertaken by Chaplin and Sezmiş (2008, Appendix 1).
- The assessment was based upon the patterns of variation at four polymorphic microsatellite loci in samples of *P. pelagicus* collected from Cockburn Sound, the Swan River Estuary and Warnbro Sound in 2007 and 2008.
- Results indicate that the genetic compositions of the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound were homogeneous at the time of sampling (2007/2008) and thus that *P. pelagicus* is represented by either a single biological stock, or a series of overlapping stocks, in these water bodies. However, the amount of gene exchange between the assemblages in Cockburn Sound, Swan River and Warnbro Sound is temporally variable and generally insufficient to have major impact on the abundances between these water bodies. On this basis the blue swimmer crab population in Cockburn Sound is managed as a single stock with limited recruitment from elsewhere.

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12.0 Appendices

12.1 Appendix 1

A genetic assessment of the relationships among the assemblages of the blue swimmer crab, *Portunus pelagicus*, in Cockburn Sound, the Swan River Estuary and Warnbro Sound

A Report Prepared for the Department of Fisheries, Western Australia. October 2008

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Executive Summary

This study provides a genetic assessment of the relationships among the assemblages of the blue swimmer crab *Portunus pelagicus* in Cockburn Sound, the adjacent Swan River Estuary and near-by Warnbro Sound in south-western Australia. It was commissioned by the Department of Fisheries Western Australia in view of recent declines in the catch rate of and recruitment in this species in Cockburn Sound.

The assessment was based upon the patterns of variation at four polymorphic microsatellite loci in samples of *P. pelagicus* collected from Cockburn Sound, the Swan River Estuary and Warnbro Sound in 2007 and 2008.

The results indicate that the genetic compositions of the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound were homogeneous at the time of sampling (2007/2008) and thus that *P. pelagicus* is represented by either a single biological stock, or a series of overlapping stocks, in these water bodies. It is not possible to use the genetic data of this study to distinguish between these alternatives (single versus overlapping stocks) because only small or occasional amounts of gene flow are required to homogenise the genetic compositions of different sub-units of a species.

On the basis of all of the available information, we have tentatively concluded that the amount of gene exchange between the assemblage of *P. pelagicus* in Cockburn Sound and those in the Swan River Estuary and Warnbro Sound is temporally variable and generally insufficient to have major impact on the abundance of this species in any of these water bodies, *i.e.,* that *P. pelagicus* is represented by a series of overlapping stocks (rather than a single stock) in these water bodies. This information consists of a simplistic assessment of the distribution of barriers to dispersal in *P. pelagicus* in south-western Australia and a combination of the genetic results of the present study and those of a previous study by Sezmiş (2004), which was based on samples of *P. pelagicus* collected from a range of water bodies, including Cockburn Sound, the Peel-Harvey Estuary and Geographe Bay in south-western Australia (but not the Swan River Estuary and Warnbro Sound) in 1999 and 2000.

In conclusion, the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound were not genetically differentiated from each other at the time of sampling and probably comprise a series of overlapping biological stocks, although we cannot exclude the possibility that they are all part of the same biological stock.

Introduction

The blue swimmer crab, *Portunus pelagicus,* is broadly distributed in the Indo-west Pacific region (Kailola *et al.*, 1993). Its distribution extends into temperate waters in some locations, including on the west coast of Australia where it maintains assemblages in a range of water bodies, including Cockburn Sound, the Swan River Estuary and Warnbro Sound (Kailola *et al.*, 1993; Kangas, 2000).

The adults and juveniles of *P. pelagicus* are bottom-dwelling and typically inhabit sheltered coastal environments (*e.g.* see Kailola *et al.*, 1993; Kangas, 2000; de Lestang *et al.*, 2003). The life cycle also includes a pelagic 'larval' phase (actually zoeae plus megalopae) that last for ~17 - 23 days at 25˚C in the laboratory (Bryars, 1997). The pelagic larvae are probably the main dispersive phase of the life-cycle, although the adults and juveniles can swim and may move extensively within a water body or between an estuary and adjacent marine embayment (Sezmiş, 2004 and references therein). The duration of the pelagic larval phase in the life-cycle of a bottom-dwelling marine species typically provides a rough but imperfect predictor of the dispersal potential of a species (reviewed by Siegel *et al.*, 2003). Thus, on the basis that the life-cycle includes a protracted pelagic larval phase, it is likely that individuals of *P. pelagicus* have the potential to disperse from their natal assemblage, but the spatial scale of dispersal cannot be resolved without additional information.

The adults and juveniles of *P. pelagicus* are mainly found in estuaries and semi-enclosed marine embayments (see Kangas, 2000; de Lestang *et al.,* 2003). While individuals will spawn within marine embayments, those within estuaries typically move into the entrance channels or adjacent marine waters to spawn (Kangas, 2000; de Lestang *et al.,* 2003), which may increase the potential for dispersal from these water bodies. Although taking place all year round in warmer waters, spawning in *P. pelagicus* typically occurs only during the warmer months in temperate waters (Kangas, 2000).

P. pelagicus is subject to exploitation by commercial and recreational fishers in many regions (Kailola *et al.*, 1993; Kangas, 2000). The commercial catches of this species in Cockburn Sound were the second largest in Western Australia, and one of the largest in Australia, until fishing for this species in Cockburn Sound was (temporally) banned in December 2006 (see Kangas, 2000). Similarly, recreational catches of this species from Cockburn Sound were also relatively high (see Kangas, 2000), although only about 15% of the commercial catch (Department of Fisheries, Media Release 15 December 2006), until the fishery was closed.

The fishery for *P. pelagicus* in Cockburn Sound was temporally closed in December 2006 in order to "give crab stocks time to recover" (Department of Fisheries, Media Release 15 December 2006); it has not yet been re-opened. The closure has come about primarily in response to a significant decline in the commercial catch rates of this species from this embayment dating back to about 2003/2004 and a suspected similar trend in recreational catches (Department of Fisheries, Media Release 15 December 2006). Although the exact reasons for the declining catch rates have not been fully elucidated, they appear to be linked to declines in the amount of recruitment in this species in this water body (Department of Fisheries, Media Release 15 December 2006), which may be associated with a combination of fluctuations in key environmental parameters and fishing pressures (Department of Fisheries, Media Release 23 November 2006).

In view of the apparent decline in the abundance of *P. pelagicus* in Cockburn Sound and uncertainty about the time-span for recovery, it seems important to understand the strength of connections between the assemblage of this species in this embayment and those in other water bodies on the west coast of Australia. Realistically, genetic data provide the only means to elucidate population (stock) structure of a species, like *P. pelagicus*, with a life-cycle that includes a potentially highly-dispersive pelagic larval phase (Cadrin *et al.*, 2005). Studies that are based upon the distribution of variation at microsatellite loci are particularly useful in this regard. This is because microsatellite loci typically have high levels of underlying polymorphism (*i.e.,* a high information content) and thus can be used to provide a relatively sensitive test for population genetic sub-division (see Hauser & Ward, 1998). Furthermore, microsatellite markers are bi-parentally inherited and so provide information about both male- and female-mediated gene flow (Hancock, 1999). They also have co-dominant expression of alleles, which means that variation can be assigned to specific loci (Queller *et al.*, 1993), increasing the precision of the resultant information.

Sezmiş (2004) compared the genetic (microsatellite) compositions of samples of *P. pelagicus* from six sites on the west coast of Australia, ranging from Exmouth Gulf in the north to Geographe Bay in the south, as a part of a larger study of the stock structure of this species in Australian waters. The results of this previous study indicate that the assemblage of *P. pelagicus* in Cockburn Sound is genetically distinctive, even in comparison with assemblages of this species at other sites, such as the Peel-Harvey Estuary (~40 km south) and Geographe Bay (~150 km south), in south-western Australia. This finding suggests that the *P. pelagicus* in Cockburn Sound constitute a separate biological stock (independent demographic unit) relative to those in these other water bodies (see Sezmiş, 2004). However, the relationship between the assemblage in Cockburn Sound and those in the adjacent Swan River Estuary and near-by Warnbro Sound has yet to be determined.

Aim: The aim of the proposed research is to determine whether the assemblages of the blue swimmer crab in Cockburn Sound, the Swan River Estuary and Warnbro Sound are genetically differentiated from each other and thus constitute separate stocks.

Material and Methods

Study Sites

Cockburn Sound is a semi-enclosed embayment on the west coast of Australia at the southern fringes of the Perth Metropolitan area. It occurs at a longitude of approximately 115°44'E and extends from about Woodman's Point (32°08'S) in the north to Cape Peron (32°16'S) in the south. Cockburn Sound is approximately 16 km long by 9 km wide, and consists of a relatively deep (16 – 20 m) central basin deep with shallow margins (Steedman & Craig, 1983; DAL Science and Engineering, 2002). It is extensively sheltered from surrounding marine waters being bound to the east by the mainland, to the west by Garden Island, and to the south-west by Cape Peron and a line of intertidal and subtidal reefs (Steedman & Craig, 1983). The northern entrance is wide but abuts a submerged, shallow sill, called Parmelia Bank (Steedman & Craig, 1983). These features, together with a rockfill causeway, broken by two trestle bridges, which connect Garden Island and the mainland, restrict water exchange between Cockburn Sound and surrounding marine waters (Steedman & Craig, 1983; DAL Science and Engineering, 2002). The flushing time of Cockburn Sound is greatest during the summer, when it is estimated to take an average of 44 days to flush 63% of the embayment (DAL Science and Engineering, 2002). This is because the prevailing winds generate circulation gyres that tend to trap waters within the embayment (DAL Science and Engineering, 2002).

The Swan River Estuary (sometimes referred to as the Swan-Canning Estuary) flows through the city of Perth. The mouth of this permanently-open estuary is located at Fremantle Harbour at a latitude of about 32°03'S and a longitude of about 115°44'E and discharges into a fairly open stretch of coastline, approximately 9 km north of Cockburn Sound (see Figure 1). The estuary consists of: (i) a long (~ 8 km) narrow inlet channel (lower estuary); (ii) a relatively deep (up to ~21 m) basin with shallow margins, which is about 12 km long by 2 km wide (middle estuary); and (iii) the tidal reaches of the Swan and Canning rivers (upper estuary) (Hodgkin, 1987; Steckis *et al.,* 1995). The hydrology of the estuary is mainly influenced by its geomorphology, tides (small amplitude and largely diurnal) and the Mediterranean climate of the region (hot, dry summers and cool, wet winters) (Stephens & Imberger, 1996; Chan & Hamilton, 2001). During the dry summer conditions, the lower reaches of the estuary are generally relatively well flushed by tidal movements and have salinities at or about that of the surrounding marine waters (Stephens & Imberger, 1996; Chan & Hamilton, 2001).

Warnbro Sound is a small marine embayment measuring approximately 7 km long by 4 km wide (Hollings, 2004). It is located at a longitude of approximately 115°44'E, between Mersey Point (32°30'S) and Becher Point (32°37'S) on the west coast of Australia, about 11 km south of Cockburn Sound (see Figure 1). It consists of a relatively deep central basin that is flanked to the north and south by broad tongues of sand forming, respectively, the North and South sands (Carrigy, 1956). The average depth of the central basin is about 17 m, while the depth ranges of the North and South sands are, respectively, 1 to 9 m and 1 to 4 m (Hollings, 2004). Warnbro Sound is protected from the open ocean by a semi-continuous line of sandstone reef at its western edge, which extends southwards and northwards, forming, among other things, the western boundary of Cockburn Sound (Carrigy, 1956). Warnbro Sound is more exposed than Cockburn Sound and its waters are likely to be regularly mixed during the summer, although there may be restrictions to circulation in the central basin (Carrigy, 1956; DEP, 1996).

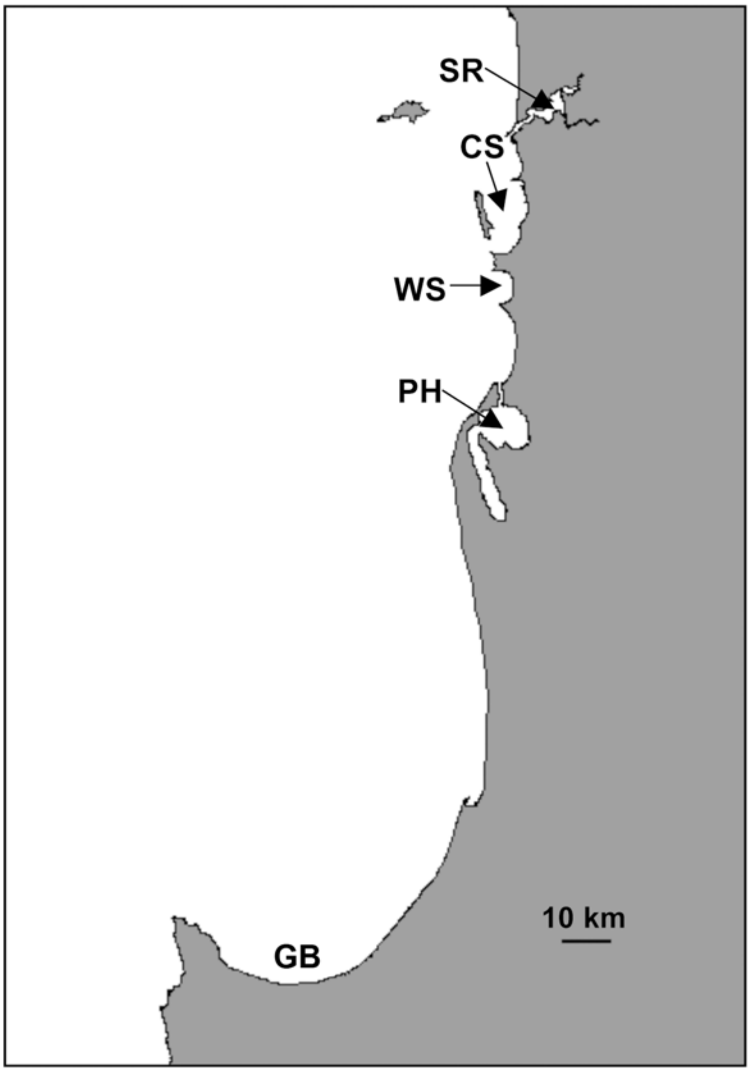

Figure 1. A map showing the locations of the Swan River Estuary (SR), Cockburn Sound (CS), Warnbro Sound (WS), the Peel-Harvey Estuary (PH) and Geographe Bay (GB) - water bodies in south-western Australia from which samples of *Portunus pelagicus* were obtained and analysed in the present study and/or the study of Sezmiş (2004).

Sampling

Forty-nine or fifty specimens of *P. pelagicus* were collected from each of Cockburn Sound and Warnbro Sound in November 2007 and January 2008, respectively. Fifty specimens of this species were also collected from the Swan River Estuary in 2007 but were poorly preserved and so ultimately replaced by a second batch of 50 specimens collected from this water body in January 2008.

The crabs were caught in commercial crab pots by commercial fishers and supplied to us via the Department of Fisheries, Western Australia. The crabs were collected from a large area within each water body. A claw was removed from each specimen and transported to the laboratory on ice and either dissected immediately or frozen for later dissection. A sample of muscle tissue was dissected from each claw and frozen at -80°C, pending DNA extraction.

Individuals of *P. pelagicus* collected from Cockburn Sound in 2000 were also assayed for the reason explained below. Muscle tissue from these individuals, which were collected as a part of the study of Sezmiş (2004), had been stored at -80°C in the interim.

Genetic Assays

DNA extractions

Total genomic DNA was extracted from approximately 5 – 10 mg of muscle tissue from each crab using a MasterPure^{TM} DNA Purification Kit (Epicentre Technologies), according to the manufacturer's protocol, except that a SDS extraction buffer (100mM NaCl, 50 mM Tris-HCl, pH 8.0, 10 mM EDTA and 0.5% SDS) was used instead of the 'Tissue Cell Lysis Buffer' and the DNA was sometimes precipitated in 100% isopropanol in the freezer overnight. The precipitated DNA was ultimately resuspended in 50 µL of TE buffer. The quality of DNA extracts was assessed by comparing the amount of high molecular weight DNA in the extracts to lambda DNA standards via agarose electrophoresis.

Microsatellite markers

This research was based on the patterns of variation at four (dinucleotide) microsatellite loci, namely *pPp02, pPp04, pPp09* and *pPp18* (see Table 1). These loci were developed for *P. pelagicus* by Yap *et al.,* (2002) and used by Sezmiş (2004) to investigate the stock structure of this species in Australian waters. An additional two loci, the dinucleotide *(pPp08)* and the tetranucleotide *(pPp19),* were also similarly developed for *P. pelagicus* and used in the previous population genetic study but not in the present study. This was because: (i) attempts to reliably amplify and score the alleles at the *pPp08* locus using the methods of the present study were not successful; and (ii) based on the results of Sezmiş (2004), the tetranucleotide locus was unlikely to reveal any population genetic sub-division in *P. pelagicus* beyond that revealed by the dinucleotide loci.

PCR

Polymerase chain reaction (PCR) was used to amplify the *pPp02, pPp04, pPp09* and *pPp18* loci from a DNA extract of each individual of *P. pelagicus,* using the primers pairs developed for each of these loci by Yap *et al.,* (2000) (see Table 1).

The optimised PCR conditions comprised: (i) an initial denaturation phase of 5 minutes at 94°C; (ii) 36 amplification cycles, with each cycle consisting of 30 seconds of denaturation at 94°C, 30 seconds of annealing at T_a°C (see Table 1), 30 seconds of extension at 72°C; and (iii) a final 5 minutes extension at 72°C. Each PCR reaction mixture had a total volume of 15 µL and contained 50 – 100 ng of DNA template, 10 mM Tris-HCl (pH = 8.3) with 50 mM KCl, 1.5 mM of $MgCl_2$, 0.2 mM of each of the dNTPs (Promega), 0.25 U of *Taq* polymerase (Roche), and 40 – 60 nmol of each primer, depending on the locus (see Table 1), with the forward primer labelled with 6-FAM (GeneWorks) fluorescent dye.

Table 1. Information about the characteristics, primer sequences and assay conditions for the four microsatellite loci that were used to investigate the relationship between the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound. T_a is the annealing temperature used for each primer pair in the PCR reactions; P_c is the concentration of each primer used in each PCR reaction mixture; *V* is the volume of PCR product per locus added to each well in the assay plate. Adapted from Yap *et al.* (2002). * = determined from the sequenced insert.

Locus (GenBank Accession no.)	Primer Sequence (5′–3′)	Repeat Unit*	T_a in °C	P_c in nM	*V* in µl
pPp02 (AF410871)	F: GTGACCAGTAGGCGACCGAG R: ACGACTGCTTGTACGACCTTCA	$(CA)_{16}$	63	40	1
pPp04 (AF410872)	F: GCCACTATCTTGCTGAGGTTGA R: GCCATAGCACGAACACTTTTGA	$(TG)_{28}$	56	40	3
pPp09 (AF410875)	F: GACTTGAGCGATGCTGAAAG R: ATGGATAGATGGAATGCAAAAT	$(TG)_{19}$	53	40	5
pPp18 (AF410877)	F: AGTAAGGGACCGTGGTGAAT R: CGTTGTCTAAAGCACATGAGATT	$(TG)_{17}$	56	60	5

Screening and scoring of alleles

Each PCR product was added to a well in a Fisher Biotech 96-well plate, along with 16 µl of (Hi-Di) formamide and 0.08 µl of 500 LIZ size standard (GeneScan). The PCR products of the loci *pPp02* and *pPp09* were multiplexed, *i.e.,* combined in the same well and analysed in the same capillary tube at the same time, as were the products for the loci *pPp04* and *pPp18*. The volume of PCR product for each locus added to each well was as indicated in Table 1. The plates were then sealed with a septum (Applied Biosystems) and the PCR products (microsatellite alleles) subject to electrophoresis and laser detection in capillary tubes and a raw chromatograph of the results produced via an Applied Biosystems 3730 DNA Analyser and associated GeneMapper (Applied Biosystems) software. The size of each allele at each locus was estimated using the software Peak Scanner™, version 1.0 (Applied Biosystems). One or more positive controls, *i.e.,* samples that had been scored as a part of a previous assay, were included in most assay plates in order to ensure internal consistency in the scoring of alleles. A negative control, *i.e.,* a PCR assay without added DNA, was also incorporated into each plate in order to check for contamination.

Comparison with previous studies

In order to place the results regarding the analysis of the relationships among the assemblages of *P. pelagicus* from Cockburn Sound, the Swan River Estuary and Warnbro Sound into context, the data analyses for this study have included, where appropriate, data generated by Sezmiş (2004) for the same for four microsatellite loci for samples of *P. pelagicus* collected from Cockburn Sound in each of 1999 and 2000, from the Peel-Harvey Estuary in each 1999 and 2000 and from Geographe Bay in 1999. However, since the present study used primers labelled with a fluorescent dye, capillary electrophoresis, laser detection and automated methods to separate and score the alleles, whereas that of Sezmiş (2004) used primers labelled with a radio-isotope, polyacrylamide-gel electrophoresis, radiographic detection methods and manual scoring, it was possible that the two studies yielded different estimates of the absolute (but not relative) sizes of the alleles at a microsatellite locus. Thus, in order to compare the allele scoring between the two data sets, it was to necessary to assay individuals using the methods of the present study and directly compare the outcomes with those obtained for these same individuals by Sezmiş (2004). This comparison was done using individuals of *P. pelagicus* collected from Cockburn Sound in 2000. The outcome was as follows: (i) the scoring of alleles at the *pPp04* and *pPp09* loci was consistent between the two methods; and (ii) the sizes of the alleles at the *pPp02* and *pPp18* loci were consistently scored as, respectively, 8 bp and 6 bp smaller using the methods of the present study. The alleles at the *pPp02* and *pPp18* loci were ultimately standardised according the scoring of Sezmiş (2004) for the data analyses.

Data Analyses

Levels of polymorphism

The level of polymorphism present at each locus in each sample of *P. pelagicus* from Cockburn Sound, the Swan River Estuary and Warnbro Sound was assessed in terms of the number of different alleles present (A) and the amount of expected heterozygosity (H_E), where $H_E = 1 - \Sigma(f_i)^2$ where f_i is the frequency of the ith allele. The level of polymorphism (diversity) provides an indication of the amount of genetic information present at these loci in these samples and hence a general indication of the level of resolution provided in the test for genetic differences.

Hardy-Weinberg equilibrium

Exact tests were used to assess the statistical significance of differences between the observed and expected numbers of homozygotes and heterozygotes at each microsatellite locus in each sample of *P. pelagicus.* These tests used the Markov chain method to estimate the exact probability of a type I error (Raymond & Rousset 1995), as implemented by GENEPOP, version 1.2 at http://genepop.curtin.edu.au (see Raymond & Roussett 1995). The iteration parameters for these, and all subsequent analyses conducted with GENEPOP, were a dememorization number of 10,000, 1,000 batches and 10,000 iterations per batch. Exact probability tests were selected for this analysis because they are not biased by small sample sizes or low frequencies of alleles or genotypes (Raymond & Rousset, 1995). Departures from Hardy-Weinberg Equilibrium conditions might indicate that the presence of null alleles (*i.e.,* alleles that do not amplify and are therefore not detected) or that the samples comprise an admixture of individuals from different populations. For this, and all subsequent analyses involving multiple tests, a sequential Bonferroni procedure was applied to assess the statistical significance of the probability values (Rice, 1989). The software Microchecker™ (Van Oosterhout *et al.*, 2004) was used to investigate the nature of any departures from Hardy-Weinberg Equilibrium conditions.

Genetic differentiation

Exact tests, as implemented by GENEPOP, version 1.2., were also used to assess the statistical significance of any differences in the genotype frequency distributions across loci between pairs of samples of *P. pelagicus*. The results of these tests are presented in terms of the probability of incorrectly rejecting the null hypothesis (no genetic differentiation). This analysis was based on genotype rather than allele frequencies because of evidence of departures from Hardy-Weinberg Equilibrium at some loci in some samples (see Results).

Relationships among samples

In order to resolve the relationships among samples of *P. pelagicus* from south-western Australia, the multi-dimensional scaling method was used to map the 'genetic distance' between pairs of samples in two-dimensional space, using the software Primer version 6 (Clark & Gorley, 2006). The 'genetic distance' between pairs of samples was estimated in terms of values of the standardised genetic variance, *i.e.,* F_{ST} (Weir & Cockerham, 1984).

Results

Sample sizes

The number of individuals of *P. pelagicus* that was genotyped ranged from 27 at the *pPp09* locus in the sample from Cockburn Sound to 46 at the *pPp18* locus in the sample from the Swan River Estuary (Table 2). The number of individuals assayed per locus for a particular site was less than the total number of individuals collected (49 – 50) from that site for a range of reasons; the largest discrepancies occurred when there were problems with the PCR assays, which were probably due to problems with primer specificity.

Table 2. The number of individuals genotyped (*n*), the number of alleles detected (*A*), the observed heterozygosity (H_O) and the expected heterozygosity (H_E) for each of four microsatellite loci in samples of *Portunus pelagicus* collected from Cockburn Sound (CS07), the Swan River Estuary (SR) and Warnbro Sound (WS) in 2007/2008. *P* is the probability that the genotype frequencies at a locus in a sample were not significantly different from those expected under Hardy-Weinberg equilibrium conditions. *P* values less than 0.05 are marked with an *, while those that were statistically significant after the significance levels were adjusted for multiple-tests, using a sequential Bonferroni procedure, are also underlined.

		LOCUS			
Sample/Site		*pPp02*	*pPp04*	*pPp09*	*pPp18*
CS					
	A	13	11	9	9
	H_O	0.56	0.83	0.74	0.54
	H_E	0.72	0.81	0.84	0.58
	P	0.0016*	0.7655	0.0047*	0.1688
	n	45	40	27	41
SR					
	A	11	11	11	9
	H_O	0.63	0.86	0.88	0.50
	H_E	0.79	0.82	0.84	0.59
	P	0.0216*	0.8604	0.4506	0.0625
	n	41	44	42	46
WS					
	A	13	12	9	8
	H_O	0.69	0.89	0.91	0.55
	H_E	0.82	0.84	0.83	0.54
	P	0.0894	0.8792	0.0330*	0.1355
	n	39	35	35	33

Levels of polymorphism

The number of alleles per locus per sample was high to moderate, ranging from 13 at the *pPp02* locus in each of the samples of *P. pelagicus* from Cockburn Sound and Warnbro Sound to eight at the *pPp18* locus in the sample from Warnbro Sound (Table 2). The values of expected heterozygosity at the *pPp02, pPp04* and *pPp09* loci were relatively high ($x \geq 0.72$), but more moderate at the *pPp18* locus (0.54 – 0.59) (Table 2). The values of both allele number and expected heterozygosity for each locus in each of these samples were within the ranges reported by Sezmiş (2004) for samples of *P. pelagicus* collected from Cockburn Sound, the Peel-Harvey Estuary and Geographe Bay in 1999 and 2000.

Hardy-Weinberg Equilibrium

The genotype frequencies in one of 12 tests (4 microsatellite loci X 3 samples) were significantly different from those expected under Hardy-Weinberg Equilibrium conditions when the significance levels were adjusted using a sequential Bonferroni procedure, and in an additional three tests without the Bonferroni adjustment (Table 2). Three of the four departures were in the form of excesses of homozygotes (Table 2), which occurred at the *pPp02* locus in the samples from Cockburn Sound and the Swan River Estuary and at the *pPp09* locus in the sample from Cockburn Sound (Table 2). Analysis of the data using the software Micro-Checker™ indicated that, when they occurred, the homozygote excesses were usually present in most genotype classes at a locus (data not presented).

Genetic differentiation

The genotype frequencies at the sampled microsatellite loci in the samples of *P. pelagicus* collected from Cockburn Sound, the Swan River Estuary and Warnbro Sound in 2007/2008 were not significantly different from each other (Table 3). On the other hand, the genotype frequencies in these three samples (assayed in the present study) were significantly different to those in samples of this species collected from the Peel-Harvey Estuary and Geographe Bay in 1999 and 2000 (assayed by Sezmiş (2004)) (Table 3). Since the assay methods of the present study and those of Sezmiş (2004) generated effectively identical data for a sample of *P. pelagicus* collected from Cockburn Sound in 2000 (Table 3), these genetic differences are not a function of the different methodologies employed by these two studies. (N.B. Slight differences in the data generated for the 2000 Cockburn Sound sample by the two different methods/studies were due to slight differences in the suite of individuals that were assayed). The extent of the differences in the genotype frequencies at the microsatellite loci between the sample of *P. pelagicus* collected from Cockburn Sound in 2007 and those collected from this water body in 1999 and 2000 approached the level expected for statistical significance, *i.e.,* the *P* values were usually less than 0.05 but not significantly different once the Bonferroni correction was applied (Table 3).

The above results are reflected in the MDS, which portrays the relationships among the samples based on the genetic distance between them. In particular, the samples from the Peel-Harvey Estuary and Geographe Bay tended to cluster together; as did those from Cockburn Sound, the Swan River Estuary and Warnbro Sound (Figure 2). The 2007 sample from Cockburn Sound was the most divergent of this latter group of samples (Figure 2).

Table 3. The outcomes of exact probability tests (*i.e., P* values) for differences in the genotype frequencies across four microsatellite loci between pairs of samples of *Portunus pelagicus* from the Swan River Estuary (SR), Cockburn Sound (CS), Warnbro Sound (WS), the Peel-Harvey Estuary (PH) and Geographe Bay (GB). The year in which the sample was collected is indicated in parentheses, such that CS(07), for example, indicates a sample collected from Cockburn Sound in 2007. The sample code CSa(00) indicates data generated via automated methods in the present study for a sample collected from Cockburn Sound in 2000; while the sample code CSr(00) indicates data generated via radiographic methods for this sample by Sezmiş (2004). Otherwise the 2007/2008 samples were collected and analysed using automated methods as a part of the present study, while the 1999/2000 samples were collected and analysed using radiographic methods as a part of the study by Sezmiş (2004). *P* values that were statistically significant after a sequential Bonferroni correction was applied are indicated in bold.

Sample Code	CS(07)	SR(08)	WS(08)	CSa(00)	CSr(00)	CS(99)	PH(99)	PH(00)	GB(99)
CS(07)	---								
SR(08)	0.568	---							
WS(08)	0.307	0.952	---						
CSa(00)	0.058	0.524	0.774	---					
CSr(00)	0.025	0.544	0.400	1	---				
CS(99)	0.049	0.129	0.296	0.071	0.069	---			
PH(99)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	---		
PH(00)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.513	---	
GB(99)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.593	0.567	---

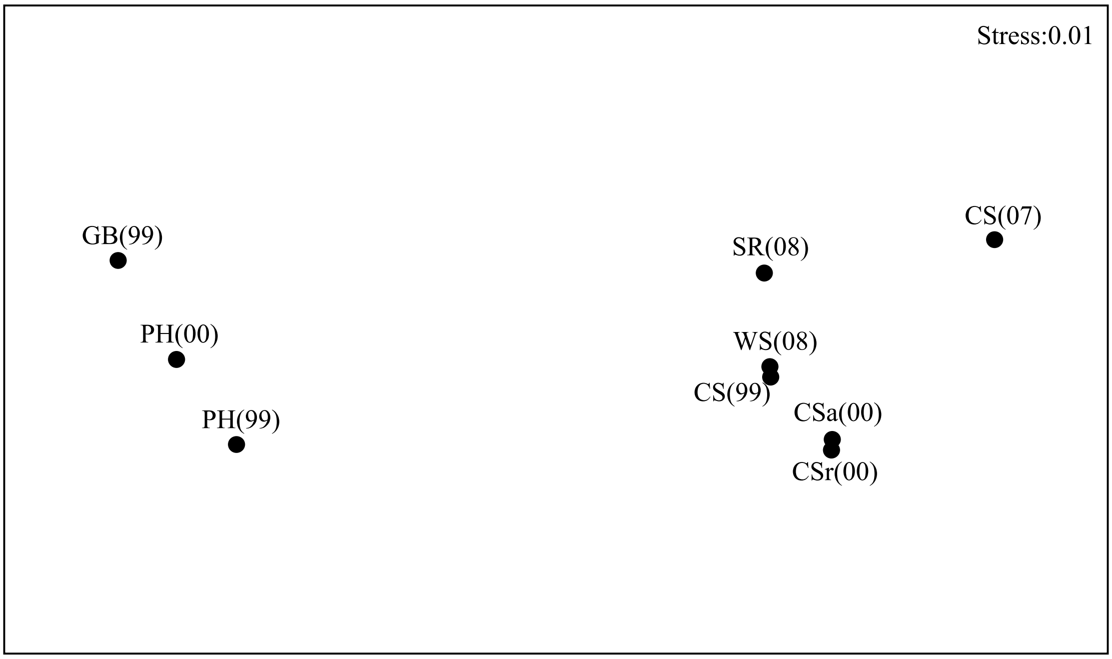

Figure 2. A two-dimensional ordination of the values of F_{ST} based on the patterns of variation at four microsatellite loci between pairs of samples of *Portunus pelagicus* from the Swan River Estuary (SR), Cockburn Sound (CS), Warnbro Sound (WS), the Peel-Harvey Estuary (PH) and Geographe Bay (GB). The year in which the sample was collected is indicated in parentheses, such that CS(07), for example, indicates a sample collected from Cockburn Sound in 2007. The sample code CSa(00) indicates data generated via automated methods in the present study for a sample collected from Cockburn Sound in 2000; while the sample code CSr(00) indicates data generated via radiographic methods for this sample by Sezmiş (2004). Otherwise the 2007/2008 samples were collected and analysed using automated methods as a part of the present study, while the 1999/2000 samples were collected and analysed using radiographic methods as a part of the study by Sezmiş (2004). A stress value of 0.01 indicates that the ordination provides a reliable representation of the relationships among samples.

Discussion

The results of this study indicate that the genetic compositions of the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound were homogeneous, at least at the time of sampling (2007/2008). Since it was based upon the patterns of variation at four microsatellite loci that exhibit high to moderate levels of polymorphism, this study has provided a relatively sensitive assay for the presence of genetic differences between these assemblages. Certainly, the amount of polymorphism at these microsatellite markers was sufficient to reveal relatively subtle genetic sub-division in *P. pelagicus* in south-western Australia, while the amount of nucleotide sequence variation in a mitochondrial DNA marker was not (Sezmiş, 2004).

The patterns of variation at the sampled microsatellite loci in *P. pelagicus* are essentially selectively neutral and hence can be interpreted in terms of the patterns of gene flow (as opposed to the outcome of locus specific selection) in this species (Sezmiş, 2004). The homogeneity of the genetic compositions of the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound indicates that there is contemporary gene flow (and so dispersal) in *P. pelagicus* between these water bodies, although not necessarily in large or temporally constant amounts.

The assemblage of *P. pelagicus* in Cockburn Sound was genetically differentiated from, among other things, those of this species in the Peel-Harvey Estuary and Geographe Bay in 1999 and 2000 (Sezmiş, 2004). This result indicates that the amount of gene flow in this species between Cockburn Sound and these other (south-west Australian) water bodies was negligible at this time (Sezmiş, 2004). The effective absence of gene flow in this situation has been attributed to the entrapment of the larvae of *P. pelagicus* in Cockburn Sound in relation to restricted rates of water exchange between this embayment and outside marine waters during the summer, when this species breeds in these waters (Sezmiş, 2004). The results of the present study indicate that the genetic compositions of the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound in 2007/2008 are different to those of the assemblages of this species in the Peel-Harvey Estuary and Geographe Bay in 1999 and 2000. The interpretation of this result in the context of spatial patterns of gene flow is confounded by the fact that it included samples collected up to nine years apart.

The results of the present study are consistent with the view that *P. pelagicus* is represented by either a single biological stock, or a series of overlapping stocks, in Cockburn Sound, the Swan River Estuary and Warnbro Sound. When these results are viewed in isolation, it is not possible to distinguish between these alternatives because only small or occasional amounts of gene flow are required to homogenise the genetic compositions of different sub-units of a species (Spieth, 1974). However, when the results are viewed in combination with those of a previous study by Sezmiş (2004) and in the context of a (simplistic) assessment of the distribution of likely barriers to the dispersal of *P. pelagicus* in south-western Australia, the latter view (overlapping stocks) seems more likely. This assessment has considered the results of an analysis of Australia-wide patterns of variation of microsatellite and mitochondrial DNA variation in *P. pelagicus* which indicate that gene flow in this species over fine and moderate spatial scales is mainly limited by restricted rates of water exchange between certain marine embayments and the ocean and significant discontinuities in the distribution of sheltered coastal environments (*i.e.,* habitat of the adults and juveniles) (Sezmiş, unpublished data).

It seems unlikely that *P. pelagicus* is represented by a single biological stock in Cockburn Sound, the Swan River Estuary and Warnbro Sound. This is because such an explanation implies, among other things, a free interchange of individuals of this species between Cockburn Sound and Warnbro Sound and so genetic homogeneity of the assemblages in these two water bodies both now and in 1999 and 2000. Hence, it also implies that the *P. pelagicus* in Warnbro Sound would have been genetically differentiated from those in the Peel-Harvey Estuary in 1999 and 2000, as were the ones in Cockburn Sound. This final implication is at odds with the apparent absence of a major barrier to gene flow in *P. pelagicus* between Warnbro Sound and the Peel-Harvey Estuary. This barrier would need to be more or less impermeable for the assemblages in these two water bodies to develop genetic differences. Instead, Warnbro Sound is adjacent to and not strongly demarcated from Comet Bay, which contains *P. pelagicus* and into which the Peel-Harvey Estuary discharges (see DEP. 1996). In this regard, it is relevant that individuals of *P. pelagicus* tend to move from estuaries into adjacent marine waters to spawn (Kangas, 2000; de Lestang *et al.,* 2003).

The explanation that *P. pelagicus* is represented by a series of overlapping biological stocks in Cockburn Sound, the Swan River Estuary and Warnbro Sound is consistent with the restricted rates of water exchange between Cockburn Sound and surrounding marine waters during the summer (see Study Sites), when *P. pelagicus* breeds in these waters (Kangas, 2000). Under this scenario, the genetic differences between the sample of *P. pelagicus* collected from Warnbro Sound in 2008 and those collected from the Peel-Harvey Estuary in 1999 and 2000 can be reconciled if one assumes that the genetic relationships between the assemblages of *P. pelagicus* in south-western Australia vary through time. In particular, the genetic differentiation of the assemblage in Cockburn Sound in 1999 and 2000 may reflect a prior period of isolation of this assemblage. However, between 2000 and the sampling for the present study, there may have been an episode(s) of gene flow that homogenised the genetic composition of the assemblage in Cockburn Sound with those in other near-by water bodies, including the Swan River Estuary, Warnbro Sound, and the Peel-Harvey Estuary. Occasional episodes of gene flow in *P. pelagicus* between Cockburn Sound and near-by water bodies might occur, for example, in response to the development of atypical patterns of water movement that allow a relatively large number of larvae to break into and/or out of this embayment during a particular breeding season. In this regard, it is interesting that the (2008) samples from the Swan River Estuary and Warnbro Sound were more similar to the 1999 and 2000 samples from Cockburn Sound than to the 2007 sample from this embayment (see Figure 2). If this pattern is real, it suggest that the assemblage of *P. pelagicus* in Cockburn Sound is once again evolving genetic differences from those of this species in near-by water bodies. If necessary, the above explanation can be tested because it predicts that the present day genetic composition of the assemblages of *P. pelagicus* in the Peel-Harvey Estuary (and the adjacent Comet Bay) will effectively be the same as those of the assemblages of this species in Cockburn Sound, the Swan River Estuary and Warnbro Sound, *i.e.,* that the genetic differences between the assemblages in Cockburn Sound and the Peel-Harvey Estuary in 1999 and 2000 have not persisted to the present day.

For the reasons outlined above, and on the basis of the results of Sezmiş (2004) that indicated that the assemblages of *P. pelagicus* in the Peel-Harvey Estuary and Geographe Bay were genetically homogeneous in 1999/2000, we predict that the assemblage of *P. pelagicus* in Warnbro Sound is more strongly connected to those in the Peel-Harvey Estuary and Geographe Bay (and other intervening water bodies, such as the Leschnault Estuary and Koombana Bay) than to that in Cockburn Sound. This may also be the case for the assemblage in Swan River Estuary because, while there appears to be a hydrological barrier to gene flow in *P. pelagicus* between Cockburn Sound and outside waters, we do not know of any such barrier between the Swan River Estuary and Warnbro Sound and these other water bodies in south-western Australia.

Homozygote excesses at most genotype classes were apparent at the *pPp02* and *pPp09* loci in the 2008 samples of *P. pelagicus* from Cockburn Sound and at the *pPp02* locus in the sample from the Swan River Estuary. Such excesses were also apparent at these and other loci in the data set of Sezmiş (2004), including in samples of *P. pelagicus* collected from Cockburn Sound in 1999 and 2000. Null alleles, *i.e.,* alleles that do not PCR amplify, and partial null alleles, *i.e.,* alleles that inconsistently amplify, are a common artefactual source of excesses of homozygotes at most genotype classes at microsatellite loci (see Shaw *et al*., 1999; Van Oosterhout *et al.*, 2004) and are probably at least partially responsible for the apparent homozygote excesses in the samples of *P. pelagicus* described above. Having said this, the fact that Bryars & Adams (1999) also found homozygote excesses at allozyme loci, which do not have a high incidence of null alleles, in independent samples of *P. pelagicus,* raises the possibility that these excesses may also (or instead) be linked to some real population-level process. In any case, it is highly unlikely that the presence of any null alleles at the assayed microsatellite loci in the samples of *P. pelagicus* have had a major influence on the outcomes of the present or Sezmiş' (2004) microsatellite-based assessments of the stock structure of this species. This is because, among other things, there is a high degree of concordance in the distribution of variation, including a strong geographic signal, at these microsatellite loci and at a mitochondrial DNA locus in *P. pelagicus* in Australian waters (Sezmiş, 2004).

Management Implications

The results of this study indicate that the genetic compositions of the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound were homogeneous at the time of sampling (2007/2008). This finding suggests that there is some gene exchange (and hence dispersal) in *P. pelagicus* among these water bodies, although it was not possible to quantify the amount of such. However, it is likely that the amount of gene exchange between the assemblage of this species in Cockburn Sound and those in the Swan River Estuary and Warnbro Sound is temporally variable and generally insufficient to have major impact on the abundance of this species in any of these water bodies. In fact, on the basis of (simplistic) deductions about the distribution of likely barriers to dispersal in this species in these waters, we predict that the assemblage of *P. pelagicus* in Warnbro Sound, and possibly also that in the Swan River Estuary, is more strongly connected to those in the Peel-Harvey Estuary and other sites in south-western Australia than to that in Cockburn Sound. In conclusion, the assemblages of *P. pelagicus* in Cockburn Sound, the Swan River Estuary and Warnbro Sound were not genetically differentiated from each other at the time of sampling and probably consist of a series of overlapping biological stocks, although we cannot exclude the possibility that they are all part of the same biological stock.

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Appendix

(ALLELE FREQUENCY DATA)

Table A.1 The allelic frequencies at the *pPp02* locus in samples of *Portunus pelagicus* collected from Cockburn Sound (CS), the Swan River Estuary and Warnbro Sound in 2007/2008.

Allele	CS	SR	WS
69	0.056	0.122	0.115
77	-	-	0.013
81	0.489	0.390	0.346
83	0.111	0.085	0.051
85	0.056	0.024	0.051
87	0.122	0.122	0.154
89	-	0.012	0.013
91	0.033	0.049	0.077
93	-	-	0.013
95	0.011	0.061	0.026
111	0.033	0.098	0.103
113	0.022	-	-
115	0.022	-	0.026
117	0.011	-	0.013
127	0.011	0.012	-
129	0.022	0.024	-

Table A.2 The allelic frequencies at the *pPp04* locus in samples of *Portunus pelagicus* collected from Cockburn Sound (CS), the Swan River Estuary and Warnbro Sound in 2007/2008.

Allele	CS	SR	WS
141	0.019	0.012	0.014
153	0.241	0.179	0.243
155	0.093	0.083	0.171
157	0.222	0.095	0.071
159	0.019	0.048	0.057
161	0.130	0.262	0.200
163	0.130	0.095	0.043
165	0.056	0.036	-
167	-	0.012	-
169	-	-	0.014
171	-	0.012	-
181	0.093	0.167	0.186

Table A.3 The allelic frequencies at the *pPp09* locus in samples of *Portunus pelagicus* collected from Cockburn Sound (CS), the Swan River Estuary and Warnbro Sound in 2007/2008.

Allele	CS	SR	WS
236	0.125	0.045	0.086
240	0.025	-	0.014
242	-	0.011	-
244	0.050	0.068	0.029
246	0.212	0.170	0.143
248	0.037	0.068	0.100
250	0.075	0.091	0.143
252	0.338	0.352	0.300
254	0.037	0.080	0.086
256	-	-	0.014
258	0.013	0.023	0.014
266	0.063	0.068	0.057
268	0.025	0.023	0.014

Table A.4 The allelic frequencies at the *pPp18* locus in samples of *Portunus pelagicus* collected from Cockburn Sound (CS), the Swan River Estuary and Warnbro Sound in 2007/2008.

Allele	CS	SR	WS
81	-	0.011	-
87	0.012	0.011	0.015
91	0.622	0.609	0.652
95	0.037	0.043	0.030
97	-	0.043	0.015
99	0.159	0.196	0.152
101	0.012	-	-
105	0.024	-	-
111	0.073	0.022	0.045
113	0.037	0.022	0.015
115	0.024	0.043	0.076