# Prawn Fishery By-catch and Discards: marine ecosystem analysis – population effects

## Ib Svane, Kate Rodda & Philip Thomas

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## Project No. 2003/023

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Signed:

Date:          21st March 2007
Distribution:  PIRSA Fisheries, South Australian Fisheries Research Advisory
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*"It's an accident that our culture invented writing and reading. It's a cultural artefact we've developed, but it's not in the nature of man. Two hundred years from now, we won't need this medium to transmit knowledge."*

Marcel Just, neuroscientist – the Bulletin, June 2006.

# TABLE OF CONTENTS

# Non technical summary

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**FRDC 2003/023 Prawn Fishery By-catch and Discards: marine ecosystem analysis – population effects**

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## Objectives

1.  To obtain measurements of the trawling catchability and population parameters of important by-catch and scavenger species, particularly including smaller sharks, skates and rays.
2.  To determine survival rates of key by-catch species using measures of physiological stress and mortality associated with capture and handling.
3.  To determine whether trawling actually attracts or substantially affects the movement of smaller sharks, skates and rays to scavenge on discarded by-catch.
4.  To incorporate the results into a marine tropho-dynamic model for sustainable resource utilization in Spencer Gulf (Ecosystem-Based Management).

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## Outcomes achieved to date

The project results and outputs have contributed to the following outcomes:
*   A comprehensive understanding and assessment of the ecological impact of prawn trawling on quantitatively important by-catch species and scavengers feeding on discarded by-catch. This information is instrumental in maintaining a sustainable development of the Spencer Gulf prawn fishery by identifying impacts on by-catch species and providing solutions for by-catch management. This approach promotes the industry as environmentally sustainable.
*   An assessment of the relationship between trawl duration and handling on the survival of by-catch species (elasmobranchs, blue crabs etc.). This information is important in the development of handling practices within the prawn fishery and contributes to reductions in by-catch mortality.
*   A methodology for the ecological application of Adenylate Energy Charge ratios (AEC) in fishery research, particularly in the assessment of sublethal effects on by-catch. This information is necessary for rigorous experimental analyses of fishing mortalities and their causes.

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- An assessment of catchability and fishing mortality of quantitatively important by-catch species by understanding movements and feeding behaviour during trawl operations. This information is important in order to reduce bias in by-catch estimates.
- Information on by-catch species composition and abundances necessary to identify indicators for the development of by-catch monitoring programs, thereby facilitating the development of a strategic approach to adaptive fisheries management.

In prawn trawl fisheries, the catch and discard of non-target species (by-catch) can have an impact on the ecosystem and species populations, particularly by the favouring of scavengers and removal of top predators. An understanding of the fished ecosystem, including species composition, abundance, catchability, and survival, is instrumental for the adoption of the principle of ecological sustainability and implementation of ecosystem-based management. The research reported here constitutes three separate studies, a) an ecological assessment of quantitatively dominant by-catch species using a consistent rigorous statistical approach for hypotheses testing applied to data sampled at five selected sites at the Spencer Gulf prawn fishing grounds with a history of different trawling intensities, b) a detailed assessment of physiological stress, and mortality of selected by-catch species during capture and handling, and c) a field and laboratory experimental study of movements and feeding behaviour of Port Jackson sharks during trawl operations.

In prawn trawls, the dominant by-catch categories on the Spencer Gulf prawn fishing grounds are benthos (sponges etc.), sand trevally, leatherjackets, blue crabs, sharks and rays. The mean catch rate of by-catch (total catch minus prawns) was found to be 117-187 kg/h with the exception of one site (Site 3) with about 30 kg/h for one standard prawn net with a headline length of 14.63 m and a 4.5 cm diamond mesh cod end. The wet weight of by-catch was significantly different between sites with no statistical difference between surveys and day/night trawling.

Twenty-two species of elasmobranchs were caught, comprising 10 shark, 8 ray, 3 skate and 1 chimera species. Port Jackson sharks were the most common species (61.6%), followed by the sparsely spotted stingaree (17.6%). Other species of importance were cobbler wobbegong, elephant shark, fiddler ray and the large stingrays. The number of species per site ranged from 8 to 15. Diversity, species richness and distribution were strongly related to habitat heterogeneity/complexity measured as the abundance of benthos biomass. Total abundance was 0.06-0.32 individuals per 100m$^2$, with the lowest abundance at the most northerly site (Western Shoal) and highest at the most southerly site (the Gutter). Seasonal variations in abundance were evident for most species.

Tagging of 1522 individual sharks, skates and rays, of nine species, was conducted in seven areas. Port Jackson sharks made up the majority of sharks tagged, and were the only species (with the exception of one stingaree) reported recaptured. A total of 79 Port Jackson sharks (6.3%) were recaptured after a period from 1 to 865 days.

Dispersal of sharks within the Gulf was low with 55% of individuals recaptured within 10 km of the original tag site, suggesting strong site fidelity.

Sexual dimorphism was found for many by-catch species. There were no seasonal effects on length but significant site effects were evident, showing that Port Jackson sharks decrease in mean length from north to south. The majority of species reached sexual maturity at a smaller size than reported elsewhere. The onset of earlier maturity may be a response to fishing pressure in conjunction with environmental and ecological factors. Mature Port Jackson sharks were found abundantly at the northerly sites, and were significantly less abundant in the south, probably as a consequence of habitat heterogeneity. Ageing studies of Port Jackson sharks indicated longevity to be 40.7 years, and age at maturity for males was 6-8 years and 8-11 years for females.

Sand trevally, bridled leatherjackets, rough leatherjackets, toothbrush leatherjackets, Degens leatherjackets, pygmy leatherjackets and blue crabs showed a significant variation between sites in size, weight, and abundance. For the 2004/05 surveys, the correlation analyses showed that biomass of rough leatherjackets and Degens leatherjackets were positive correlated to trawl hours and negatively correlated with benthos biomass. Excluding the Oct 2003 survey, sand trevally showed a negative correlation with historical trawl hours and no correlation with benthos biomass, which can be explained by a pelagic existence. The correlations for blue crabs with trawl hours and benthos biomass were mixed. The observed patterns of abundance suggest that habitat affects abundance but the strongest correlation is with trawl hours. Trawl hours were negatively correlated to habitat complexity, which explains 23.3% of the variance for the Oct 2004 survey and 28.6% for the Jan 2005 survey.

Little is known about the physiological state of by-catch species following trawl capture. Although some species appear alive when discarded, stress is known to cause major physiological changes, which may render them vulnerable to predation or increased mortality. We examined the stress level of the quantitatively important species, blue crab, sparsely spotted stingaree, Port Jackson shark, and Degens leatherjacket in order to establish the relationship between trawl time and stress and where possible, survival. Additional species were opportunistically sampled and assessed as well. Animals exposed to trawl times of 15, 30 and 45 minutes were compared to a base-line group that had been line or drop net captured specifically in order to minimise capture stress prior to sampling. Our data shows that, irrespective of time, trawl capture results in a response that is consistent with what is recognised in animals subject to stress. In the blue crab, post capture mortality was due almost totally to damage that caused holes in the carapace, rather than the commonly occurring limb loss. The sparsely spotted stingaree showed a clear stress response to trawl capture, and had a low probability of survival. Carapace damaged crabs and all but one stingaree died or were eaten by sea lice during a 3-day sea cage survival test. Port Jackson sharks appeared unaffected by trawl capture, and no death occurred during trawls or when kept in tanks. Of trawl captured Degens leatherjacket, approximately 46% subjected to 15-minute trawls and 78% to 45-minute trawls did not survive. The results indicate that discarded Degens leatherjackets are subjected to considerable stress during trawling, which increases with trawl time, and is likely to contribute to mortality in discarded fish.

Field experiments using Vemco® Radio Acoustic Position Telemetry (VRAPT) showed there was no effect of either by-catch or prawn trawl operations on the movements of Port Jackson sharks. Laboratory experiments showed that sharks were attracted to by-catch, but that by-catch composition and volume had little influence on attraction.

Stable isotopes can be used to determine trophic linkages because of their ability to concentrate at increasing higher trophic levels. Stable isotopes ($^{13}$C and $^{15}$N) of quantitatively important by-catch species showed a distinct trophic fractionation separating the species into six trophic groups, with crustaceans at the lowest level and Port Jackson sharks at the highest. The primary food source of crustaceans was found to be detritus. Stable isotope values for most species declined from the northern site towards the southern site, and therefore were not consistent within a species. Disregarding Port Jackson sharks at the highest trophic level, there was a general trend of negative correlation between stable isotope concentrations and trawl hours, and a positive correlation with benthos abundance, implying that discarded by-catch and/or habitat heterogeneity/complexity affects diet composition. The studied species are omnivorous with more than 10 diet categories. Port Jackson sharks had a higher occurrence of blue crabs and calamary in their diet during periods of trawling, and prawns during periods of no trawling, demonstrating that discarded by-catch affects the diet.

## Keywords

Spencer Gulf, prawn fishery, by-catch, discards, by-catch mortality, AEC-ratios, stress physiology, stable isotopes, by-catch populations, scavengers, elasmobranches.

## Acknowledgements

# CHAPTER 1: GENERAL INTRODUCTION

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## Background

The concepts of Ecosystem-Based Management (EBM) and Ecologically Sustainable Development (ESD) are important for fishery management to implement precautionary fishing, thereby protecting both the fished resources and the environment. There are scientific challenges associated with many aspects of ecosystem-based fisheries management including definitions of concepts, policy and workable approach (see Ward *et al.* 2002, Browman & Stergiou 2005, Fletcher *et al.* 2005, Scandol *et al.* 2005). However, there is a consensus that an important part of this work is to incorporate ecosystem impacts of by-catch and discards into fishery management models and operational practices, which requires that knowledge be obtained on how fishing affects ecosystem structure and function. The Standing Committee on Fisheries and Aquaculture (SCFA) and FRDC have recognised the scientific challenge of ecosystem-level goals because of the complex nature of exploited marine ecosystems and developed a national ESD reporting framework (FRDC 2000/145, 2002/86, Fletcher *et al.* 2005), which provides guidelines for a formal risk assessment process consistent with the Australian Standard (AS/NZS 4360:1999 Risk Management).

The management model and the operational practices used in the Spencer Gulf prawn fishery are well adapted to this framework, and at an international level are more technically advanced than most fisheries, allowing for the utilisation of complex ecological data, particularly data on by-catch species importance, distribution and abundance. Unlike most, the Spencer Gulf prawn fishery is highly amenable to experimental evaluation of ecosystem effects, an aspect of fisheries impact study that has received relatively little practical attention, yet considerable speculation.

The project reported here builds upon the results of FRDC project 98/225: *Prawn fishery by-catch and discards: fates and consequences for a marine ecosystem* (Svane 2003). The important results from this project included an understanding of the major components of the food chain in the trawled areas of the Gulf and the identification and quantification of the consumption rates of the major scavenger groups on discarded by-catch.

To implement EBM principles, ecological information on important by-catch elements and scavenger groups is needed for decision-making. Applying statistical comparisons between different habitats exposed to different levels of trawling activities is likely to demonstrate effects of trawling if any. Such research needs to be a continuous process to ensure that data on temporal environmental variability and by-catch dynamics are in phase with the fishery. Accordingly, we proposed three subprojects: 1) Obtain population data on the species at the top of the food chain, which constitutes smaller sharks, skates and rays, because population effects are more likely to be observed there. Population data on the other quantitatively important scavenger and by-catch species such as Degens leatherjackets and sand trevally, will be obtained simultaneously and possibly also include sealice as a separate student project. 2) Experimentally test effects of trawl duration and handling on stress and survival of major by-catch species. Stress or sub-lethal effects (catch species most likely experience elevated mortality rates after being trawled and discarded alive) will be studied using AEC-ratios (Adenylate Energy Charge) obtained using HPLC. The results of this component and its ecological application incorporates specific industry needs that will complement national projects on assessment of by-catch, and operational practices linked to the evaluation of so-called "hopper conveyors", coordinated nationally by Oceanwatch. 3) To experimentally test whether larger scavengers (sharks, rays) are attracted to trawling and discarded by-catch. If so, this may affect catchability, survival and other population characteristics. The first sub-project is for collecting general population parameters; the second is to specifically study trawl and handling stress using competence, newly developed methods, and equipment based at Lincoln Marine Science Centre; and the third, to estimate bias in mortality estimates and size of by-catch feeding guilds.

**Need**

1) The ecosystem effects of fishing need to be understood in order to work towards an EBM model for Spencer Gulf. The South Australian government's Marine and Coastal Manager's Forum has identified Spencer Gulf as the first area in SA for the development of a marine management plan. Better information on impacts of fishing will reduce the reliance of managers on the precautionary principle in developing management plans.

2) The important results from FRDC project 98/225*: Prawn fishery by-catch and discards: fates and consequences for a marine ecosystem,* are an understanding of the major components of the food chain in the trawled areas of the gulf and the identification of the major scavenger groups on discarded by-catch. There is now a need to obtain data on population impacts in order to develop tropho-dynamic models.

3) A prerequisite for energy-flow models is population data outlining life history parameters, growth, migration and immigration and standing stock of, in principle, <u>all</u> the major species.

4) Previous research shows that the quantitatively important scavengers on discarded by-catch in the Spencer Gulf prawn fishery are Degens leatherjackets, sealice (non-parasitic highly aggressive isopods and amphipods), blue crabs, large stingrays and Port Jackson sharks. The effects of discarded by-catch on the population structure of these species need to be understood with the aim of developing an ecosystem model.

5) The impact of fishing on marine populations (target and by-catch) including sharks, skates and rays is a worldwide problem. 50% of the global catch is taken as by-catch. The catch of non-target species can have an impact at the population and ecosystem levels, particularly by the removal of top predators. Elasmobranchs are at the top of the food chain in Spencer Gulf and are important by-catch in the prawn fishery. The ecosystem level effects of fishing on populations of these species are unknown. Discarded by-catch is susceptible to mortality. This mortality, including sub-lethal effects and post discard mortality, needs to be measured in order to predict ecosystem effects.

6) To adopt the principle of ecological sustainability in the prawn fishery, change negative public perceptions of environmental impacts, and improve fishing practices.

7) To enhance the research capacity in environmental research with emphasis on biological resource utilisation.

## Objectives

1. To obtain measurements of the trawling catchability and population parameters of important by-catch and scavenger species, particularly including smaller sharks, skates and rays.

2. To determine survival rates of key by-catch species using measures of physiological stress and mortality associated with capture and handling.

3. To determine whether trawling actually attracts or substantially affects the movement of smaller sharks, skates and rays to scavenge on discarded by-catch.

4. To incorporate the results into a marine tropho-dynamic model for sustainable resource utilization in the Spencer Gulf (EBM).

## Overview

The environmental effects of trawl fishing are primarily on structure, function and habitat of the exploited ecosystem (Dayton *et al.*1995, Jennings *et al.* 2001, Burridge *et al.* 2003, Tanner 2003). The impact on structure and function often translate into changes in food webs and removal of biomass, affecting populations and diversity of the ecosystem in a complex way (Jennings & Kaiser 1998, Hall 1999, Bianchi *et al.* 2000, Graham *et al.* 2001). However, the impact on the habitat is by removal of sessile fauna and epifauna, thereby fundamentally changing habitat heterogeneity and ecosystem function (Watling & Norse 1998, Jennings *et al.* 2001, Thrush *et al.* 2001, Kaiser *et al.* 2006, Queirós *et al.* 2006). The discard of by-catch impacts on the ecosystem by providing increased feeding opportunities for scavengers (Andrew & Pepperell 1992, Kennelly 1995). Some discarded by-catch, whether it is discarded underwater through modification of the net or cod-end, or by returning unwanted catch to the sea, may survive, but smaller species and juveniles do not or become vulnerable to predation because of fatigue or trawl injury (Bottari *et al.* 2003, Ryer 2004, Miller *et al.* 2005). Discarding of by-catch is affected by by-catch composition, landing restrictions and economic factors, and is usually not viewed positively by both fishermen and the public (Gillespie 2002, Catchpole *et al.* 2005). It is therefore important to understand ecosystem effects of discarded by-catch and find ways to minimize risk for by-catch and improve survival (Broadhurst 2000, Fletcher *et al.* 2005, Hall & Mainprize 2005, Harrington *et al.* 2005).

In terms of prawn catch, the Spencer Gulf prawn fishery has been operating sustainable for more than thirty years, with an annual catch varying from 1,048 t in 1986/87 to 2,522 t in 2000/01. The average catch for the last five years was 2,006 t, approximately 5% greater than the mean historical catch of 1,910 t (Carrick 2003, Dixon *et al.* 2005). However, commercial effort has declined steadily and during 2003/04 was 20,193 hours, which is 44% of the maximum recorded in 1978/79 (Dixon *et al.* 2005). Although no continuous by-catch recording is available, anecdotal information indicates that over the last 10 years quality and quantity of by-catch has remained fairly constant, depending on the area trawled, suggesting a fairly constant biomass over time representing a steady state of a fished ecosystem (see Carrick 1997, Dixon *et al.* 2005 for by-catch information).

The Standing Committee on Fisheries and Aquaculture (SCFA) and FRDC has recently developed a national ESD reporting framework (FRDC 2000/145, 2002/86, Fletcher *et al.* 2005), identifying eight major ESD components relevant for fisheries. Three of these address the contribution of a fishery to ecological wellbeing namely, retained species, non-retained species and the general ecosystem. For these environmental issues, the national ESD reporting framework provides guidelines for a formal risk assessment process consistent with the Australian Standard (AS/NZS 4360:1999 Risk Management) using ecological consequence tables that cover five areas:
1. Target species/major non-retained species,
2. By-product/minor non-retained species
3. Protected species
4. Habitat issues and
5. Ecosystem effects.

The important results from FRDC project 98/225 are an understanding of the major components of the food chain in the trawled areas of the gulf and the identification and quantification of the consumption rates of the major scavenger groups on discarded by-catch. FRDC 98/225 primarily contributed to point 4 and 5 in the ecological consequence table of the risk assessment. The project reported here is focused on the by-catch/by-product species of the Spencer Gulf prawn fishery, which

may include protected and endangered species. The project thus contributes to points 1, 2 and 3, excluding the fishery target species, western king prawn.

The implementation of EBM in the Spencer Gulf prawn fishery requires a shift from managing a single species as an isolated component to managing a complex multi-species system, which is a scientific challenge (see Scandol *et al.* 2005). In many fisheries such a shift has been accomplished by using "mass-balance" trophic-based modelling (environmental stoichiometry), which assumes a simplified structure of the ecosystem to be described as energy flow between the major groups exploited (see Pauly *et al.* 2000, Christensen & Walters 2004, Scandol *et al.* 2005). This type of modelling may be beneficial for predicting possible outcomes of management actions such as implementation of by-catch reducing devices and spatial restrictions (Gibble 2003, Criales-Hernandez *et al.* 2006). However, simplification of ecosystem effects of fishing through trophic modelling can be misleading because of the complexity of the ecosystem being modelled and unrealistic parameterisations (Fulton *et al.* 2003, Fulton & Smith 2004). In addition, ecosystem effects of fishing involve a number of elements other than biomass balances, such as impacts on population structures and habitat (Poiner *et al.* 1998, Blaber *et al.* 2000, Jennings *et al.* 2001, Burridge *et al.* 2003, Tanner 2003, Kaiser *et al.* 2006, Queirós *et al.* 2006). In order to develop a tropho-dynamic (mass-balance) model describing energy-flow, population variables (population biomass, growth, mortality, immigration/migration) of all interacting species obtained need to be estimated. Substantial research effort would be necessary to obtain and maintain such data sets and may not be cost effective.

The results obtained from FRDC 98/225 and the project reported here provide a data set that allows for the development of a simplified mass-balance (ECOPATH) model with the prospect of further development through ECOSIM and ECOSPACE by making certain assumptions and providing additional ecological data as well as fishery data (Christensen *et al.* 2005, Gribble 2000, 2003, Christensen & Walters 2004). Additional ECOPATH data requirements are available from stock assessment, other ecological studies, or through the literature. The data required are biomass estimates, total mortality estimates, consumption estimates, diet compositions, and fishery catches. ECOPATH data additionally requires regular updates to capture temporal variability. The sampling program undertaken in FRDC 98/225 and in this

project is designed for hypothesis testing using fixed sites with known environmental and fishery characteristics. For a mass-balance model, a fully replicated stratified or randomised sampling program is required, covering a considerable area of Spencer Gulf, in order to obtain spatial and temporal data on population abundances and distributions.

ECOPATH with ECOSIM is intended to lead to policy exploration for ecosystem-based fisheries management. One of the objectives for the project reported here was to incorporate the results into a marine tropho-dynamic model for sustainable resource utilisation in Spencer Gulf (EMB). In the project agreement, development of a mass-balance model was not an output or an outcome, and therefore awaits further development provided the fisheries managers and the fishing industries wish to pursue this path. However, the ecological assessment provided here, and by FRDC 98/225, will allow the development of a formal risk assessment in line with the national ESD reporting framework using ecological consequence tables by interpolation of the results from the five sites, which are believed to reflect representative habitat types and fishing grounds found in Spencer Gulf.

## The study sites

This study was conducted over a limited geographical area within Spencer Gulf, covering five representative fishing grounds (Figure 1, Table I). The depth at the five sites was between 21-33 meters. Coastal areas down to this depth were not sampled, nor were other large areas within Spencer Gulf. Diversity, richness, distribution and abundance data does not describe the entire gulf (to do so is beyond the scope of this project), but reflect what species are likely to be encountered by operational prawn trawlers in different areas of the gulf, and thus provides the means of hypotheses testing. One of the aims of the study was to obtain measurements of the trawling catchability and population parameters of important by-catch and scavenger species, particularly the smaller sharks, skates and rays. The five sites chosen were characterised by varying sediment types, environmental parameters and history of annual fishing effort. It was therefore expected to observe differences in species richness, distribution, abundance and reproductive patterns based on habitat and environmental heterogeneity and exposure to varying intensity of fishing. Seasonal

variation in abundance is not uncommon. Most species are distributed heterogeneously both spatially and temporally, because of the diversity of habitats and the requirements of individual species lifecycles (Poulard & Léauté 2002). Fishing activity also varies spatially and temporally. Hence, interactions between prawn trawling and by-catch species are not likely to be consistent in both space and time (see Burridge *et al.* 2003, 2006).

The analysis of the effort of the major fishing grounds shown in Figure 2 shows that most effort is concentrated in the areas Shoalwater/Middlebank and Wallaroo. Historically effort has been more dispersed, but due to spatial management, the areas fished have been reduced through time because of a strategy of targeting larger prawns and prawn concentrations (Figure 3). The five selected sites thus represents 73% of all effort expended during the 5-year period from 1999-2004.

Table I. Positions of the five sites selected for the study of by-catch on the Spencer Gulf prawn fishing grounds.

Site 1: $33^o$ 11.1 S; $137^o$ 36.0 E (Western Shoal)

Site 2: $33^o$ 25.8 S; $137^o$ 29.6 E (Plank Point)

Site 3: $33^o$ 36.3 S; $137^o$ 29.0 E (Middlebank)

Site 4: $33^o$ 53.0 S; $137^o$ 28.8 E (Wallaroo)

Site 5: $34^o$ 06.5 S; $136^o$ 56.3 E (the Gutter)

Figure 1. Map showing the Spencer Gulf fishing blocks, the major fishing regions and the positions ● of the five selected sites for this study (after Dixon *et al.* 2005). Northern region = site 1, Shoalwater region = site 2, Middlebank region = site 3, Wallaroo region = site 4, and Gutter region = site 5.

## Effort 1999/2000-2003/2004



Figure 2. Effort on the major fishing grounds in the Spencer Gulf prawn fishery during the periods of 1999-2004.



Figure 3. Historical trawl hours from fishing blocks around the five selected survey sites in Spencer Gulf. Northern region = site 1, Shoalwater region = site 2, Middlebank region = site 3, Wallaroo region = site 4, and Gutter region = site 5.

## Structure of the report

This report provides a comprehensive written assessment of the population level impacts on key consumer species feeding on discarded by-catch in the Spencer Gulf prawn fishery, consistent with the performance assessment that ESD and EBM principles demand. The report is written as a series of individual papers, which cover the four objectives divided into the three subprojects stated in the project agreement.

1) To obtain measurements of the trawling catchability and population parameters of important by-catch and scavenger species particularly including smaller sharks, skates and rays.

The results of this subproject are presented in Chapters 2, 3 and 4 with additional analyses in Chapter 7.

2) To determine survival rates of key by-catch species using measures of physiological stress and mortality associated with capture and handling.

The results of this subproject are presented in Chapter 5.

3) To determine whether trawling actually attracts or substantially affects the movement of smaller sharks, skates and rays to scavenge on discarded by-catch.

The results from this subproject are presented in Chapter 6, and finally

4) To incorporate the results into a marine tropho-dynamic model for sustainable resource utilisation in Spencer Gulf (EBM).

This subproject is described above and in Chapter 7. The objective of this subproject was not to develop a tropho-dynamic model, and therefore it was not listed as an output or an outcome or described in the methods. The purpose was to integrate the information collected from the research and from previous work to develop a framework for EBM. The assumption was that the fisheries managers and the prawn fishing industry concurrently would advance EBM through the 3-year project period by implementing DEH recommendations of establishing a by-catch sampling program. It is anticipated that this will occur in the near future, allowing for a formal risk assessment with the option of developing an ECOPATH/ECOSIM model. This project and FRDC 98/225 will allow this.

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# CHAPTER 2: FISHERY INDEPENDENT SURVEYS OF THE BY-CATCH OF THE SPENCER GULF PRAWN FISHING GROUNDS

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# Abstract

The by-catch on the Spencer Gulf prawn fishing grounds was studied at five representative sites using data from two fishery independent surveys with the aim of providing a broad overview of the by-catch composition at the five studied sites. At each site and survey the catch of eight 30-min trawl shots (four during the day and four at night) were sorted into dominant species and groups and weighed. The results showed that the wet weight of by-catch was significantly different between sites, with lower by-catch catch rates at Site 3 (Middlebank channel) than elsewhere. The mean catch rate of by-catch (total catch minus prawns) was found to be 146.27 kg/h for one standard prawn net with a 4.5 cm diamond mesh in the cod end. No statistical difference between surveys and day/night trawling was found. The species composition and the relative weight of the by-catch components varied between sites, but were dominated by benthos (sponges etc.), sand trevally, blue crab, and sharks and rays. The relative weights were affected by the occurrence of large numbers and sizes of sharks and rays dominating the catch in some samples. The catch rates for one net over all sites were within the range of 117-187 kg/h with the exception of Site 3 with about 30 kg/h. Disregarding depletion effects, the annual volume of by-catch caught on the Spencer Gulf prawn fishing grounds by the prawn fishing fleet for the period 1999 to 2004 was estimated to be 4,249 t per year. The largest volume was caught at the Wallaroo region (1,906 t) followed by the Corny Point region (555 t) and the Main Gutter (411 t), calculated for two nets.

## Introduction

Commercial prawn trawl fisheries are generally known to impact on the coastal ecosystem where they occur (Watling & Norse 1998, Lindegarth *et al.* 2000, Pitcher *et al.* 2000, Jennings *et al.* 2001). Common to all prawn fisheries are environmental issues in relation to discarded by-catch, which is a challenge to fishery management (Andrew & Pepperell 1992, Ortiz *et al.* 2000, Hall & Mainprize 2005, Harrington *et al.* 2006). In the South Australian prawn fisheries, by-catch usually constitutes a mix of benthic sessile organism (benthos) and a diverse group of pelagic and demersal fish and invertebrates, some exploited by other commercial and recreational fisheries. In addition, prawn-trawling by-catch may also include species of ecological significance such as protected and endangered species (Dixon *et al.* 2005). Overall, the species composition, abundance and distribution of by-catch are poorly documented. Carrick (1997) reported the by-catch composition in commercial trawls at 32 stations in Spencer Gulf, and further data has been collected haphazardly from 2000-2005 (reviewed by Dixon *et al.* 2005). This report provides a broad overview of the by-catch on five sites of the Spencer Gulf prawn fishing grounds, obtained from data collected during two fishery independent surveys. The report provides data on the major species groups in the form of catch rates (wet weight), and compares the relative distributions of these groups at five sites representing areas of different fishing intensity. Detailed analyses of important by-catch species and their trophic relationships are provided elsewhere in this report. Detailed analyses of the fate and consumption of discarded by-catch has previously been reported by Svane (2003, 2005).

## Materials and Methods

Two fishery-independent surveys were carried out in Spencer Gulf using the RV Ngerin. The first one took place in October 2004 (7-15/10) and the second one in January 2005 (23-28/1). At five sites (refer to Figure1 and Table I in Chapter 1), eight 30-minute trawl shots (four during the day and four at night) were carried out using one standard prawn trawl with a headline length of 14.63 m and a 4.5 cm diamond mesh cod end. Commercial trawling occurs only during night so daytime sampling was done for comparison and to optimise the boat time available. The speed was

maintained at 3 knots. The eight trawl transects at each site were selected haphazardly in the vicinity of the positions shown in Table I (Chapter 1), avoiding trawling over transects previously sampled during that survey. The five sites selected represent the major prawn fishing grounds, each with different historical fishing activity.

After each 30-minute trawl shot the catch was dropped on a sorting table and sorted into groups. All species of quantitative importance were identified, sorted into groups, and weighed using a spring scale for larger species and a Marel series 2000 electronic marine scale for the remainder. Sponges were not identified and the cryptofauna not extracted. By-catch was defined as all catch exclusive of prawns and thus contains the by-product slipper lobsters "bugs" (*Ibacus peronii*) and southern Calamary (*Sepioteuthis australis*).

The data were analysed using a three-way ANOVA with the fixed factors site, day/night and time (survey) and wet weight (kg) as the dependent variable using the statistical software package SPSS 14.0. Homogeneity of variances and normality were tested using Levene's test and the Kolmogorov-Smirnoff test, respectively, as provided in SPSS 14.0. Post-hoc comparisons was done using Tukey HSD post-hoc test. Partial eta squared values are provided as an alternative measure for effect size (Pallant 2005). Individual species are not analysed here but are dealt with in details in Chapters 3 and 4.

## Results

The list of species and groups is shown in Table I. Variability in by-catch composition, distribution and abundance was evident. In particular, variability in the catches of large elasmobranchs, particularly smooth stingrays and Port Jackson sharks was found. Overall homogeneity of variances could not be achieved irrespective of transformation. However, a detailed analysis showed that all variables were normally distributed and that non-significant homogeneity of variances was only evident for the factor site. Because ANOVA's are generally robust against non-homogeneous variances, the non-transformed data were analysed regardless (see Underwood 1997, Quinn and Keough 2002). Catch rates showed a significant effect of site ($F_{[4, 78]}=11.871$, $P<0.001$, partial eta squared $= 0.450$) (Figure 1), but no significant effect

of day/night ($F_{[1, 78]}$=0.186, P=0.668), time ($F_{[1, 78]}$=0.071, P=0.791) (Figures 2 and 3) with no significant interactions ($F_{[4, 78]}$=0.795, P=0.533). The partial eta squared for the factor site was large (0.450) with a power of 1.0, while partial eta squared values for the factors day/night and time were small (0.003 and 0.001) giving a power of 0.071 and 0.058, respectively, increasing the probability of Type 1 errors for these two latter factors. Accordingly, no significant differences in catch rate were found between surveys and between night and day (Figure 1 and 2). High variability between trawl transects was evident, caused by the occasional capture of large stingrays leading to the low statistical power. However, a statistically significant difference between sites was evident (Figure 3). A Tukey HSD post hoc test separated Site 3 from Site 2 and Sites 1, 4 and 5 combined. No significant differences were found between Sites 1, 4 and 5 (Figure 3). The mean level of catch rates of by-catch was thus Site 2>Site1≥Site 4≥Site 5>Site 3 with 187 kg/h at Site 2, 146 kg/h at Site 1, 126 kg/h at Site 4, 118 kg/h at Site 5, and 30 kg/h at Site 3 caught with one net only. The mean catch rate of by-catch for all sites was 146 kg/h for one net.

Figure 1. The mean catch rates (kg/h wet weight) of by-catch obtained during two surveys of five major Spencer Gulf prawn fishing grounds. The prawn catch is excluded. Error bars are 95% CI.

Table I. List of species groups and single species collected during two fishery independent surveys on the Spencer Gulf prawn fishing grounds.

| | |
|---|---|
| Benthos* | sponges, bryozoans, molluscs etc. |
| Blue crab (*Portunus pelagicus*) | single species |
| Bugs (*Ibacus peronii*) | single species |
| Prawn (*Melicertus latisulcatus*) | single species |
| Degens leatherjacket (*Thamnaconus degeni*) | single species |
| Sand trevally (*Pseudocaranx wrighti*) | single species |
| Snapper (*Pagrus auratus*) | single species |
| Cephalopods (*Sepioteuthis australis, Sepia apama*) | southern calamary and cuttlefish |
| Miscellaneous fish | bullseye, hardyhead, soldierfish, striped trumpeter etc. |
| Port Jackson shark (*Heterodontus portusjacksoni*) | single species |
| Australian angel shark (*Squatina australis*) | single species |
| Elephant fish (*Callorhinchus milii*) | single species |
| Cobbler wobbegong (*Sutorectus tentaculatus*) | single species |
| Melbourne skate (*Dipturus whitleyi*) | single species |
| Fiddler ray (*Trygonorrhina fasciata*) | single species |
| Smooth stingray (*Dasyatis brevicaudata*) | single species |
| Black ray (*Dasyatis thetidis*) | single species |
| Eagle ray (*Myliobatis australis*) | single species |
| Miscellaneous elasmobranchs | stingarees, shovelnose ray |

* The group "benthos" includes a high diversity assemblage of benthic organisms dominated by sponges, sponge crypto-fauna, bryozoans and bivalves.

Figure 2. The mean catch rates (kg/h wet weight) of by-catch obtained at both day and night during two surveys of five major Spencer Gulf prawn fishing grounds. The prawn catch is excluded. Error bars are 95% CI.



Figure 3. The mean catch rates (kg/h wet weight) of by-catch at five sites obtained during two surveys of the major Spencer Gulf prawn fishing grounds. The prawn catch is excluded. Error bars are 95% CI.

Figure 4. The relative abundance (%) by weight of by-catch on the Spencer Gulf prawn fishing grounds at all five sites.

The five sites also showed differences in species composition and relative abundance. A broad overview of the relative abundance of by-catch, excluding prawns, in terms of wet weight is shown in Figure 4. These results showed that at Sites 1 and 2, benthos, prawns and blue crabs dominated (Figure 4). At Site 3, the abundance of benthos was low (presumably due to high tidal currents and hard gravel bottoms), thus increasing the relative abundance of particularly sand trevally, Port Jackson sharks and miscellaneous fish (a group of smaller fish). The four dominant groups and species by weight at Site 4 were benthos, blue crabs, Port Jackson sharks and miscellaneous fish. At Site 5, the abundance changed to five major groups and species namely, sand trevally, prawns and smooth stingray followed by Degens leatherjacket and miscellaneous fish.

The relative abundances are affected by the occurrence of large elasmobranchs dominating by weight in some samples. The catch rates of benthos, elasmobranchs (sharks and rays), blue crabs and prawns are shown in Figures 5, 6, 7 and 8. The catch rates of benthos by weight were highest at Sites 1 and 2. At these two sites, sponges and patches of the mytelid bivalve *Trichomya hirsutus* (bearded mussel) were abundant. Further south, at Sites 4 and 5, patches of sponges and bryozoans dominated the benthos. The recorded catch rates at Site 1 and 2 were 85 and 109 kg/h, respectively. The catch rates of sharks and rays were highest at Sites 2, 4 and 5, but with a large variability between samples (Figure 6). The highest catch rate was recorded at Site 4 with 41 kg/h. The catch rate of blue swimmer crabs (*Portunus pelagicus*) was highest at Site 4 with 30 kg/h (Figure 7). The catch rate of prawns (*Melicertus latisulcatus*) during the two surveys was highest at Site 1 and 2 with 51 and 44 kg/h. However, at these two sites the prawns were small although abundant (Figure 8).

Figure 5. The mean catch rates of the group "benthos" in by-catch at five sites obtained during two surveys of the major Spencer Gulf prawn fishing grounds. Error bars are 95% CI.



Figure 6. The mean catch rates of the group "sharks and rays" in by-catch at five sites obtained during two surveys of the major Spencer Gulf prawn fishing grounds. Error bars are 95% CI.

Figure 7. The mean catch rates of blue swimmer crabs in by-catch at five sites obtained during two surveys of the major Spencer Gulf prawn fishing grounds. Error bars are 95% CI.



Figure 8. The mean catch rates of prawns at five sites obtained during two surveys of the major Spencer Gulf prawn fishing grounds. Error bars are 95% CI.

Figure 9. The mean catch rates of Cephalopods (southern calamary and cuttlefish) in by-catch at five sites obtained during two surveys of the major Spencer Gulf prawn fishing grounds. Error bars are 95% CI.

The catch rates of cephalopods (southern calamary = *Sepioteuthis australis* and cuttlefish = *Sepia apama*, combined), slipper lobster (*Ibacus peroni*) and snapper (*Pagrus auratus*) are shown in Figures 9, 10 and 11, respectively. Of theses species southern calamary and slipper lobster, also referred to as "bugs", are by-product in the Spencer Gulf prawn fishery. Cephalopods occurred in the by-catch throughout the gulf but were more abundant at Site 4 with a mean of 2.45 kg per hour wet-weight. Slipper lobster was also caught throughout the gulf but with highest numbers at the two northern (Sites 1 and 2), with 1.48 and 1.16 kg per hour, respectively (Figure 10). Snapper was caught at Sites 1 and 2 only, predominantly as juveniles, with 3.66 and 0.33 kg per hour, respectively (Figure 11).

Figure 10. The mean catch rates of slipper lobster (*Ibacus peroni*) in by-catch at five sites obtained during two surveys of the major Spencer Gulf prawn fishing grounds. Error bars are 95% CI.



Figure 11. The mean catch rates of snapper (*Pagurus auratus*) in by-catch at five sites obtained during two surveys of the major Spencer Gulf prawn fishing grounds. Error bars are 95% CI.

Table II. Estimates of the annual by-catch per region and total annual by-catch in the Spencer Gulf prawn fishery based on mean trawl hours for the fishing season 2004-05 and mean by-catch catch rates.

| Region | Mean trawl hours (2004-05) | Standard Error | Site for by-catch survey | Mean by-catch catch rate (kg/h/net) | Total annual by-catch (kg/net) |
|---|---|---|---|---|---|
| Northern region | 1,045.1 | 520.3 | 1,2 | 166.9 | 174,385 |
| Wallaroo | 7,562.8 | 250.2 | 4 | 126.0 | 952,913 |
| Shoalwater/Middlebank | 4,580.3 | 246.6 | 3 | 30.1 | 137,821 |
| Wardang | 874.2 | 245 | 5 | 117.6 | 102,762 |
| Main Gutter | 1,747.3 | 146.4 | 5 | 117.6 | 205,395 |
| Cowell | 707.8 | 185 | 5 | 117.6 | 83,201 |
| West Gutter | 269.5 | 63.6 | 5 | 117.6 | 31,680 |
| South Gutter | 658.8 | 146.6 | 5 | 117.6 | 77,442 |
| Corny Point | 2,359.7 | 470.2 | 5 | 117.6 | 277,383 |
| Thistle Island | 694.1 | 333.9 | 5 | 117.6 | 81,592 |
| Total (h and kg) | 20,499.6 | | | | 2,125 |
| Total for two nets (t) | | | | | 4,250 |

## Discussion

The distribution and abundance of by-catch on the Spencer Gulf prawn fishing grounds is variable and reflects different habitats of a fished ecosystem. The overview of the results of the two surveys presented here only takes into account variability between surveys three months apart. During that time, the Spencer Gulf prawn fishery completed the pre-Christmas fishing period of 5,509 hours of trawling from the 14th November to the 7th December 2004.

The composition of by-catch varied with sites .The catch rates between sites are within the range of 117-187 kg/h with the exception of Site 3 with about 30 kg/h for one net. The trawl grounds with the highest number of trawl hours are historically

Sites 2, 3 and 4. However, this is dependent on time of year and the current fishing strategy. The fishing fleet operates at about 20,000 trawl hours per year distributed over 39 fishing vessels for about 60 days using two nets.

In prawn and shrimp trawling, the proportion of non-target species (by-catch) can be high, but with substantial variation between and within geographical regions (Alverson *et al.* 1994, Andrew & Pepperell 1992). The annual biomass of by-catch caught on the Spencer Gulf prawn fishing grounds by the prawn fishing fleet can be estimated by using the mean catch rates obtained from the fishery independent by-catch surveys and the mean trawl hours. The estimates by region and the total estimated by-catch are shown in Table II. The total volume of by-catch for the period 1999 to 2004 caught by the Spencer Gulf prawn fishing fleet was estimated to be 4,250 t per year. The largest biomass was caught in theWallaroo region (1,906 t) followed by the Corny Point region (555 t) and the Main Gutter (411 t). These estimates, however, ignore depletion effects, which are likely to occur during fishing periods. Carrick (1997) reported the overall catch/by-catch ratio to be 1:3.5, which implies that with an annual prawn catch of 2000 t then the by-catch volume should be about 7000 t.  McShane *et al.* (1998) reported a catch/by-catch ratio for the Spencer Gulf prawn fishery to be 2:1 implying an annual by-catch volume of 1000 t. In the tropical Queensland prawn fishery, the reported catch/by-catch ratio is 1:4.3 and is accordingly much higher (Poiner *et al.*1998). However, generalisations based on catch, effort and/or catch/by-catch volumes are simplified descriptors of by-catch. Rochet & Trenkel (2005) found that the assumption, that discards are proportional to catch or to effort, is generally not supported by the available evidence, because both environmental conditions and fishing methods and strategies influence the amounts and composition of discards.

Few attempts have been made to manage by-catch in prawn trawl fisheries effectively through spatial management, and the major focus has been on by-catch reducing devices (BRD's) or modifications to mesh configurations of the cod end (Broadhurst *et al.* 2004, Hall and Mainprize 2005, Criales-Hernandez 2006). However, while modification of fishing gear may differentiate selectivity and thereby meet catch and by-catch reference points set by management, it might do little to mitigate ecosystem effects of trawling because of damage to escaping fish and depletion of benthic fauna

(Wassenberg & Hill 1993, Farmer *et al.* 1998, Burridge *et al.* 2003). In this study a rigorous statistical sampling program was undertaken at five sites representing different historical levels of effort in the Spencer Gulf prawn fishery. In order to develop a realistic by-catch risk assessment for management purposes, by-catch composition, abundance and distribution at the full spatial and temporal scale of the fishery, including estimates of depletion, have to be obtained. A simple by-catch management system can be developed using spatial catch rates for dominant groups calculated as probabilities of by-catch load measured at the start of the fishing season applying risk assessment principles (http://www.fisheries-esd.com/c/implement/implement0200.cfm). Because the spatial and temporal fishing pattern in the Spencer Gulf prawn fishery are fairly constant between years, depletion rates of benthic fauna and habitat risks may be measured once with subsequent between years temporal adjustments, and repeated if changes of the general ecological conditions occur.

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# CHAPTER 3: POPULATION PARAMETERS OF KEY ELASMOBRANCH BY-CATCH SPECIES IN SPENCER GULF

## Section I. General Introduction

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## Background

Elasmobranchs are a diverse group of animals. Worldwide there are 1025 species, of which 300 are found in Australian waters. These are made up of 167 true sharks, 117 ray species and 13 chimeras. More than half of these are endemic to Australia. Elasmobranchs can be found in a broad range of habitats from shallow coastal waters, across the continental shelf and slope, deep oceans and even in river systems. They can be pelagic (mid-upper water), or benthic, living at or near the seafloor. Many species are highly migratory and travel long distances across the world's oceans.

The diversity and wide distribution of elasmobranchs mean that they are exposed to many fisheries and likely to interact with fishing operations as both target and non-target species. Global commercial landings are approximately 800 000 metric tonnes and it is estimated that the unreported by-catch is similar (Stevens *et al.* 2000). Absolute numbers or weight taken as by-catch are not known because there is no requirement for fisheries to record such data. However, where it has been recorded, it has been described as constituting a large quantity of the catch.

In Australia, elasmobranchs are taken by commercial, indigenous, recreational and game fishers as well as in beach control programs for bather protection. Elasmobranchs can be taken as target species (that species which the fisher purposefully targets), or as incidental catch, which is either retained (and called by-product) or discarded (referred to as by-catch). There is currently little or no requirement for fisheries to record the by-catch element of their catch.

Elasmobranchs play an important role in ecosystem structure and function and concerns about declining population sizes have been raised internationally. Sharks are often the top order predator and their removal, whether it is targeted or incidental, can trigger widespread changes in the overall marine ecosystem.

There are direct and indirect consequences of fishing activities on populations of elasmobranchs. Direct effects through the capture of individual species can lead to changes in abundance, size structure of the population and density-dependent life history parameters (Rago *et al.* 1998, Walker and Hislop 1998, Walker *et al.* 1998,

Stevens *et al.* 2000 and Graham *et al.* 2001). When fishing gear selects for larger, mature animals, a change in size structure and reproductive strategy (i.e. smaller size at maturity) may result. Indirect effects of fishing include the selective removal of predator or prey species, removal of competitors, species replacement, and even positive effects on populations due to the enhanced food supplies through discarded by-catch (Stevens *et al.* 2000).

Current catches of elasmobranchs, as target and non-target species are widely considered unsustainable. Elasmobranchs are long-lived, slow growing, late to mature and have a low fecundity making them susceptible to over exploitation by commercial fisheries. The selective removal of larger mature animals by virtue of the gear selectivity, adds to the risk of over-exploitation by further decreasing reproductive rates. These life history traits mean that populations are often slow to recover if they are overfished.

In response to worldwide concerns over increases in shark fishing, vulnerability of sharks to overfishing, and the lack of management by the majority of nations involved in this activity, an International Plan of Action for the Conservation and Management of Sharks (IPOA) was developed by the Food and Agriculture Organisation of the United Nations in 1999 (FAO 1999). The IPOA suggests that those nations that target shark, or regularly catch shark, develop a National Plan of Action for sharks (NPOA). Australia ratified its NPOA nationally in April 2004 (NPOA 2004). The Shark-plan was developed to ensure that all Australia's shark species are managed sustainable at a national level. The Shark-Plan will ensure that special conservation and management needs of sharks are not overlooked in managing the impacts of all resource users on the marine environment.

We need to improve our knowledge of the biology and catch vulnerability of the wide variety of elasmobranch species that are caught as by-catch or in target fisheries. Knowledge of the reproductive biology of a species is essential to understanding its ecology, and to implementing effective management and conservation measures. We also need to understand the status of the populations in those areas where encounter with fishery gear is likely, and understand the impacts of fishing on populations and mortality rates as a consequence of capture. There is little information on the

percentage of these animals that survive their encounter with fishing gear. A study of the Northern Prawn Fishery estimated a 66% mortality rate, while sharks are likely to have an even higher mortality rate if caught on long-lines or in gill nets (. Understanding these issues may lead to changes in the operational practices of commercial fishing vessels to reduce by-catch or increase survival of those caught.

A rapid assessment of the risk to elasmobranch species due to fishing has been developed by Walker (2004). It is based on a small data series over a short time frame and ranks various biological and ecological parameters in order to identify those species most likely to need precautionary management. The approach involves monitoring all species impacted by fishing and is an interim measure for management. While long-term monitoring and complex data sets are required for a full understanding of the impacts (Walker 2004), this allows a quick evaluation of the effects of fishing on populations while longer-term research is carried out. A similar assessment has been utilized by Stobutzki *et al.* (2001, 2002) for teleost and elasmobranch by-catch in the tropical prawn fisheries. Biological parameters include productivity (based on reproductive rate and natural mortality), abundance over time and position in the food web. In addition, the catch susceptibility of a species is determined through availability, encounterbility, selectivity of the gear and post-capture mortality. In general, elasmobranchs are thought to be susceptible to overfishing due to their life history traits, however the ability of a species to be fished sustainable is highly variable. Some are particularly resilient, while others have high mortality rates. The ability to examine each species individually and assess the risk on an individual species basis allows more precise management decisions to be made.

**Current knowledge of key elasmobranch by-catch species in Spencer Gulf**
Many species of elasmobranch can be found in Spencer Gulf (Last and Stevens 1994). Those that have preferred habitats that overlap with the traditional fishing grounds are likely to interact with the commercial prawn fishery at some point in time, for example, those species that are bottom dwelling and prefer open, sandy environments. The biological database for these species is generally poor. However because some of the species are commonly found and are known to interact with fishing operations in other state waters, some information is available for the most commonly caught species. This information is summarised below.

*Port Jackson shark*

One of 3 species of Heterodontidae found in Australian waters, *Heterodontus portusjacksoni* has a distribution covering the southern half of Australia, from Byron Bay (NSW) to the Houtman Abrolhos (WA). It is a small, common shark that has a high affinity for interactions with a wide range of commercial and recreational fisheries due to its scavenging nature. The maximum size of this species is not clear, ranging from 1120 – 1650 mm total length (TL) (Last and Stevens 1994, Izzo 2005). The largest animal collected during this three-year study was 970 mm TL and discussions with fishers operating in the Spencer Gulf prawn fishery confirm that they have not seen animals larger than this. Females attain a much larger size than males, by at least 250 mm. Most of the ecological and biological information known about this species comes from a study by McLaughlin and O'Gower in the late 1960's on the east coast populations. It is oviparous, laying 10-16 egg capsules in a breeding season lasting 2-3 months. The species is highly migratory, with females returning to the same egg laying sites each year (Mclaughlin and O'Gower 1971). The eggs have a long incubation of 12 months (Rodda 2000), after which time the young shark tends to remain in or near a nursery area for several years in mixed sex populations. At the onset of sexual maturity, the population segregates into males and females. Clasper growth is rapid during the maturation process and males become mature at 700-800 mm TL while females become mature at 800-950 mm TL. After breeding, the males tend to move into deeper waters, while the females remain to lay the eggs.


*Stingarees*

The Urolophidae (stingarees and round stingrays) are comprised of approximately 40 species, most of which belong to the genus *Urolophus*. The remaining six species are endemic to Australia and belong to the genus *Trygonoptera* (Last and Stevens 1994). Five species are found in gulf waters in South Australia, *Urolophus expansus, Urolophus gigas, Urolophus orarius, Urolophus paucimaculata* and *Trygonoptera mucosa.* Little is known about the biology of either genus, although they are described as viviparous or live bearing. White *et al.* (2001) conducted detailed research on the reproductive biology, age and growth in the Western Australian stingaree, *Urolophus lobatus*, while the reproductive biology of North American round stingray, *Urobatis halleri* (previously known as *Urolophus halleri)* was the subject of a Ph.D. dissertation by Babel in 1967. Vaudo and Lowe (2006) used conventional and

acoustic tags to track populations of *U. halleri* to examine movement patterns in California, showing that migration was seasonal and related to environmental factors, particularly water temperature.  Populations aggregated in areas where water temperature was significantly elevated to breed, and quickly left the area soon after. The widely distributed and commonly caught *U. paucimaculata* has received little attention until recently. Aspects of the population dynamics and ecology were reported by Edwards (1980), and only recently has more intensive research been carried out in Victoria and Western Australia (Trinnie, pers. comm., White and Potter 2005). White also described the reproductive biology, age and growth in *T. personata* and *T. mucosa* (White *et al.* 2002) and was the first to report embryonic diapause in members of the *Trygonoptera* genus.

Females reach greater maximum size and size at maturity than males for all four species of Urolophidae found in south-western Australia.  Size at maturity first occurs at 242 and 310 mm TL, and maximum length is attained at 365 and 435 mm TL for male and female *U. lobatus* (White *et al.* 2001). *U. halleri* reaches a greater size than *U. lobatus* but is similar to *U. paucimaculata* (males – 450 mm TL and females – 530 mm TL). Both sexes become sexually mature at 265 mm TL (Babel 1967). White and Potter  (2005) report that *U. paucimaculata* conceives early/mid summer and gives birth late spring/ early summer, similar to *U. lobatus*. Gestation can last 10-12 months and fecundity ranges from 2-6 (Babel 1967, Edwards 1980, White *et al.* 2002, White and Potter 2005, Trinnie, pers. comm.), however, as gestation proceeds, White *et al.* (2001) believe that some of the embryos may perish, such that only one or two are born. Size at birth is typically large for these species, with newborns approximately 33-52% of their maximum adult size (White *et al.* 2001, 2002, White and Potter 2005). Large size at birth necessitates a low fecundity.

A more detailed examination of *U. paucimaculata* has been the focus of a recent Ph.D. study in Victoria (Trinnie, University of Melbourne). Sexual maturity is attained at a TL of 280 mm and 270mm TL for males and females respectively, although Edwards (1980) did not observe pregnant females until 340 mm TL. They have a relatively low fecundity of around 6 that is likely to increase with maternal size (Edwards 1980, Sosebee 2005). After a long gestation (12 months) the young are born during spring at 155 mm TL. White (unpublished data in White *et al.* 2001) reports a

similar birth size of 158 and 154 mm TL for Western Australian populations of *U. paucimaculata* and *U. lobatus,* respectively.

Abundances during winter and summer in Port Philip Bay differed significantly, possibly as populations moved into deeper water in winter or the reduction in activity during winter reduced the potential for capture because they were less likely to be stirred by the trawl net (Edwards 1980). There has been no research on South Australian populations, despite this species being a significant proportion of elasmobranch by-catch in prawn trawl fisheries (estimated at 10% by number or approximately 25,000 caught per year in Spencer Gulf).

*Large stingrays (Genus Dasyatis and Myliobatitis)*
The large stingrays *Dasyatis* spp. and *Myliobatitis* spp. are viviparous with a relatively short gestation of 4-4.5 months (Snelson *et al.*1988, Capapé 1993). Fecundity is low (1-6) and the young born at 10-37% of adult size.

*D. brevicaudata* (smooth stingray) and *D. thetidas* (black ray) have wide distributions, ranging from Shark Bay, WA to southern Queensland, including Tasmania. Both species are also common around NZ and off the coast of southern Africa. *D. brevicaudata* is the largest of all stingrays, reaching a disc width (DW) of at least 210 cm and TL of 430 cm. It has a relatively small birth size of 17% of maximum size (or 360 cm DW) (Last and Stevens 1994). Of the 24 individual smooth stingrays caught during this project, only one was small enough to be considered newborn (344 mm DW caught in January 2005). *D. thetidas* can also reach large size, reportedly reaching 180 cm DW and 400 cm TL. Black rays have been observed free swimming at 35 cm DW (Last and Stevens 1994). Our records include only 14 black rays, with the smallest being 33 cm DW in November 2004.

Amongst the Myliobatididae (eagle rays), only the southern eagle ray, *M. australis,* can be found in southern Australian waters. It is a common species, found in shallow sandy areas from Jurien Bay (WA) to Moreton Bay (Qld), including the east coast of Tasmania (Last and Stevens 1994). A smaller ray than the smooth and black rays, it reaches approximately 120 cm DW and 190 cm TL and is born at about 32 cm DW.

We captured only 14 eagle rays during this project the smallest being 60 cm DW, therefore no information on parturition seasons for this species could be gathered from our data.

*Shovelnose rays*

Little is known about the shovelnose ray family (Rhinobatidae) in Australian waters. Being demersal in nature and preferring sandy substrata, they are susceptible to being caught in bottom trawl fishing operations and have been recorded in gillnet and long line fisheries (Walker and Hudson 2005). The western shovelnose ray, *Aptychotrema vincentiana*, is found in the relatively shallow waters of southern and western Australia, from Bass Strait to Port Hedland (Western Australia). A relatively small ray, it reaches about 79 cm TL. The larger eastern shovelnose ray, *A. rostrata*, is restricted to the east coast of Australia and reaches 120 cm TL. The southern fiddler ray, *Trygonorrhina fasciata*, is common in the shallow coastal waters from eastern Bass Strait to Lancelin (WA) (Last and Stevens 1994). It is a medium sized ray, reportedly reaching 126 cm TL.

The first reported description of the reproductive potential of Australian rhinobatids (western shovelnose rays and southern fiddler rays) was carried out in 1885 by Haacke (see Kyne and Bennet 2002). Since then there has been nothing published until Kyne and Bennet (2002) who described the reproductive biology of the eastern shovelnose ray. Shovelnose rays are aplacental viviparous, that is live bearing with the developing embryo utilising a food source such as ova, yolk reserves or placental analogues (Carrier *et al.* 2004). In general, gestation in rhinobatids is relatively long (between 4-12 months) (Wenbin and Shuyuan 1993, Abdel-Aziz *et al.* 1994), however the eastern shovelnose ray (*A. rostrata*) appears to have a shorter gestation of 3-5 months (Kyne and Bennet 2002). The only recorded information on the western shovelnose ray, *A. vincentiana*, does not indicate a gestation period or a reproductive season but suggests that males mature at about 65 cm TL and a fecundity of 14-16 young (Last and Stevens 1994). Reported fecundity of the *A. rostrata* is similar (4-18) but the data may be incomplete, not taking into account environmental factors and maternal size. Fecundity in the fiddler ray is much lower (1-6), and after a 12 month gestation, the young are born in April (Readon pers. comm.). Both males and females reach maturity at about 750-800 mm TL.

*Elephant shark*

The elephant shark, *Callorhinchus milii*, is closely related to sharks and rays. It is the only recorded species within the family Callorhinchidae that inhabits Australian waters. The maximum recorded size for females is 1200 mm TL and males 700 mm TL. Males mature at about 550-650 mm TL (Last and Stevens 1994, Bell pers. comm.), while females mature at about 700-731 mm TL (Walker and Hudson 2005, Bell pers. comm.). The species is oviparous, laying 16-24 large leathery egg capsules in sandy and muddy substrates over a 2-3 month period. Eggs are laid in shallow, soft and bare habitats. Sticky hairs on the outer surface of the capsule result in sediment holding onto and burying the capsule for the incubation period (Bell pers. comm.). Incubation lasts 5-10 months, depending on environmental conditions, after which time, an 80-120 mm long hatchling emerges sometime during August to November.

Significant catches of elephant shark are recorded from southern Australia. Over the last 10 years, commercial catches have been relatively stable at around 50-60 tonnes, prompting the assessment of the species resilience to fishing (Walker and Hudson 2005). While common in South Australian gulf waters, no comparative studies have taken place.

*Angel sharks*

Angel sharks belong to the family Squatinidae, of which 4 species are found in Australian waters. The Australian angel shark, *Squantina australis*, is the largest of the four, reaching at least 152 cm in length (Last and Stevens 1994) and has the greatest distribution. *S. australis* is found in relatively shallow water on the continental shelf along the southern half of Australia, from Rottnest Island, Western Australia to near Sydney, New South Wales. This species is targeted for its flesh by some fisheries, and due to its demersal nature, constitutes a substantial proportion of the by-catch in bottom trawl fisheries (Jones pers. comm., this study).

Despite the importance of angel sharks as a commercial fishery and by-catch species, little research has been conducted on the reproductive biology of this species. In order to assess the ecological risk of fishing on *S. australis*, a rapid risk assessment was conducted (Tovar-Avila *et al.* 2004), which found that females mature at 800-1000 mm TL, reproduce every 2-3 years and after a long gestation of 12-14 months,

produce up to 14 pups. According to the risk assessment, *S. autralis* was classified as a medium biological risk. But with all the components of the risk assessment considered, the species had an overall low ecological risk. However, breeding every 2-3 years increases the risk to populations in Australia because if mortality is high enough, populations may eventually be unable to replenish themselves under a constant fishing pressure.

*S. australis* forms a substantial proportion of the by-catch in trawl fisheries operating in Western Australia (Jones pers. comm.). Biological information of collected specimens shows that male angel sharks mature at about 750 mm TL, while females mature at 850 mm TL, similar to the Victorian populations. Sexual maturity is similarly late in the Pacific angel shark, *S. californica* (Natanson and Calliet 1986), occurring between 900-1000 mm TL, but fecundity is lower and breeding occurs annually. Late size at sexual maturity and annual cycles are also reported in angel sharks from Brazil, *S. guggenheim* and *S. occulta* (Sunye and Vooren 1997).

An estimated 13,000 angel sharks are caught by the Spencer Gulf prawn fishery each year, representing 5% of the elasmobranch by-catch. While it was not possible to assess mortality due to trawling in this study, based on observations on captured individuals the species appeared quite resilient and post trawl survival is likely to be high. It is possible that unknown long-term effects may increase the mortality rate.

*Skates*

Skates are small to large rays whose disc is more circular or rhomboidal in shape. The snout varies in shape and can be rather pointed and supported by a central cartilage (Last and Stevens 1994). There are 38 species from at least five genera that occupy Australian waters. They are largely tropical or subtropical species. Spencer Gulf is home to four species, the peacock skate, *Pavoraja nitida,* the pygmy thornback skate, *Raja* spp., the white spotted skate, *Raja cerva,* and the Melbourne skate, *Raja whitleyi,* the latter two being the most common. *R. whitleyi* is the largest skate, reaching at least 170 cm TL and over 50 kg. This skate is found on the continental shelf from Albany (WA) to Wollongong (NSW) where it prefers shallow water but has been recorded from depths of 170 m (Last and Stevens 1994).

Reproductive information is scarce for all skate species and there is virtually no information on the biology of skate species in Australia.  There are some data on size at maturity in males that indicates a very late maturity relative to maximum length, consistent with international studies. In general, male skates do not reach sexual maturity until approximately 60-90% of maximum TL (Last and Stevens 1994, Mabragaña and Cousseau 2004, Gedamke *et al.* 2005, Oddone *et al.* 2005, Whittamore and McCarthy 2005). It can be estimated that male *R. whitleyi* are not mature until at least 120 mm TL, assuming the maximum of 170mm TL reported for females (Last and Stevens 1994) is similar to that reached by males because sexual dimorphism appears to be less obvious in skate species (Last and Stevens 1994, Gedamke *et al.* 2005, Oddone *et al.* 2005, Whittamore and McCarthy 2005). Male *R. cerva* are reportedly not mature until at least 450 mm TL (Last and Stevens 1994), although our data suggest that this is an overestimate, with males apparently mature at about 285 mm TL.

Skates are becoming increasingly targeted overseas as fisheries economics drive the retaining of skate species rather than discarding them (McFarlane and King 2006, Ruocco *et al.* 2006). Researchers in these areas have urged governments to support research on these species in order to develop fishery management plans.

**Aims of this study**

Given the biological characteristics of elasmobranchs (late maturity, slow growth, low fecundity and slow recovery rate), and the conservation status of some and unknown status of many, it is vital for the fishing industries and research to work together to reduce the incidence of by-catch and increase the survival of those species that are caught.  The Spencer Gulf and West Coast prawn fishery is regarded as one of the best-managed prawn fisheries in the world. The prawns of Spencer Gulf have been fished sustainable for many years and contribute more than $30 million annually to the South Australian economy. Many fisheries around the world have significant levels of by-catch. This fishery is unique in that is has a relatively low by-catch: prawn catch ratio (3-3.5:1) compared to tropical prawn and shrimp fisheries around the world (Carrick 1997, Svane 2003, Stobutzki *et al.* 2001, this study). The aim of this study was to establish population parameters of the key elasmobranch species caught as by-catch in the Spencer Gulf prawn fishery.   This study builds upon the

results of the FRDC project 98/225*: Prawn fishery by-catch and discards: fates and consequences for a marine ecosystem* conducted recently in Spencer Gulf, South Australia. The important outcomes from this project were an understanding of the major components of the food chain in the trawled areas of the gulf and the identification and quantification of the consumption rates of the major scavenger groups on discarded by-catch.

Spencer Gulf is an ecologically rich environment that supports a large variety of marine fauna and flora. Among the extensive fauna, elasmobranchs make up an important component. Spencer Gulf also supports several commercial and recreational fisheries that interact with the fauna and flora to varying degrees. Elasmobranchs are an important top predator in Spencer Gulf, and constitute a major component of the by-catch in the commercial prawn fishery. The ecosystem level effects of fishing on the populations of these species are unknown. The activity of the Spencer Gulf prawn fleet was assessed in terms of the number of hours fished within blocks in and immediately surrounding our five research sites. In the last seven years, fishing effort in the gulf has been subtly changed, such that effort has increased in the south and decreased in the north. The mean annual effort for the five sites varied such that the majority of fishing effort concentrated around Wallaroo (Site 4 - 665.2 hours) and Middle Bank (Site 3 - 483.6 hours), with comparatively little effort occurring around The Gutter (Site 5 - 112.8 hours), Western Shoal (Site 1 - 44.4 hours) and Plank Point (Site 2 - 49.1 hours). The five sites were also characterised by varying sediment types and environmental parameters. Sediment changed from fine sand at Sites 1 and 2, hard coarse sand and gravel at Site 3, mixed sand and patches of gravel at Site 4 to sandy plains at Site 5 (Svane 2003). Physical parameters such as salinity, temperature and water currents also differed between sites. During the summer months, the two northern-most sites (1 and 2) experienced slightly higher temperatures and salinity than sites located further south (Bye 1981, Smith and Veeh 1989). Tidal currents at Sites 3 and 4 were also stronger than other sites (Svane 2003). Differences in species richness, distribution, abundance and reproductive strategies based on habitat and environmental complexity and exposure to varying intensity of fishing were therefore expected. Most species are distributed heterogeneously both spatially and temporally because of the diversity of habitats and the requirements of individual species lifecycles (Poulard and Léauté 2002). Fishing activity also varies spatially and

temporally. Hence, interactions between the fishing fleet and by-catch species are not likely to be consistent. Knowledge of the seasonal and spatial variability in population parameters will lead to better predictions of the interactions that may occur.

It was the aim of this study to determine if they were any correlation between fishing activity and population dynamics of the major elasmobranch by-catch species. Each area of study is reported in separate sections. The first is this section. Section II describes the distribution, diversity and abundance of key elasmobranch by-catch species at the five sites in Spencer Gulf. Section III discusses movement within the gulf through tagging studies. Morphometric comparisons between sites are made in Section IV including allometric relationships, size structure and sex ratios. Section V describes reproduction in the two most common species and how fishing activities overlap with reproductive strategies for the key species. Age and growth relationships in the Port Jackson shark are examined in Section VI. The final section includes an overall summary and general conclusions regarding the population parameters of the key elasmobranch by-catch species encountered in this project.

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# Section II. Diversity, Distribution and Abundance

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# Abstract

The spatial and temporal abundance and distribution of 1428 sharks, skates and rays caught in trawls in Spencer Gulf was determined from five sites and seven seasons between October 2003 and October 2005. The five sites chosen were representative of commercial fishing blocks and were characterized by varying fishing intensities, habitat and environmental parameters.  Overall diversity was low. A total of 22 species of sharks, skates and rays were caught, comprising 10 shark, 8 ray, 3 skate and 1 chimera species.  Five of these species were considered unique, being represented by only one individual and a further four considered rare.  The number of species per site ranged from 8 at Site 3 to 15 at Site 5.  Site 5 was the preferred site for many species, and was the site with highest overall abundance  (mean = 0.32 individuals per 100m$^2$). Site 1 had the lowest abundance (0.06 individuals per 100m$^2$). Season effects on abundance were evident for most species.  Abundance was highest in January 2005 and October 2005 and lowest in April 2004 and 2005, however variability amongst the species was high, reflecting species biological characteristics.  The physical and environmental attributes of the five sites vary considerably and it is likely that habitat complexity, reproductive cycles and food availability were major contributors to the observed patterns of abundance and distribution.

## Introduction

Spencer Gulf is home to a diverse group of marine fauna and flora. It also represents an environmentally complex system with high habitat complexity and changing physical attributes. The five sites chosen in this project are characterised by varying sediment types, environmental parameters, habitat complexity and fishing effort. Sediment changes from fine sand at Sites 1 and 2 (Western Shoal and Plank Point), hard coarse sand and gravel at Site 3 (Middle Bank), mixed sand and patches of gravel at Site 4 (Wallaroo) to sandy plains at Site 5 (The Gutter) (Svane 2003). Physical parameters such as salinity, temperature and water currents also differ between sites. During the summer months, the two northern-most sites (1 and 2) experience slightly higher temperatures and salinity than sites located further south (Bye 1981, Smith and Veeh 1989). Tidal currents at Sites 3 and 4 are also stronger than at other sites (Svane 2003). Habitat complexity is defined by the biomass of bycatch and this varies substantially between sites and season (Chapter 2). It is likely therefore that there will be differences in species distribution and abundance among the sites according to each species habitat preferences. It was the aim of this project to determine which species were present in Spencer Gulf and were likely to interact with commercial prawn trawlers, and to determine the effects of site and trawl activity on the distribution and relative abundance of elasmobranchs.

## Materials and Methods

### Sampling

Population parameters of sharks, skates and rays in Spencer Gulf were determined from five sites (Site 1 -Western Shoal, Site 2 -Plank Point, Site 3 -Middle Bank, Site 4 -Wallaroo and Site 5 - the Gutter) and over seven sampling periods hereon referred to as seasons (October 2003, April 2004, October 2004, January 2005, April 2005, July 2005 and October 2005). The five sites are representative of commercial fishing blocks and are characterized by varying fishing intensities. The mean annual effort (in hours) between the years 1999 and 2004 in and around these sites is shown in Figure 1.

Trawling was carried out from the research vessel *Ngerin*, using a standard otter trawl with a headline length of 14.63 m and a minimum mesh size of 4.5 cm. The trawl

consisted of a cone shaped net closed at one end by a cod-end, and lateral wings extending forward from the opening that are held open horizontally by two otter boards. The net typically opens to 80% of its capacity during fishing.  Only one net was deployed. At each site for each of the seven seasons, 4 day and 4 night trawls were carried out, each 30-minutes in duration. The exception was Site 2 where damage to the net during trawling in January 2005 resulted in two fewer trawls being conducted at this site. All sharks, skates and rays were identified and counted.



Figure 1. The annual mean fishing effort (hours) reported within the vicinity of the five research sites in Spencer Gulf during 1999 and 2004. Error Bars are 95% Confidence Intervals.

**Diversity**

In order to estimate diversity patterns and compare the five sites, diversity estimates were calculated using Simpson's Index of Diversity and Evenness according to Krebs (1999). Simpson's Index of Diversity is based on the probability that two randomly chosen individuals will be different species.  Evenness illustrates how evenly the species are distributed among the total number of individuals. The actual number of species caught in the individual trawl samples was correlated to historical fishing effort estimated as the 6-year mean trawl hours (1999-2004) for a particular site using Spearman Rank Correlation. To determine whether species were distributed evenly at the five sites, Goodness of Fit tests were carried out for each species. The null hypothesis was that all species were distributed equally among the five sites.

**Abundance**

Abundance of the most common species was calculated as the number caught per $100m^2$. The area covered by each trawl was calculated as:

Area ($100m^2$) = speed of trawl (metres per hour) x width of net opening (14.63m x 0.8) x time (hour) x $10^{-4}$

Where speed (m per h) = speed (knots) x 1842. The net typically opens to only 80% during trawling operations hence the available area is only 14.63 x 0.8 m. The dimension of height of the net is ignored. Typically, the research vessel travelled at 3 knots and the trawl area covered by the one net in 30 minutes was $0.032 \text{ km}^2$.

Biomass (weight per $100m^2$) was not examined in this section because abundance was considered a better measure of any effect on the population of the species. The biomass of elasmobranchs is treated with other by-catch species in Chapter 2.

**Data analysis**

The individual species abundance data from replicated trawl samples was tested using a three-way MANOVA with site, time of day and season as fixed factors for the nine most common species. Five sites, two trawl times (night or day) and seven seasons were included in the analyses. Significant interactions between factors led to further analysis using three-way ANOVAs for each species with abundance as the dependent factor and site, time of day and season as fixed factors. Multiple comparisons were made with the Dunnett C test (if there were no interactions between the factors), or with one-way ANOVA's comparing one factor separately at each level of the corresponding interacting factor (if significant interactions were found). Homogeneity of variances was tested using Levene's test, however normality and homogeneity of variances within the data were rare because of the large number of zero values and patchiness of distribution of the species. Transformations on the data were carried out in an attempt to achieve homogeneity of variances but these were largely unsuccessful. Because ANOVA is robust against violations of assumptions if samples are large enough (> 30) and of approximately equal size (Underwood 1997, Zar 1999), an analysis was performed on non-transformed data regardless.

## Results

### Diversity

1428 sharks, skates and rays were caught in 278 trawls from 5 sites over three years.
Overall diversity was low. Across the five sites, 22 species of sharks, skates and rays
were caught between October 2003 and October 2005, comprising 10 shark, 8 ray, 3
skate and 1 chimera species (Table I, Figure 2). Five of these species were considered
unique, being represented by only one individual (gulf catshark, ornate wobbegong,
broadnose sevengill shark, black ray and the coastal stingaree), and a further four
considered rare with less than 5 individuals (common sawshark, pygmy thornback
skate, white spotted skate and gummy shark).  The number of species per site was low
(Table II), ranging from 8 (Site 3) to 15 (Site 5).  53% of the species represented at
any one site were considered unique or rare for that site, but they were generally
found at more than one site thus increasing their overall numbers.  Simpson's Index of
Diversity and Evenness was calculated for each site (Table II, Figure 3). Sites 1, 2 and
5 had similar diversity, while Sites 3 and 4 were substantially lower. There was a 0.66
probability that two individuals chosen at random from Site 5 would be different
species, whereas two individuals from Site 3 had a 0.31 probability that they would be
different species. Seasonal variation in Simpson's Index of Diversity at each site was
highly variable (Figure 4). Sites 1 and 5 appeared to be relatively constant between
seasons while the remaining sites were variable and no seasonal trends could be
identified. Species at Sites 1, 3 and 5 were distributed more evenly than those at Sites
2 and 4, although the variability was high (Figure 3b) and the differences are not
likely to be significant. There was no correlation between the number of species
present and mean fishing effort for the period 1999/2004 (rho=0.052, p=0.393,
n=276).

### Distribution

Table III shows the percentage of individuals from each species found at each site.
Many of the species were found at some but not all sites, others at all sites but not
equally.  For example, Port Jackson sharks were found at all sites but demonstrated a
preference for Site 5 (31.9%). Site 5 was also the preferred site for stingarees (98.8%),
elephant sharks (67.3%) and skates (86.4%), while 97.6% of angel sharks were found
at Site 2. Southern fiddler rays were found at all sites but showed a preference for Site

4 (40.6%).  While Site 5 was the preferred site for many species, some (cobbler wobbegong and western shovelnose rays) were noticeably absent from this most southern site while relatively evenly distributed further north. For each species where sufficient data existed, Goodness of Fit tests confirmed an uneven distribution across the five sites (Table IV).

Table I. A list of the species of sharks, skates and rays caught at the five sites between October 2003 and October 2005. * designates those species that were considered unique and only one individual was encountered.  ** identifies rare species, less than 5 in total number.

|  | Scientific name | Common name |
| --- | --- | --- |
| Sharks | *Heterodontus portusjacksoni* | Port Jackson shark |
|  | *Squatina australis* | Australian angel shark |
|  | *Sutorectus tentaculatus* | Cobbler wobbegong |
|  | *Orectolobus ornatus* | Ornate wobbegong * |
|  | *Squalus megalops* | Piked spurdog |
|  | *Asymbolus vincentii* | Gulf catshark  * |
|  | *Pristiophorus cirratus* | Common sawshark ** |
|  | *Pristiophorus nudipinnis* | Southern sawshark |
|  | *Notorynchus cepedianus* | Broadnose sevengill shark  * |
|  | *Mustelus antarcticus* | Gummy shark  ** |
| Rays | *Aptychotyrema vincentiana* | Western shovelnose ray |
|  | *Trygonorrhina fasciata* | Southern fiddler ray |
|  | *Dasyatis brevicaudata* | Smooth stingray |
|  | *Dasyatis thetidis* | Black ray  * |
|  | *Myliobatis australis* | Eagle ray |
|  | *Urolophus paucimaculatus* | Sparsely spotted stingaree |
|  | *Urolophus orarius* | Coastal stingaree  * |
|  | *Hypnos monopterygium* | Coffin ray |
| Skates | *Dipturus whitleyi* | Melbourne skate |
|  | *Raja cerva* | White spotted skate  ** |
|  | *Raja spp.* | Pygmy thornback skate  ** |
| Chimeras | *Callorhinchus milii* | Elephant shark |

Port Jackson shark

Elephant shark

Ornate wobbegong

Cobbler wobbegong

Figure 2. Some of the sharks,
skates and rays caught during
trawling in Spencer Gulf.

Sparsely spotted stingarees

Numbfish

Australian angel sharks

Figure 2 continued. Some of the sharks, skates and rays caught during trawling in Spencer Gulf.

Melbourne skate

Broadnose sevengill shark


Western shovelnose ray


Southern fiddler ray

Figure 2 continued. Some of the sharks, skates and rays caught during trawling in Spencer Gulf.


Eagle rays

Table II. Simpson' measure of diversity and evenness at the five sites in Spencer Gulf.

| Site | Number of species | Total number of individuals | Simpson's Index, 1-D | Simpson's Evenness |
|------|------|------|------|------|
| 1 | 11 | 101 | 0.67 | 0.71 |
| 2 | 10 | 241 | 0.61 | 0.53 |
| 3 | 8 | 139 | 0.31 | 0.66 |
| 4 | 11 | 227 | 0.43 | 0.52 |
| 5 | 15 | 720 | 0.66 | 0.62 |



Figure 3. Species diversity at the five sites, shown as Simpson's Index of Diversity (a) and Evenness (b). Error bars are 95% CI.

Figure 4. Species diversity at the five sites for the seven seasons shown as Simpson's Index of Diversity (1-D).

Table III. The percentage of each species found at each site

| Species | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 |
|---|---|---|---|---|---|
| Port Jackson shark | 8.1 | 21.9 | 15.3 | 22.8 | 31.9 |
| Stingaree | 0.4 | 0 | 0.4 | 0.4 | 98.8 |
| Elephant shark | 6.9 | 15.5 | 0 | 10.3 | 67.3 |
| Southern fiddler ray | 21.9 | 17.2 | 15.6 | 40.6 | 4.7 |
| Melbourne skate | 0 | 0 | 4.5 | 9.1 | 86.4 |
| Angel shark | 2.4 | 97.6 | 0 | 0 | 0 |
| Cobbler wobbegong | 39.0 | 36.6 | 4.9 | 19.5 | 0 |
| Smooth stingray | 20.0 | 6.6 | 13.4 | 20.0 | 40.0 |
| Western shovelnose ray | 10.0 | 60.0 | 20.0 | 10.0 | 0 |

Table IV. Results from the chi-square analysis testing equal distribution among the five sites. P values in bold are significant.

| Species | $\chi^2$ | P |
|---|---|---|
| Port Jackson shark | 103.98 | **P<0.001** |
| Stingaree | 1010.23 | **P<0.001** |
| Elephant shark | 84.59 | **P<0.001** |
| Southern fiddler ray | 22.09 | **P<0.001** |
| Melbourne skate | 61.18 | **P<0.001** |
| Angel shark | 154.24 | **P<0.001** |
| Cobbler wobbegong | 25.95 | **P<0.001** |

**Abundance**

Of the 22 species, only nine were found in sufficient numbers to conduct any statistical analysis. The remaining species were found infrequently (less than 5 in total), or only encountered on one occasion (e.g. black ray, broadnose sevengill shark, coastal stingaree, gulf catshark, ornate wobbegong and piked spurdog), and were excluded from the analyses. Figure 5 shows the overall abundance for the nine most abundant species (5a), species abundance as a function of site (5b), time of trawl (5c) and season (5d). The abundance for the nine individual species at each site and season are shown in Figures 6 and 7 respectively.

The results from the three-way MANOVA (Table V) reveal a statistical difference among the variables. A Pillai's trace value of 0.642 with a significance of P<0.001 shows a statistically significant difference in abundance between seasons. Similarly, statistically significant differences due to site and time of day were found (Pillai's trace value =1.204, P<0.001 and 0.149, P<0.001 respectively). Significant interactions between season and site, and site and time were also found (Pillai's trace value =1.684, P<0.001 and 0.410, P<0.001 respectively). Consequently, three-way ANOVA's were performed for each of the nine species with abundance as the dependent variable and season, site and time of day fixed factors (Table VI). Site had a significant effect on abundance for all but one species (smooth stingrays). A significant effect of time was found for three species (fiddler and shovelnose rays, and elephant sharks), and seasonal changes in abundance were significant for six species

(stingarees, elephant sharks, fiddler rays, Melbourne skates, cobbler wobbegongs and smooth stingrays). However, there were significant interactions between site and season for three of the nine species (Table VI), suggesting the effect of site was inconsistent across season for these species. Significant interactions between site and time, and season and time were also found for a few species (Table VI). The interactions between variables are shown as interaction graphs (Figures 8 and 9), and can be further analysed using one-way ANOVA's. The complex interactions made patterns in abundance difficult to interpret; however some generalisations can be made. Abundance across the five sites varied with species and was inconsistent with season and time. A peak in abundance during summer (January 2005) was observed for seven of the nine species, especially at Site 5. A smaller peak in abundance occurred during October 2004 for some species but generally only at one site. April 2005 recorded a low abundance for almost all species at all sites.



Figure 5. Mean abundance (number per 100m$^2$) of the nine most common elasmobranch species in Spencer Gulf. Effect of species (a), site (b), time of day trawled (c) and season (d) are shown. Error bars are 95% CI. Key: PJS = Port Jackson shark, S=stingaree, E = elephant shark, SS = smooth stingray, WSR = western shovelnose ray, AAS = Australian angel shark, CW = cobbler wobbegong, FR = fiddler ray and MS = Melbourne skate.

*Port Jackson sharks*

Port Jackson sharks were the most abundant species (Figure 5a), with an abundance of $0.727 \pm 0.174$ per 100m$^2$ (mean $\pm$ 95% CI). Abundance was highly variable across all sites and seasons (Figures 6 and 7). The three-way ANOVA showed a significant effect of site but not season on the abundance (Table VI). However, interaction between site and season was significant, indicating that the two factors were not independent of each other in regard to their effect on abundance. Interactions were high (Figure 8), with only 6% of the variation explained by site (partial eta squared 0.058). One-way ANOVA's comparing abundance across the five sites for each season, demonstrated that abundance varied significantly during April 2004, October 2004 and January 2005. In April 2004 and January 2005, abundance was lowest at Site 1 and highest at Site 5, while in October 2004, Site 2 had the lowest abundance and the highest abundance of Port Jackson sharks was recorded at Site 4. For all other seasons, the abundances across the five sites were similar.

*Stingarees*

Stingarees were the second most abundant species (Figure 5a), with $0.284 \pm 0.106$ individuals per 100m$^2$. They were found almost exclusively at Site 5 (Figure 6), and during all seasons, but had a substantially higher abundance during January 2005 and October 2005 (Figure 7). Consequently, the three-way ANOVA showed a significant effect of both site and season, and interactions between them (Table VI). Seasonal (11.0%) and site (52.3%) effects explained a large amount of the variance. One-way ANOVA's showed that the abundance across sites varied significantly for each season ($F_{(4,39)}$ =7.198, 9.428, 6.058, 109.451, 21.763, 18.066 and 8.909, p<0.001), due to a significantly higher abundance at Site 5 ($F_{(6,55)}$ =4.896, p=0.001) (Figure 8).

*Elephant sharks*

Results from the three-way ANOVA revealed significant effects for all main factors (Table VI). However, significant interactions between site and season, season and time, and site and time were also found (Figures 8 and 9), suggesting that the effects on abundance attributed to one factor was not independent from the other main effects. Site and season contributed to a large proportion of the variation (30.8 and 17.4% respectively). Elephant sharks were only captured during winter and spring (July and October), and were noticeably absent during summer and autumn (January

and April) (Figure 7). Elephant sharks were also patchy in their distribution. Elephant sharks did not occur at Site 3 and were only rarely observed at Sites 1, 2 and 4. The majority of individuals (67%) were captured from Site 5 (Figure 6). One-way ANOVA's showed that seasonal abundance varied significantly for each of the individual sites where elephant sharks were found (Site 1: $F_{(6,53)}=6.670$, p<0.001; Site 2: $F_{(6,55)}=2.338$, p=0.046; Site 4: $F_{(6,55)}=2.289$, p=0.050; Site 5: $F_{(6,55)}=3.445$, p=0.006). This was due to the absence of elephant sharks in January and April. With the exception of October 2005 ($F_{(4,39)}=1.470$, p=0.232), abundance varied significantly across all sites for each season that elephant sharks were caught (October 2003: $F_{(4,39)}=3.432$, p=0.018; October 2004: $F_{(4,39)}=4.633$, p=0.004; July 2005: $F_{(4,39)}=6.828$, p<0.001), due to the majority of elephant sharks being caught at Site 5. There were significant changes in seasonal abundance for both night and day trawls (night: $F_{(6,136)}=2.223$, p=0.045; day: $F_{(6,140)}5=2.598$, p=0.021), due to the absence of elephant sharks in three seasons (Figure 9). However, abundance at night or day did not vary significantly for each season, that is, for each season, abundance was similar during night and day (October 2003: $F_{(1,39)}=1.218$, p=0.277; October 2004: $F_{(1,39)}=3.200$, p=0.082; July 2005: $F_{(1,39)}=3.294$, p=0.077; October 2005: $F_{(1,39)}=0.68$, p=0.415). Abundances varied significantly across the five sites at night ($F_{(4,136)}=10.856$, p<0.001), but not during day trawls ($F_{(4,140)}=1.714$, p=0.150). This difference was only apparent at Site 5 ($F_{(1,55)}=11.506$, p=0.001), with strong overlap between night and day at all other sites (Figure 9). Abundance was higher at night at Site 5.

*Smooth stingrays*

Significant seasonal effects for smooth stingrays (Table VI) were due to their absence during four seasons (Figure 7). A Dunnett C post hoc test on season showed abundance in January 2005 was higher than all other seasons. Abundance did not vary with site or time of day trawled. However, significant interactions between time of trawl and site were found. One-way ANOVA's showed that abundance across sites did not vary for night or day (night: $F_{(4,140)}=2.316$, p=0.060; day: $F_{(4,136)}=0.259$, p=0.903), however the abundance between night and day did vary significantly at Site 5 ($F_{(1,55)}=5.277$, p=0.026). This can be seen in Figure 9 where abundance was higher during daytime trawls at this site.

*Western shovelnose rays*

There was a significant effect of site and time of trawl on abundance (Table VI). No significant interactions were found. However, due to the small number of individuals caught (20) and low power of detection, these data should be treated with caution. A Dunnett C post hoc test did not reveal any site to be significantly different from any other, but a trend for higher abundance at Site 2 was observed during most seasons, with few individuals caught at Sites 1, 3 and 4. None were caught at Site 5 (Figure 6).

*Angel sharks*

Angel sharks were only caught at the two northern-most sites (Figure 6) and in all seasons (Figure 7). There was a significant effect of site, but not of season or time on the abundance of angel sharks, and no significant interactions between any of the main effects (Table VI). A Dunnett C post hoc test on site confirmed the observation that abundance at Site 2 was significantly higher than Site 1, the only other site at which angel sharks were caught.

*Cobbler wobbegongs*

Significant effects of site and season were found for cobbler wobbegongs (Table VI). Interaction between them was also significant, with 13% of the variance attributed to site. Hence, abundance varied with site but not consistently between seasons. One-way ANOVA's showed seasonal abundance varied significantly for two of the four sites at which cobbler wobbegongs occurred (Site 2: $F_{(6,55)} = 2.562$, p=0.033; Site 4: $F_{(6,55)} = 2.289$, p=0.050). Abundance at Sites 1 and 3 did not vary across seasons ($F_{(6,53)} = 1.204$, p=0.321; $F_{(6,55)} = 0.838$, p=0.547 respectively). At Site 2, the highest abundances were recorded in October 2003 and April 2004, and the lowest in October 2004 and January 2005, with no wobbegongs caught in July 2005 or October 2005 (Figure 8). The pattern was different at Site 4, where the highest abundance was recorded in April of 2004 and 2005, the lowest in October 2003, with no individuals caught in October 2004 and 2005 and July 2005. For each individual season, abundances across the five sites varied significantly on three occasions (October 2003: $F_{(4,39)} = 4.296$, p=0.006; January 2005: $F_{(4,37)} = 2.867$, p=0.038; July 2005: $F_{(4,39)} = 6.971$, p<0.001). In October 2003, the highest abundance was at Site 2, while in January 2005 and July 2005, Site 1 had the highest abundance (Figure 8). For each occasion, few individuals were found at Site 3 and none at Site 5. Abundance across

the five sites did not vary significantly for the remaining seasons (April 2004: $F_{(4,39)}$ =2.059, p=0.107; October 2004: $F_{(4,39)}$ =1.444, p=0.240; April 2005: $F_{(4,39)}$ =1.612, p=0.193; October 2005: $F_{(4,39)}$ =2.332, p=0.075).

*Southern fiddler ray*

Fiddler rays were found at all sites, with the majority caught at Site 4, and during all seasons (Figures 6 and 7). Significant effects of site, season and time of trawl were found (Table VI). Interaction between site and season was also significant, with 6-7% of the variance attributed to both site and seasonal effects, due to high abundance at Site 4 during October 2004 when abundance increased markedly (Figure 8). One-way ANOVA's showed seasonal abundance varied significantly only at Site 4 ($F_{(6,55)}$ =2.892, p=0.017), and differences between sites were only significant in October 2004 ($F_{(4,39)}$ =8.349, p<0.001). Abundance at all other sites and seasons did not vary significantly (Site 1: $F_{(6,53)}$ =1.215, p=0.316; Site 2: $F_{(6,55)}$ =1.051, p=0.405; Site 3: $F_{(6,55)}$ =1.116, p=0.340; Site 5: $F_{(6,55)}$ =0.751, p=0.612; October 2003: $F_{(4,39)}$ =2.520, p=0.059; April 2004: $F_{(4,39)}$ =0.075, p=0.989; January 2005: $F_{(4,37)}$ =1.585, p=0.201; April 2005: $F_{(4,39)}$ =0.078, p=0.989; July 2005: $F_{(4,39)}$ =0.885, p=0.483; October 2005: $F_{(4,39)}$ =0.505, p=0.732).

*Melbourne skates*

Melbourne skates were found predominantly at Site 5 and were absent in the northern Spencer Gulf (Sites 1 and 2)(Figure 6). Skates were captured during all but one trip but were most abundant in January 2005 (Figure 7). Significant site, seasonal and interaction effects were found (Table VI), suggesting that the effect of site were inconsistent with season. Site explained a large amount of the variance (partial eta squared =0.225), while season was responsible for 7.3% of the variance. One-way ANOVA's showed that seasonal abundance varied at two of the three sites where skates were found (Site 4: $F_{(6,55)}$ =2.332, p=0.046; Site 5: $F_{(6,55)}$ =3.585, p=0.005). Significant differences across the five sites were found during October 2003 ($F_{(4,39)}$ =5.703, p=0.001) and January 2005 ($F_{(4,37)}$ =7.724, p<0.001). Figure 8 demonstrates these patterns, showing an increase in abundance at Site 5 during October 2003 and January 2005, and a smaller increase at Site 4 during October 2004. Abundance at Site 3, the remaining site where skates were found, did not vary seasonally ($F_{(6,55)}$ =1.000, p=0.436). Similarly, abundances across the sites were similar for the most of

the seasons (April 2004: $F_{(4,39)} = 1.000$, p=0.421; October 2004: $F_{(4,39)} = 1.168$, p=0.342; April 2005: $F_{(4,39)} = 2.332$, p=0.075; October 2005: $F_{(4,39)} = 1.000$, p=0.421).



Figure 6. Mean abundance (# per 100m$^2$) for each species per site. Data are pooled for season. Error bars are 95% CI.

Figure 6 continued. Mean abundance (# per 100m$^2$) for each species per site. Data are pooled for season. Error bars are 95% CI.



Figure 7. Mean abundance (# per 100m$^2$) for each species per season. Data are pooled for site. Error bars are 95% CI.

Figure 7 continued. Mean abundance (# per 100m$^2$) for each species per season. Data are pooled for site. Error bars are 95% CI.

Figure 8. Interaction graphs for individual species of mean abundance (numbers per 100m²), site and season. Error bars are omitted for clarity. Key: closed circle = Site 1, open square = Site 2, open triangle = Site 3, cross = Site 4 and asterisks = Site 5.

Figure 9. Interaction graphs for elephant sharks and smooth stingrays showing mean abundance (numbers per 100m$^2$) for season and time, and site and time. Error bars are 95% CI. Key: closed circle = night, open circle = day.

Table V. Results from a three-way MANOVA with species abundance as the dependent variable and time of day, site and season as fixed factors for the nine most common species. Results are from Pillai's trace multivariate test.  P values in bold indicate a significant effect.

| Effect | Value | F | Hypothesis df | Error df | P | Partial eta squared | Noncent. Parameter | Power |
|---|---|---|---|---|---|---|---|---|
| season | 0.642 | 2.728 | 54.00 | 1230.00 | **<0.001** | 0.107 | 147.31 | 1.000 |
| site | 1.204 | 9.717 | 36.00 | 812.00 | **<0.001** | 0.301 | 349.81 | 1.000 |
| time | 0.149 | 3.896 | 9.00 | 200.00 | **<0.001** | 0.149 | 35.066 | 0.994 |
| season * site | 1.684 | 1.996 | 216.00 | 1872.00 | **<0.001** | 0.187 | 431.03 | 1.000 |
| season * time | 0.287 | 1.145 | 54.00 | 1230.00 | 0.223 | 0.048 | 61.84 | 0.994 |
| site * time | 0.410 | 2.577 | 36.00 | 812.00 | **<0.001** | 0.103 | 92.77 | 1.000 |
| season*site*time | 1.076 | 1.176 | 216.00 | 1872 | **0.049** | 0.120 | 254.08 | 1.000 |

Table VI. Results from a three-way ANOVA with time of day, site and season as fixed factors for the nine most common species. P values in bold indicate a significant effect at 0.05.

| Species | Source | SS | df | MS | F | P | Partial eta squared | Power |
|---|---|---|---|---|---|---|---|---|
| Port Jackson shark | season | 20.253 | 6 | 3.375 | 1.788 | 0.105 | 0.049 | 0.663 |
| | site | 24.287 | 4 | 6.072 | 3.209 | **0.014** | 0.058 | 0.821 |
| | time | 1.494 | 1 | 1.494 | 0.821 | 0.366 | 0.004 | 0.143 |
| | season * site | 85.367 | 24 | 3.557 | 1.877 | **0.010** | 0.178 | 0.988 |
| | season * time | 17.814 | 6 | 2.969 | 1.572 | 0.157 | 0.043 | 0.596 |
| | site * time | 7.913 | 4 | 1.978 | 1.036 | 0.390 | 0.020 | 0.326 |
| | season*site*time | 53.673 | 24 | 2.236 | 1.182 | 0.261 | 0.120 | 0.871 |
| | Error | 393.97 | 208 | 1.894 | | | | |
| Stingaree | season | 9.914 | 6 | 1.652 | 4.296 | **0.000** | 0.110 | 0.980 |
| | site | 87.752 | 4 | 21.938 | 57.036 | **0.000** | 0.523 | 1.000 |
| | time | 0.447 | 1 | 0.447 | 1.163 | 0.282 | 0.006 | 0.189 |
| | season * site | 40.640 | 24 | 1.693 | 4.402 | **0.000** | 0.337 | 1.000 |
| | season * time | 0.662 | 6 | 0.110 | 0.287 | 0.943 | 0.008 | 0.128 |
| | site * time | 1.546 | 4 | 0.387 | 1.005 | 0.406 | 0.019 | 0.315 |
| | season*site*time | 2.608 | 24 | 0.109 | 0.283 | 1.000 | 0.032 | 0.221 |
| | Error | 80.005 | 208 | 0.385 | | | | |
| Elephant shark | season | 0.788 | 6 | 0.131 | 7.312 | **0.000** | 0.174 | 1.000 |
| | site | 1.667 | 4 | 0.417 | 23.189 | **0.000** | 0.308 | 1.000 |
| | time | 0.359 | 1 | 0.359 | 20.007 | **0.000** | 0.088 | 0.994 |
| | season * site | 2.620 | 24 | 0.109 | 6.074 | **0.000** | 0.412 | 1.000 |
| | season * time | 0.457 | 6 | 0.076 | 4.242 | **0.000** | 0.109 | 0.978 |
| | site * time | 1.342 | 4 | 0.336 | 18.677 | **0.000** | 0.264 | 1.000 |
| | season*site*time | 2.522 | 24 | 0.105 | 5.848 | **0.000** | 0.403 | 1.000 |
| | Error | 3.737 | 208 | 0.018 | | | | |
| Fiddler ray | season | 0.398 | 6 | 0.066 | 2.125 | **0.052** | 0.058 | 0.753 |
| | site | 0.498 | 4 | 0.125 | 3.987 | **0.004** | 0.071 | 0.904 |
| | time | 0.198 | 1 | 0.198 | 6.322 | **0.013** | 0.029 | 0.706 |
| | season * site | 1.322 | 24 | 0.055 | 1.763 | **0.019** | 0.169 | 0.981 |
| | season * time | 0.174 | 6 | 0.029 | 0.927 | 0.477 | 0.026 | 0.363 |
| | site * time | 0.028 | 4 | 0.007 | 0.221 | 0.927 | 0.004 | 0.097 |
| | season*site*time | 0.774 | 24 | 0.032 | 1.031 | 0.428 | 0.106 | 0.806 |
| | Error | 6.501 | 208 | 0.031 | | | | |
| Melbourne skate | season | 0.167 | 6 | 0.028 | 2.736 | **0.014** | 0.073 | 0.867 |
| | site | 0.614 | 4 | 0.153 | 15.083 | **0.000** | 0.225 | 1.000 |
| | time | 0.010 | 1 | 0.010 | 0.948 | 0.331 | 0.005 | 0.163 |
| | season * site | 0.738 | 24 | 0.031 | 3.022 | **0.000** | 0.259 | 1.000 |
| | season * time | 0.007 | 6 | 0.001 | 0.117 | 0.994 | 0.003 | 0.079 |
| | site * time | 0.024 | 4 | 0.006 | 0.601 | 0.662 | 0.011 | 0.196 |
| | season*site*time | 0.041 | 24 | 0.002 | 0.169 | 1.000 | 0.019 | 0.138 |
| | Error | 2.117 | 208 | 0.010 | | | | |

Table VI continued. Results from a three-way ANOVA with time of day, site and season as fixed factors for the nine most common species. P values in bold indicate a significant effect at 0.05.

| Species | Source | SS | df | MS | F | P | Partial eta squared | Power |
|---------|--------|-----|-----|-----|-----|-----|-----|-----|
| Cobbler wobbegong | season | 0.153 | 6 | 0.026 | 2.101 | **0.054** | 0.057 | 0.748 |
| | site | 0.378 | 4 | 0.094 | 7.781 | **0.000** | 0.130 | 0.997 |
| | time | 0.003 | 1 | 0.003 | 0.214 | 0.644 | 0.001 | 0.075 |
| | season * site | 0.504 | 24 | 0.021 | 1.731 | **0.022** | 0.166 | 0.979 |
| | season * time | 0.036 | 6 | 0.006 | 0.500 | 0.808 | 0.014 | 0.200 |
| | site * time | 0.112 | 4 | 0.028 | 2.312 | 0.059 | 0.043 | 0.666 |
| | season*site*time | 0.201 | 24 | 0.008 | 0.690 | 0.859 | 0.074 | 0.576 |
| | Error | 2.525 | 208 | 0.012 | | | | |
| Angel shark | season | 0.115 | 6 | 0.019 | 1.085 | 0.372 | 0.030 | 0.424 |
| | site | 2.011 | 4 | 0.503 | 28.578 | **0.000** | 0.355 | 1.000 |
| | time | 0.028 | 1 | 0.028 | 1.598 | 0.208 | 0.008 | 0.242 |
| | season * site | 0.462 | 24 | 0.019 | 1.095 | 0.351 | 0.112 | 0.837 |
| | season * time | 0.164 | 6 | 0.027 | 1.550 | 0.163 | 0.043 | 0.591 |
| | site * time | 0.147 | 4 | 0.037 | 2.085 | 0.084 | 0.039 | 0.614 |
| | season*site*time | 0.651 | 24 | 0.027 | 1.543 | 0.057 | 0.151 | 0.959 |
| | Error | 3.656 | 208 | 0.018 | | | | |
| Shovelnose ray | season | 0.016 | 6 | 0.003 | 0.735 | 0.622 | 0.021 | 0.288 |
| | site | 0.039 | 4 | 0.010 | 2.622 | **0.036** | 0.048 | 0.728 |
| | time | 0.017 | 1 | 0.017 | 4.730 | **0.031** | 0.022 | 0.581 |
| | season * site | 0.054 | 24 | 0.002 | 0.613 | 0.922 | 0.066 | 0.511 |
| | season * time | 0.004 | 6 | 0.001 | 0.198 | 0.977 | 0.006 | 0.101 |
| | site * time | 0.014 | 4 | 0.004 | 0.969 | 0.425 | 0.018 | 0.304 |
| | season*site*time | 0.076 | 24 | 0.003 | 0.859 | 0.658 | 0.090 | 0.704 |
| | Error | 0.766 | 208 | 0.004 | | | | |
| Smooth stingray | season | 0.123 | 6 | 0.020 | 4.449 | **0.000** | 0.114 | 0.983 |
| | site | 0.024 | 4 | 0.006 | 1.290 | 0.275 | 0.024 | 0.400 |
| | time | 0.016 | 1 | 0.016 | 3.409 | 0.066 | 0.016 | 0.451 |
| | season * site | 0.148 | 24 | 0.006 | 1.341 | 0.141 | 0.134 | 0.920 |
| | season * time | 0.030 | 6 | 0.005 | 1.074 | 0.379 | 0.030 | 0.419 |
| | site * time | 0.048 | 4 | 0.012 | 2.582 | **0.038** | 0.047 | 0.721 |
| | season*site*time | 0.093 | 24 | 0.004 | 0.845 | 0.677 | 0.089 | 0.695 |
| | Error | 0.957 | 208 | 0.005 | | | | |

## Discussion

### Diversity, richness and distribution

The 278 standard 30-minute trawls were conducted over a limited geographical distribution within Spencer Gulf, covering commercial prawn trawl grounds only. Coastal areas up to 15 m in depth were not sampled, nor were large areas within the gulf. Thus the diversity, richness and distribution data does not describe the entire gulf (to do so is beyond the scope of this project), however it does reflect what species are likely to be encountered by operational prawn trawlers in different areas of the gulf.

Overall diversity was low. Across the five sites, 22 species of sharks, skates and rays were caught between October 2003 and October 2005, comprising 10 shark, 8 ray, 3 skate and 1 chimera species. Five of these species were considered unique, being represented by only one individual (gulf catshark, ornate wobbegong, sevengill shark, black ray and the coastal stingaree), a further four were considered rare with less than 5 individuals (common sawshark, pygmy thornback skate, white spotted skate and gummy shark). The number of species per site was low, ranging from 8 (Site 3) to 15 (Site 5), with 53% of the species represented at a site considered unique or rare at that site. The number of elasmobranch species was greatest at Sites 2 and 5, and lowest at Site 3. Similarly, diversity indices were highest at Sites 1, 2 and 5, with Site 3 demonstrating the lowest diversity. In a previous study, Svane (2003) measured species richness at the same sites within Spencer Gulf including all faunal groups (pelagic and demersal fish, mobile epifauna and sessile benthos). He found highest species richness at Site 2, followed by Sites 1, 5, and 4. As was found for elasmobranchs in this study, Site 3 demonstrated the lowest species richness.

Diversity, number of species and distribution at the five sites are strongly related to the habitat complexity (as defined by the volume of biomass), which in turn is negatively correlated to effort. Sites 1, 2 and 5 are characterised by sandy sediment, while Sites 3 and 4 are characterised by a coarse sand or gravel bottom, with strong tidal flow. Many of the sharks, skates and rays species encountered in fishing operations in Spencer Gulf are generally small (under one metre in length), bottom dwelling and spend considerable time buried or at least partially buried in the

82

sediment. The dorso-ventrally flattened shape of many species facilitates immersion in the sandy substrate as a predatory strategy. These species also rely on the macro-fauna that reside in the sediment for at least part of their dietary requirements. A predominance of species at the sites with a sand substrate is expected. The sandy sediments of Sites 1, 2 and 5 provide a habitat for burrowing below the sand and demersal species such as angel sharks, stingarees, skates and shovelnose rays were found to occupy these sites preferentially. Other species with greater mobility and a laterally compressed body shape are less likely to require soft sediment in which to bury. These species (Port Jackson sharks, cobbler wobbegongs, elephant sharks and fiddler rays) and the large stingrays tend to have a wider distribution and are found at most sites.

Low diversity and richness at Site 3, where the substrate is hard and tidal currents are strong, is a reflection on the unsuitable habitat type for many of the species recorded and the reduction in food availability (Svane 2003) at this site. It is not surprising to observe most species at Sites 1, 2 and 5 and fewest at Site 3 where the habitat provides little protection from predators and poor availability of food. The strong tidal currents typical at this site are also likely to incur greater energetic costs for sedentary species trying to maintain position in or near the seafloor.

Commercial fishing data in the last 6 years (Figure 1) indicates that 36% of total fishing effort is concentrated around Site 4 and 20% around Site 3. Comparatively, little effort has occurred around Sites 5 (5%), 1 (2%) and 2 (2%). The spatial distribution of fishing effort is related to prawn population dynamics and by-catch. By-catch biomass is highest in the northern Spencer Gulf at Sites 1 and 2, lowest at Site 3 and intermediate in southern Spencer Gulf at Sites 4 and 5 (see Chapter 2, Svane 2003). Therefore the fishing fleet avoids the collection of excessive by-catch by expending little effort north and more effort south. There was no correlation between the number of species present and fishing effort, and it is likely that habitat complexity has the greatest influence on the diversity patterns observed in this study.

**Abundance**

Variation in seasonal abundance is not uncommon. Most species are distributed heterogeneously both spatially and temporally because of the diversity of habitats and

the requirements of individual species lifecycles (Poulard and Léauté 2002). Fishing activity also varies spatially and temporally. Hence, interactions between the fishing fleet and by-catch species will not be consistent. Knowledge of the seasonal and spatial variability in abundance will lead to better predictions of the interactions that may occur.

It was not surprising to observe that different species occurred in varying abundances. This effect reflects the variability in species distribution that in turn is related to habitat type. The abundances recorded in this study only reflect the species caught by the trawl net. This study did not aim to account for every species that can be found in Spencer Gulf, nor their relative abundances. Rather, it proposed to estimate the abundances of species that interact with the prawn trawl fishery. Species will vary in their ability to avoid capture in the net. While we recorded very low abundances of species such as skates, shovelnose rays, angel sharks and large stingrays, it is highly probable that these species can avoid capture by actively swimming away from the net as it approaches or by burrowing deeply. The natural abundances of these species in Spencer Gulf are likely to be much higher than recorded here, but assessing this was beyond the capability of this study. However we can predict from our results that there is a lower probability of the commercial fleet encountering these species than the more common species.

In Spencer Gulf, Port Jackson sharks were the most common species of elasmobranch encountered, making up 61.6% of all species caught. Port Jackson sharks are highly successful scavengers with a high resilience to fishing mortality (Chapter 5). During the commercial fishing season, there is strong evidence that Port Jackson sharks feed heavily on discarded by-catch (Chapter 5) and tagging studies (Chapter 3, Section III) demonstrate that individuals are often caught on more than one occasion. They have the capability to be mobile, yet their nature makes them seemingly prone to capture in nets. Stingarees are also caught in large numbers and constitute 17.6% of the elasmobranch by-catch. While stingarees have the ability to bury, being small (generally under 150 g) they remain close to the surface and this may limit their ability to avoid capture.

*Site, season and time of day*

The abundance of most species was affected by both site and season, with complex and inconsistent interactions between them that varied between and within species. Time of day also influenced abundance in three species, with catches significantly higher in the night trawls than in the daytime trawls, perhaps reflecting the nocturnal nature of these species. These interactions, in part due to a high variability and small sample size for some species, make general discussion difficult, but some overall patterns emerged from the data. Overall abundance (all species combined) across the five sites was significantly different with high abundance at Sites 5 and 2, and low abundances at Sites 4, 3 and 1. This was due to clear site preferences (and subsequent higher abundance), shown by angel sharks and western shovelnose rays for Site 2 and stingarees, elephant sharks, skates and smooth stingrays for Site 5. No single species demonstrated a clear preference for Sites 1 or 3, and hence abundances recorded at these sites were lower. Only the fiddler ray showed some preference towards Site 4 (where 40% of individuals were found), otherwise low abundances were recorded at this site for all species encountered. The similarities in abundances at Sites 2 and 5, and 3 and 4, are not surprising given the similar sediment types of the matched sites (sandy or gravel respectively) that are discussed above. For all species but one, there were significant effects of site on species abundance. The exception was the smooth stingray. This species was caught in low numbers and the data need to be treated cautiously, however, it is a large, highly mobile species that consumes a wide variety of prey. It is not surprising to observe its presence over a large spatial scale. For the remaining species, habitat and food availability are likely to limit their distribution and abundance as discussed above.

Seasonal changes in the occurrence of different species are not surprising, given the wide variety of reproductive strategies employed by the sharks, skates and rays in Spencer Gulf, from laying their egg capsules in specific habitats to giving birth to live young. The patterns between species are likely to differ as well, reflecting the timing of various reproductive activities. A significant effect of season was found for six species. The effect was strong for elephant sharks, smooth stingrays and stingarees and less so for cobbler wobbegongs, southern fiddler rays and Melbourne skates. Elephant sharks and smooth stingrays were absent in three and four sampling trips respectively, thus contributing to the seasonal effects. Elephant sharks were only

recorded during winter-spring (July-October) and in some surveys during November. The elephant shark is known to migrate into large estuaries and inshore bays to breed during spring (Last and Stevens 1994). It is likely that, in Spencer Gulf, this migration takes place late spring/summer when they presumably move into shallower water to breed and then lay their eggs over a protracted season (February-May). Migration back into the deeper gulf waters takes place after this period. The absence of smooth stingrays during the cooler months (April and July) may reflect a migration to warmer shallower waters, but further information on movement through tag recaptures and reproductive data on this species is required. Stingarees were found in all sampling trips but were more abundant during spring/summer (October and January) than during autumn/winter (April and July). Similar seasonal effects were observed on the same species in Port Phillip Bay, Victoria (Edwards 1980). These variations suggest a seasonal migration, or changes in stingaree behaviour that alter their catchability or detectability, such as a reduction in activity during the colder months that reduced the potential for capture because they were less likely to be stirred by the trawl net. Tagging studies carried out in California on the related round stingray, *Urobatis halleri*, showed a migration towards warmer waters where breeding took place. This migration resulted in seasonal aggregations that were quickly dispersed once breeding was completed. Males appeared to exhibit greater site fidelity while females remained for only a short period of time at the breeding site (Vaudo and Lowe 2006).

For the remaining three species in which significant seasonal effects were found, one-way ANOVA's revealed no consistent seasonal patterns for individual sites in cobbler wobbegongs or Melbourne skates. Seasonal effects were apparent for fiddler rays in October 2004, due to a ten-fold increase in catch at Site 4, but they were not consistent, with no observed increase in abundance in October 2003 or October 2005.

Natural migration and reproductive strategies such as aggregation at pre-determined breeding sites at different times of the year are likely to influence the seasonal appearance of species at sites in Spencer Gulf. The tagging study conducted as part of the current project did not recover any tagged species other than Port Jackson sharks and one stingaree. Further study is required to understand migration patterns in these species.

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# Section III.  Tagging and Movement in Spencer Gulf

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# Abstract

Tagging of 1522 individual sharks, skates and rays, comprising nine species, was conducted at seven areas in Spencer Gulf between October 2003 and October 2005. Port Jackson sharks made up the majority of sharks tagged, and were the only species (with the exception of one stingaree) that was reported recaptured. A total of 79 Port Jackson sharks were recaptured, seven of which were recaptured on more than one occasion. The time at liberty ranged from 1 to 865 days, and the distance travelled varied between 1 and 78 km. In general, dispersal of sharks within the gulf was low with 55% of individuals recaptured within 10 km of the original tagging site. Few individuals made excursions greater than 20 km. Tagging was conducted at several tag centres within the gulf and analysis of direction was not possible, however trends in movement were apparent. Most individuals demonstrated strong site fidelity, not moving far from the original capture site. Those that made longer treks from Plank Point, Cowell and the Gutter appeared to be heading towards the Wallaroo region. More data are required to fully understand the movements of this and other species and the collection of recapture information is ongoing, with industry maintaining participation in the coming years.

## Introduction

Tagging studies can provide valuable key information on various aspects of elasmobranch biology, including ageing and growth, mortality, stock size, behavioural and distribution patterns and migration patterns (McFarlane *et al.* 1990, Kohler and Turner 2001). Elasmobranchs have been successfully tagged with external identification tags since 1936. Since then there have been more than 60 tagging studies worldwide reported in the literature, some of which have been ongoing for 30 years (see review by Kohler and Turner 2001). The majority of these studies investigate shark movements both spatially and temporally. Spencer Gulf is known to provide habitat to many sharks, skates and rays, some of which interact with commercial and recreational fishing operations. Knowledge of movements of a species through known fishing grounds will allow a better understanding of potential interactions and development of management strategies. This tagging study was designed to determine the movements and recapture rates of the more common species of elasmobranchs that are captured during trawling within the gulf.

## Materials and Methods

Trawls were conducted at the five research sites detailed in Chapter 3, Section II and from other two areas within Spencer Gulf (Cowell, Corny Point) on board commercial prawn trawlers during pre-fishing surveys. All sharks, skates and rays that were captured were sexed, measured and weighed. Each individual was allocated a unique identification code (for example, PJ45 for Port Jackson shark number 45 and SSS34 for the 34th sparsely spotted stingaree captured). Animals were held in large aerated tanks on board the vessel until we processed them, which generally took less than 30 minutes.

### Tagging

There are many considerations in choosing a tag type for a tagging study, such as the size and appearance of the tag, the initial and potential maximum size of the animal, and ease of attachment. Tags need to be bright and large enough to be noticed but small enough that they do not create drag and impair natural movement. There needs to be potential for growth around the tag (Heupel *et al.* 1998), and attachment must be quick and relatively painless. The potential for fouling needs to be considered as well

(Thorstad *et al.* 2001). Two types of tags were used in this study; a fin tag (rototag) and a plastic dart tag (Figure 1), both of which are widely used in elasmobranch tagging around the world (Heupel *et al.* 1998, Kohler and Turner 2001, Stevens *et al.* 2000, Merson and Pratt 2001). The rototag (Dalton) comprised male and female halves that were inserted in the first dorsal fin. The rototag was applied with an applicator through a hole punched near the centre and towards the leading edge of the first dorsal fin by a leather punch (Figure 2). The female half was imprinted with a unique tag number (0000-2000), the male half with tag return details. The dart tag (Hallprint) was composed of a plastic dart head, monofilament line and a vinyl plastic covering that was imprinted with a unique tag number (0000-2000) and tag return details. The dart tags were implanted into the subdermal musculature below the first dorsal fin or in the middle to proximal region of the wing (Figure 3) with the aid of a sharpened stainless steel needle. The dart was inserted at an angle toward the head of the animal so that the tag trailed along the body to minimise drag. Once tagged, the animals were released as near to the capture location as possible. Tag numbers, animal ID number, weight, TL and release position were recorded. Forty-five Port Jackson sharks were double tagged with a rototag and a dart tag to examine tag loss. The site of tag attachment was assessed visually in all tag recaptures.

The minimum size of animals tagged was based on the size of the tag relative to the animal and the method of tag attachment. Port Jackson sharks under 275 mm TL were not tagged because their dorsal fin was too small to carry the rototag. Dart tags were not placed into juvenile stingarees (<115 mm DW), fiddler rays (<120 mm DW), western shovelnose rays (<160 mm DW) and angel sharks (<165 mm DW) because the dart head was too large relative to the thickness of the wings.

Some species (Port Jackson sharks and angel sharks) were injected with a dose of Engemycin antibiotic that contained Oxytetracycline (OTC) at a concentration of 25mg/kg body weight. OTC acts to mark the vertebrae with a fluorescent band and can be used to validate growth from recaptures in these species. More detail is provided in Chapter 3, Section VI.

Figure 1. The tags used in this study. The rototag is shown at the top with the two halves separate, and the dart tag is shown at the bottom.



Figure 2. A tagged Port Jackson shark with a rototag through the first dorsal fin and a dart tag below it.



Figure 3. Two eagle rays tagged with dart tags in the wing.

Initially, the commercial prawn industry was requested to collect and freeze any tagged individuals, recording position, tag number, length and weight if possible. After the first year, the industry was instructed to re-release the shark if it had been at liberty less than one year after recording recapture and re-release details. Outreach was extended to the general public through articles in the local fishing magazines and papers.

**Data analysis**

*Tag recovery*

Tag recoveries were expressed as the percentage of tagged animals recaptured divided by the total number of animals tagged by fishing area, and as a total.

*Distance, displacement rate and direction of movement*

To reduce any effect of the tagging event on the distance travelled and rate of displacement, individuals that had been at liberty less than 7 days were excluded from the analysis.  The distance travelled (in km) from the tag site to the site of recapture was calculated as a straight line using a model developed by Greg Collings (SARDI) comparing two GPS points.  Rate of displacement (in km per day) was calculated as the distance travelled divided by the number of days at liberty.  Spearman's Rank Order Correlation (rho) was used to calculate the strength of the relationship between days at liberty and distance travelled, and days at liberty and rate of displacement of travel (log transformed) for males and females separately.  Correlations were categorised as weak (rho = 0.10 – 0.29), medium (0.3-0.49) or strong (0.5-1.0) as described by Pallant (2001).  Differences in mean distance and rate of displacement between the sexes and maturity stages (immature v mature) were compared with ANOVA's.  Maps of the tag release and recapture locations were produced by A. Doonan (SARDI) using a spatial GIS system.  Directional movement was analysed with Rayleighs test to determine if movement was random (no preferred direction) or non-random.

## Results

A total of 1522 individual sharks, skates and rays were tagged, comprising nine species. Table I details the number of each species caught and tagged, and the tag type used during this project. Port Jackson sharks made up the majority of animals tagged (83%). Only 17 % of the stingarees that were caught were tagged due to the small size of individuals. The sites in Spencer Gulf at which the tagged Port Jackson sharks were released are shown in Figure 4.

Table I. The number of each species captured and tagged in Spencer Gulf. The tag type used for each species is given.

| Species | Number captured | Number tagged | Tag type |
|---|---|---|---|
| Port Jackson shark | 1380 | 1261 | rototag and dart |
| Sparsely spotted stingaree | 394 | 66 | dart |
| Southern fiddler ray | 122 | 51 | dart |
| Australian angel shark | 48 | 45 | dart |
| Melbourne skate | 63 | 39 | dart |
| Smooth stingray | 24 | 24 | dart |
| Eagle ray | 14 | 14 | dart |
| Black ray | 14 | 14 | dart |
| Western shovelnose ray | 25 | 8 | dart |

**Recaptures**

*Port Jackson shark*

A total of 79 recaptures were recorded over 3 years, comprising 19 males and 60 females. The location of these recaptures is shown in Figure 5.  The majority of these were captured by the commercial fishing fleet (74), with a small number caught during our sampling cruises on the SARDI research vessel *Ngerin* (3) and two by recreational fishers. 72 were recaptured once, 6 on two occasions and one three times over a period of one month. Multiple recaptures were all female. Time at liberty ranged from 1-865 days. Seven individuals were recaptured after less than 7 days at liberty, a further nine less than 30 days after being tagged. Eleven had been at liberty for a year or more. The range of lengths of recaptured sharks relative to the range of lengths of sharks tagged is shown in Figure 6.  In general, the larger individuals made up the majority of recaptures. Over 85% of recaptured sharks were over 500 mm TL. While 228 sharks (18 %) tagged were less than 400 mm TL, only 2 (0.9 %) were recaptured, suggesting that the length of individual tagged fish does influence the likelihood of being detected by the fishermen.  Small individuals may be missed in the sorting process.

Figure 4.  Sites in Spencer Gulf where tagged Port Jackson sharks were released.



Figure 5.  Sites in Spencer Gulf where tagged Port Jackson sharks were recaptured.

*Other species*

Only one stingaree was recaptured during the three years, caught by the MRV *Ngerin* after 364 days at liberty and only 2 km from the original tag site. No other tagged sharks, skates or rays were recorded as being recaptured.



Figure 6. The lengths of all tagged and recaptured male (a) and female (b) Port Jackson sharks.

**Site of tag attachment**

The site of tag attachment in recaptured sharks and the one stingaree was generally considered to be in fair condition. In some, where the tag was placed too close to the musculature of the fin, there was some ulceration, however in most this did not occur (Figure 7).



Figure 7. Site of tag attachment for Port Jackson sharks (left) after 105 days and of stingarees (right) after 364 days at liberty.

**Recapture site and rates**

The overall recapture rate for Port Jackson sharks in this study was 6.3%. Recapture rates of the two sexes varied with 7.9% of females and 3.7% of males recaptured to date. Table II shows the number of Port Jackson sharks tagged and recaptured as a percentage of the total and for each area. More than half the total recaptures of Port Jackson sharks were recorded from the Wallaroo area (52.2%), despite less than 20% being tagged in this area. The majority of Port Jackson sharks were tagged in The Gutter and surrounding waters (38.6%) but only 17.9% of all sharks recaptured originated from there. The number of sharks tagged at each site varied markedly (range 35-487), hence recapture rates were reported at the site level (Table II). As a percentage of animals recaptured from each area in the gulf, the highest tag return rate came from the Wallaroo area (15.1 % of animals tagged in this area were recaptured), followed by Middle Bank (7.5%). The remaining areas recorded return rates of less than 3%. The most northern site, Western Shoal (Site 1), did not record any returns. Fishing effort in the seven areas also varied and is shown in Table II. Wallaroo was fished most intensely, followed by Middle Bank and Corny Point. Little effort was concentrated in the northern Spencer Gulf, with less than 700 hours spent at Sites 1

and 2. A Pearson Correlation revealed a strong correlation between effort and % recapture rate at the seven areas (r=0.921, P= 0.003).

Table II. The number of Port Jackson shark tagged and recaptured as a percentage of the overall total and totals for each area. Fishing effort for each of the areas is the annual mean for 2003-2005.

| Site | Area | Number tagged | % of total tagged | Number recaptured | % recapture rate (of site) | % recapture rate (of total) | Fishing effort (h) |
|------|------|---------------|-------------------|-------------------|---------------------------|----------------------------|--------------------|
| 1 | Western Shoal | 50 | 4 | 0 | 0 | 0 | 539.5 |
| 2 | Plank Point | 157 | 12.5 | 2 | 1.3 | 3 | 153.0 |
| 3 | Middle Bank | 200 | 15.9 | 15 | 7.5 | 22.4 | 4386.0 |
| 4 | Wallaroo | 232 | 18.4 | 35 | 15.1 | 52.2 | 8674.5 |
| 5 | The Gutter | 487 | 38.6 | 12 | 2.4 | 17.9 | 1104.5 |
| | Cowell | 99 | 7.9 | 2 | 2.0 | 3 | 261.5 |
| | Corny Point | 35 | 2.7 | 1 | 2.9 | 1.5 | 2477.5 |

**Distance travelled**

Tag recapture data shows Port Jackson sharks moved a net distance of between 1-78 km over 7-865 days. In general, Port Jackson sharks did not appear to move very far from the original tag site (Figure 8). 55.5% of recaptured sharks travelled a net distance of 10 km or less, 25% travelled between 10 and 20 km and less than 20% travelled a net distance of 20 km or more. The longest net distance covered by one shark was 78 km in 233 days. The shark that had been at liberty the longest (865 days) had travelled a net distance of 8.2 km from the original tagging site. The pattern was similar for males and females (Figure 9), with the exception that a greater proportion of recaptured males had travelled less than 10 km and none between 20-50 km from the tag site.

The relationship between days at liberty and overall distance travelled between tagging site and recapture site is shown in Figure 10. There was a weak positive correlation between days at liberty and distance travelled at the 1% level but it was not significant (Spearman's rho = 0.253, p =0.311, df =18; and rho = 0.145, p =0.310, df =51, for males and females respectively).

Figure 8. Distance moved by recaptured Port Jackson sharks.



Figure 9. Movement of female and male Port Jackson sharks.

The mean distance ($\pm$ 95% CI) travelled was 11.38 km $\pm$ 7.94 (18) and 15.70 km $\pm$ 4.09 (51) for male and female Port Jackson sharks, respectively. There was no significant difference between the sexes (ANOVA, $F_{(1,62)}$= 1.029, p=0.31). The state of maturity (immature vs. mature) had no significant effect on the distance moved for male Port Jackson sharks. Immature males (<550 mm TL) travelled a mean distance of 17.66 km $\pm$ 17.11 (8), while mature males travelled 6.35 km $\pm$ 2.72 (10), however due to the high variability in the data, these differences were not statistically different (ANOVA, $F_{(1,16)}$= 2.05, p=0.17). In contrast, there was a significant effect of state of maturity on distance travelled for females (ANOVA, $F_{(1,44)}$= 4.957, p=0.031). Immature females (<700 mm TL) travelled 9.88 km $\pm$ 4.55 (19), while mature females travelled significantly longer distances of 19.16 km $\pm$ 5.66 (32).

100

**Rate of displacement**

There was an inverse exponential relationship between days at liberty and rate of displacement at which the shark travelled (Figure 11). Sharks recaptured after less than 7 days from tagging travelled up to 3.3 km/day (mean 1.86 km per day ± 0.84, n=7). This rate of movement decreased markedly after the first week. The overall mean rate of displacement after 7 days was 0.19 km per day ± 0.07, n= 64. At around 30 days at liberty, the sharks were travelling at rate of displacements up to 1.25 km/day. After 50 days at liberty, this was reduced to less than 0.5 km/day. To reduce bias caused by capture and tagging, recoveries from the first 7 days were excluded from the analyses. There was a strong and significant negative correlation between days at liberty and rate of displacement (Spearman's rho = -0.676, p =0.002, df =18; and rho = -0.646, p <0.001, df =51, for males and females respectively).

Excluding the first week of liberty, the overall mean rate of displacement (± 95% CI) was 0.12 km per day ± 0.09 (18) and 0.21 km per day ± 0.16 (50) for male and female Port Jackson sharks respectively. An ANOVA showed no significant difference between the sexes (ANOVA, $F_{(1,66)}$= 1.39, p=0.24). The mean rate of displacements for immature and mature male and female Port Jackson shark were 0.21 ± 0.18 (8), 0.06 ± 0.04 (10), 0.17 ± 0.14 (18) and 0.23 ± 0.12 (32) respectively. State of maturity had no effect on the mean rate of displacement (km per day) for either sex (ANOVA, $F_{(1,16)}$= 3.06, p=0.09 and $F_{(1,48)}$= 0.64, p=0.43 for males and females respectively).

**Direction of movement**

There was no preferred direction of movement for any gender or maturity group (Rayleigh's z=1.164, 0.003, 0.353 and 0.945, P>0.05 in all cases). This was not surprising since individuals were not tagged at a single source site but from as many as seven different areas. Of those individuals that made significant movements, there was a trend for movement in a south-west direction (Figures 12-15). Overall, females were more mobile than males. Immature females appeared to stay in the same general area with the exception of 3 individuals, two of which made north-easterly treks from The Gutter and the other who moved 42 km south from Plank Point (Figure 12). Mature females were more mobile (Figure 13). Most were tagged in the Wallaroo area and half of these were recaptured south-west of their tag location. Three sharks were tagged outside the Wallaroo area (Plank Point, Cowell and The Gutter) but were

recaptured within Wallaroo. Two of these travelled in a north-easterly direction, the other south to finish at Wallaroo. Male Port Jackson sharks tended to be remain more local. All but one of the immature males were caught within 10 km from the tag site (Figure 14). The exception was PJ361, a 426 mm male that travelled the greatest distance of all our recaptures. Over 233 days, he travelled 78.3 km in a north-easterly direction from The Gutter towards the Middle Bank/Wallaroo area. All of the mature males were found within 12 km of the original tag site, and no pattern in direction could be identified (Figure 15).



Figure 10. Relationship between days at liberty and distance travelled for male and female Port Jackson sharks.



Figure 11. Relationship between days at liberty and the rate of displacement of travel for male and female Port Jackson sharks.

Figure 12. A spatial representation of movements of immature female Port Jackson sharks (<700 mm TL) from tag recaptures in Spencer Gulf.



Figure 13. A spatial representation of movements of mature female Port Jackson sharks (>700 mm TL) from tag recaptures in Spencer Gulf.

Figure 14. A spatial representation of movements of immature male Port Jackson sharks (<550 mm TL) from tag recaptures in Spencer Gulf.



Figure 15. A spatial representation of movements of mature male Port Jackson sharks (>550 mm TL) from tag recaptures in Spencer Gulf.

104

## Discussion

### Recapture rates

More than half of the 66 shark tagging studies reviewed by Kohler and Turner (2001) reported tag returns of less than 5%. Tag recovery rates are influenced by a wide variety of factors such as tag type, reporting rate, fishing effort, outreach activities and a variety of logistical issues related to the size of the study area (Stevens *et al.* 2000, Ortiz *et al.* 2003, Bolle *et al.* 2005). In addition there is evidence that the presence of external tags can lead to an increase in the recapture rate at least in some teleost species (Rikardsen and Thorstad 2006), and subsequently an overestimate of abundance. The tag return rate in this study was comparable to other studies (6.3%). There was good cooperation from the commercial fleet, with tagged sharks reported by 21 of the 39 commercial vessels. In addition, the research vessel *Ngerin* and two recreational fishers reported recaptures. While 18% of the Port Jackson sharks tagged were less than 400 mm TL, only two of these (representing 0.9%) were recaptured. This low recapture rate for small sharks may have been because they did not survive the tagging process, moved quickly out of the area once released, or that fishers overlooked smaller individuals during their retrieval and sorting process. It is unlikely that the presence of tags increased the likelihood of capture because they were relatively small compared to the animal and were placed flush along the dorsal fin.

The recapture rates reflected the fishing intensity in different areas of Spencer Gulf. There was a strong correlation between effort and recapture rate for the seven areas in which tagging occurred. Wallaroo is the most heavily fished, followed by Middle Bank, while little fishing effort was expended in the north (Western Shoal and Plank Point) (Table II). More than half the total recaptures of Port Jackson sharks were recorded from the Wallaroo area (52.2%), despite less than 20% being tagged in this area, reflecting the greater intensity of fishing in this area. Middle Bank had the second highest recapture rate (by total and by site), indicative of the second highest fishing intensity. The majority of Port Jackson sharks were tagged in The Gutter and surrounding waters (38.6%) but only 17.9% of all sharks and 2.4% of sharks tagged from the area were recaptured because The Gutter is exploited less frequently. The remaining areas recorded return rates of less than 3% and the most northern site, Western Shoal, did not record any returns. At these sites (Western Shoal, Plank Point,

Cowell, Corny Point) fewer animals were tagged. In addition, fishing effort was low at three of these sites, therefore it was not expected to have high recapture rates from these areas.

Females tended to be recaptured more often than males, with 60 females and 19 males recaptured to date. The higher recapture rate of females may be related to their higher mobility compared to males that may result in them being more exposed to interactions with the fishery.

**Movement of Port Jackson sharks in Spencer Gulf**

The time an individual was at liberty had no effect on the distance it was recaptured from the original tag site, suggesting strong site fidelity in this species. Animals that had been at liberty for more than one year were found an average of 22 km from the initial tag site (range 2.5 - 65.9 km). Therefore, not surprisingly, there was a strong negative correlation between rate of displacement and days at liberty, with rate of displacement decreasing the longer an individual was at large. If individuals demonstrate low dispersal from the original tagging site and instead remain within a 'home range', the resultant rate of displacement (km/day) will decrease the longer that individual is at liberty. However, the concept of rate of displacement should be treated carefully because the data reports the distance travelled between tag and recapture sites, with no knowledge on what movements occurred in the interim.

No single pattern of directional movement for Port Jackson sharks could be identified because the data reflected variable tagging and fishing effort throughout the gulf. Tagging was conducted at multiple sites within the gulf and the number of individuals varied at each site. Difficulties in standardising the data and low returns meant only generalisations were possible. Port Jackson sharks made slow, short excursions within Spencer Gulf, the greatest distance travelled being 78 km over 233 days. More than half of all animals were recaptured within 10 km of the original tag site, even after more than one year. Of those that were recaptured at distances greater than this, there was a general trend for a southwest movement. The longer treks (more than 20 km) showed a tendency for movement towards the Wallaroo area from other areas such as The Gutter, Cowell and Plank Point. This pattern was observed for both sexes and both maturity stages. Port Jackson sharks are known to aggregate in large

numbers and in all sizes for several months prior to and during breeding in winter (McLaughlin and O'Gower 1970). This behaviour has been observed along the west coast of Yorke Peninsula by local fishermen. It is likely that Wallaroo is a preferred area for breeding, and that this preference drives the patterns observed. While males did not tend to move far compared to females, Wallaroo was still considered the "hotspot" for Port Jackson sharks. All but two of the ten mature male Port Jackson sharks recaptured were originally caught and tagged in the Wallaroo area and moved less than 12 km. One of the remaining two was heading south from Middle Bank towards Wallaroo.

Females tended to be more mobile than males. The effect of state of maturity (immature versus mature) on the net distance moved for female Port Jackson sharks was significant (p=0.031), with larger mature females travelling greater distances. Mature animals are likely to be more mobile in search of breeding grounds, mates and egg laying habitat. The reverse was found for males, where immature males appeared to travel greater distances than mature males, but the differences were not significant due to the high variability in the data and the small number of male recaptures.


**Implications for fishing fleet**

The fleet operates approximately about 20,000 hours per year, and observations from 86 trawls on commercial surveys recorded an average of 6.965 Port Jackson sharks caught per hour (Table III). This equates to an annual catch of 139,000 fish. A recapture rate of 6.3% implies that 8,750 of these are caught on more than one occasion. Assuming no depletion of Port Jackson shark stocks in the gulf through fishing, it is then estimated that approximately 130,000 individual Port Jackson sharks are caught annually. If depletion occurs through fishing mortality, then the consequences for the species are likely to be more severe. Fortunately for this species, relatively high recapture rates and low mortality (see Chapter 5) suggest that populations of Port Jackson sharks in Spencer Gulf are relatively stable. There are no tag return data on the remaining elasmobranch species, significant numbers of which are caught each year by commercial trawlers (Table III). The future of other elasmobranch species remains uncertain until more information is obtained.

Table III. Estimated numbers of sharks, skates and rays caught on commercial prawn trawlers. The number caught per hour is based on observations from 86 commercial trawls in Spencer Gulf. Annual catches are calculated from an average of 20,000 hours of commercial effort per year. * A 6.3% recapture rate for Port Jackson sharks is taken into account.

| Species | Number of sharks, skates and rays caught per hour | Approximate number of sharks, skates and rays caught per year |
|---|---|---|
| Port Jackson shark | 6.965 | 130000 * |
| Sparsely spotted stingaree | 1.256 | 25000 |
| Elephant shark | 0.419 | 8350 |
| Smooth stingray | 0.116 | 2325 |
| Western shovelnose ray | 0.267 | 5350 |
| Angel shark | 0.651 | 13000 |
| Cobbler wobbegong | 0.535 | 10700 |
| Fiddler ray | 0.860 | 17200 |
| Coffin ray | 0.093 | 1850 |
| Melbourne skate | 0.512 | 10200 |
| Southern sawshark | 0.047 | 930 |
| Eagle ray | 0.163 | 3250 |
| Gummy shark | 0.023 | 470 |
| Black ray | 0.186 | 3720 |

**Limitations to tagging studies**

The data on movement of tagged sharks is a function of the distribution of tag and recapture fishing effort (Stevens *et al.* 2000, Ortiz *et al.* 2003, Bolle *et al.* 2005), and thus may not completely describe movements within the gulf. For example, movements closer to shore in shallow non-fishing grounds are not examined and may constitute a significant proportion of the population's activities. The movement of individuals is influenced by a great many variables, including competition for space or food, reproductive requirements for mating and egg laying habitats, and environmental factors such as water temperature (McLaughlin and O'Gower 1971, Vaudo and Lowe 2006). In addition, the place and time of tagging, period at liberty, season of recapture, size and sex may affect movement (Stevens *et al.* 2000). It was

not possible to examine each of these variables and data presented here are general movements of Port Jackson sharks in Spencer Gulf. It is further recognised that data from recaptured conventional tags such as rototags or dart tags is limited to the date and position at release and at capture. Information on where the animal moved in between is unknown. However recapture events can provide evidence that the individual survived the capture, handling and tagging processes, and data on general movements within the gulf.

The effect of the capture and tagging process on the shark was not examined, and the data from recaptures after less than 7 days was excluded to reduce the error in describing movement patterns. For example, the animals may respond by just swimming as far away as they can from the area once released, as suggested by initial rate of displacements (mean 1.86 km per day). Animals recovered after one week travelled at much lower rate of displacement (mean 0.19 km per day) in comparison.

**Site of tag attachment**

Histological examination of the tissue surrounding the tag insertion site after recapture has demonstrated that the healing process from tagging is normal but occurs slowly because of a decreased blood flow to the area (Heupel *et al.* 1998). Even though tissue disruption was recorded in almost all fins, there was no sign of infection in any of the three shark species studied by Heupel *et al.* (1998). Tissue samples removed from tag sites in epaulette sharks, *Hemiscyllium ocellatum*, showed initial acute responses of localised tissue breakdown and haemorrhaging (Heupel and Bennet 1997). Chronic responses included fibrous tissue formation around the tag site, thus anchoring the tag and isolating the tag site area from the surrounding tissue. Tissue repair continued over time and there were no secondary infections at the site. Tagging with dart tags did not appear to affect the long-term health of the animals. However, Manire and Gruber (1991) questioned the effect of tagging on growth because growth rates in juvenile lemon sharks, *Negraprion brevirostis* were negatively impacted by tagging with dart tags. The single stingaree recaptured after approximately one year in this study was tagged with a dart tag and had grown only 1mm. The individual was not a juvenile (243 mm TL) but the tags were quite large compared to the animal, hence growth may have been affected.

**Tag shedding**

Too few double-tagged animals have been recovered to make any definitive statement on tag retention. Only two of the 45 individuals that were double tagged have been recaptured, however, each of these retained both tags. It is possible that rototags placed too close to the leading edge of the dorsal fin may be lost if the fin rips (as was the case for two individuals kept in captivity), but it was not possible to account for these losses in the field. To minimize potential tag loss, we placed the tags as near to the middle of the fin as possible. Stevens *et al.* (2000) believed that tag loss was greater when tags were placed too close to the leading edge of the dorsal fin and therefore tended to be ripped out easily through the thinner tip. Alternatively tags could be lost if they became embedded in the dorsal fin musculature and, due to growth and thickening of the fin, were lost as the animal grew.

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# Section IV: Morphometrics

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# Abstract

Morphometric analysis of the sharks, skates and rays in Spencer Gulf was undertaken at five sites (Western Shoal, Plank Point, Middle Bank, Wallaroo and The Gutter) over seven seasons (October 2003, April 2004, October 2004, January 2005, April 2005, July 2005 and October 2005). A total of 2239 sharks, skates and rays were examined from 337 trawls in Spencer Gulf, comprising 22 species. In all, 963 males, 1222 females and 54 of undetermined sex were weighed and measured. Strong allometric relationships were identified between length and weight for all species. For the majority of species, males and females were similar in morphological shape. Only the Port Jackson shark and fiddler ray showed differences between the sexes, with females being heavier for any given length than males. A clear sexual dimorphism in maximum size was found for many species, with females attaining greater size than their male counterparts. In those species for which there were sufficient data, there were no seasonal effects on size frequencies and mean total length. Site differences were apparent only for Port Jackson sharks. A clear decline in mean length from north to south was observed. Sex ratios were largely 1:1, but Port Jackson sharks, fiddler rays and eagle rays showed a female bias.

## Introduction

Length and weight are integral components of any model describing fish populations. These data are essential in the understanding of growth, age structure, survivorship and other aspects of population dynamics (Kohler *et al.* 1995). These parameters can be inherently difficult to measure accurately at sea, particularly weight, and the development of allometric relationships that describe the growth of one parameter (e.g. weight) in relation to another (e.g. length or width) has practical value.

It is well established that populations of elasmobranchs are distributed unevenly both spatially and temporally due to habitat preferences, including environmental and ecological needs, prey availability and migrational patterns (Heithaus 2001, Henderson *et al.* 2001). In addition, many species of elasmobranch are segregated by size and sex for at least part of their life history (McLaughlin and O'Gower 1971, Francis and Duffy 2005, Martins *et al.* 2005). The onset of sexual maturity is often the instigating factor for changes in the distribution and behaviour of sharks (Francis and Duffy 2005). Immature animals are often found in mixed sex groups, but sexual segregation is often observed in mature animals. Accordingly, it may be reasonable to expect seasonal and site differences in the size of individuals within Spencer Gulf, delineating nursery areas or breeding sites. It was the aim of this study to describe the morphometric differences at the five research sites exposed to varying fishing pressures and to identify nursery sites or sites where adults aggregate prior to breeding, based on size frequency data.

## Materials and Methods

### Sampling

Population parameters of elasmobranchs in Spencer Gulf were determined at five sites (Western Shoal, Plank Point, Middle Bank, Wallaroo and The Gutter) over seven seasons (October 2003, April 2004, October 2004, January 2005, April 2005, July 2005 and October 2005). Trawling was carried out from the research vessel *Ngerin*, using a standard otter trawl with a headline length of 14.63 m and a minimum mesh size of 4.5 cm. The trawl consisted of a cone shaped net closed at one end by a cod-end, and lateral wings extending forward from the opening that are held open horizontally by two otter boards. Only one net was deployed. At each site for each of

the seven seasons 4 day and 4 night trawls were carried out, each 30 minutes in duration. In addition, several more trawls were run to determine the effect of trawl time on stress related parameters. These were of 15 and 45 minutes duration. Sampling was also conducted opportunistically from commercial prawn trawl vessels during commercial prawn surveys.

**Morphometrics**

Immediately upon retrieval of the trawl net, every elasmobranch specimen (with the exception of large stingrays) was placed in a circulating holding tank until the entire catch was sorted. Large stingrays were examined immediately and once measurements were complete, they were released. This process often took less than five minutes. All remaining individuals were then identified to species, sex determined and weighed (to the nearest gram) using a Marel Series 2000 electronic marine scale. Length measurements (to the nearest mm) varied depending on the species, total length (TL) was taken for all individuals, fork length (FL) for Port Jackson sharks, disc width (DW) for all rays, skates and some shark species and body length (BL) for Stingarees. Total length was defined by the straight-line distance from the end of the snout to the posterior edge of the upper lobe of the caudal fin as measured with a steel ruler mounted to a wooden board. Fork length was defined as the straight line distance from the tip of the snout to the notch in the caudal fin separating upper and lower lobes, disc width as the greatest distance from the edge of one wing or modified pectoral fin to the other. Body length was defined as the straight line distance between the tip of the snout to the posterior edge of the pelvic fin (Figure 1). Males were identified by the presence of two external claspers attached to the inside edge of the anal fins.



Figure 1. Length measurements taken from sharks and rays

An allometric equation (power function) was calculated by fitting a nonlinear regression model by least squares. The form of the equation is y=$ax^b$, where y was the independent variable, x was the dependent variable, and *a* and *b* constants relating to the intercept and slope of the relationships. A highly significant correlation was found between weight and length or width for all species (Table I). Therefore, with weight as the dependent variable and length or width the covariate, a one-way ANCOVA in SSPS 14 (SPSS Inc., Chicago) was performed on log-transformed data in SPSS to test the effect of sex for the nine most common species for which sufficient data were available. Six species were found at too few sites and in insufficient numbers to assess the effect of site on the length–weight relationship. Therefore two-way ANCOVA's with weight as the dependent variable, total length the co-variate and site and sex the fixed factors was performed for only three species (Port Jackson shark, fiddler rays and cobbler wobbegong).

The mean total length (MTL) was calculated from the size frequency for each species and the differences between sexes were tested with t-tests in SSPS 14. The effects of sex, site and season on TL were investigated using a three-way ANOVA in SSPS. Homogeneity of variances was tested using Levene's test and multiple comparisons made with the Dunnett C test. Several transformations (log10, square root, fourth root and square root log) were performed in an attempt to achieve normality. Failure to achieve homogenous variances and normality because the data were skewed did not prevent the use of ANOVA tests because these statistical analyses are robust against violations of the assumption of equal variance (Underwood 1997). Stingarees were found at only one site (with the exception of three individuals), hence site effects were not examined for this species. Instead, a two-way ANCOVA with TL as the dependent variable, and weight as the covariate was performed to test the effect of season and sex (fixed factors).

Any significant interactions between factors arising from the multiple ANOVA's were further investigated with one-way ANOVA's comparing one factor separately at each level of the other factor (Underwood 1997).

**Sex ratios**

The ratio of total number of males to females was determined for each species. Where sufficient data were available, significant deviation from unity was tested using a chi-squared test with a Yates correction. Data were treated separately based on site and season for each species in Spencer Gulf and the effect of site or season on sex ratios was assessed with a Goodness of Fit test. The null hypothesis was that the sex ratio was equal across the five sites and seven seasons.

## Results

**Morphometrics**

A total of 2239 sharks, skates and rays were examined from 337 trawls in Spencer Gulf over three years, comprising 22 species. In all, 963 males, 1222 females and 54 of undetermined sex were weighed and measured. For those species where sufficient data were available (sample size greater than four), there was a strong relationship between allometric variables. Table I reports the parameters from the power equations that described the relationships for the ten most common species. Data are pooled for site and season and presented for females and males. Values for $b > 3$ reflect a positive allometric growth with weight increasing rapidly with growth. Fiddler rays angel sharks and male shovelnose rays demonstrate negative allometric growth ($b<3$) with length increasing more rapidly than weight with growth.

*Effect of sex*

A significant effect of sex on the length–weight relationship was found for six species (cobbler wobbegongs, elephant sharks, fiddler rays, Melbourne skates, Port Jackson sharks and stingarees)(Tables II and III). For all species except elephant sharks, females were heavier for any given length than males. Male elephant sharks were heavier the females for any given length. For the three remaining species (angel sharks, smooth stingrays and shovelnose rays), males and females were, for the measured parameters, morphometrically similar (Table II).

Table I. Allometric relationship between total length (TL) or disc width (DW) and weight (Wt) for females (F) and males (M) in the ten most common species of sharks, skates and rays in Spencer Gulf. Values for '$a$' and '$b$' are derived from the power equation $y=ax^b$. Data are untransformed. P values in bold are significant.

| Species | Sex | x | y | a | b | $r^2$ | n | P |
|---|---|---|---|---|---|---|---|---|
| Port Jackson shark | F | TL | Wt | 2.00E-06 | 3.2339 | 0.9942 | 655 | **<0.001** |
| Port Jackson shark | M | TL | Wt | 6.00E-06 | 3.0466 | 0.9813 | 452 | **<0.001** |
| Stingaree | F | DW | Wt | 2.00E-05 | 3.1118 | 0.9675 | 147 | **<0.001** |
| Stingaree | M | DW | Wt | 2.00E-05 | 3.1024 | 0.9531 | 134 | **<0.001** |
| Elephant shark | F | TL | Wt | 4.00E-07 | 3.4052 | 0.9706 | 40 | **<0.001** |
| Elephant shark | M | TL | Wt | 3.00E-07 | 3.4696 | 0.8629 | 42 | **<0.001** |
| Fiddler ray | F | TL | Wt | 7.00E-06 | 2.984 | 0.9919 | 82 | **<0.001** |
| Fiddler ray | M | TL | Wt | 1.00E-05 | 2.8336 | 0.9783 | 40 | **<0.001** |
| Cobbler wobbegong | F | TL | Wt | 3.00E-07 | 3.4564 | 0.9637 | 28 | **<0.001** |
| Cobbler wobbegong | M | TL | Wt | 4.00E-07 | 3.4005 | 0.9734 | 24 | **<0.001** |
| Melbourne skate | F | TL | Wt | 5.00E-06 | 3.1112 | 0.9908 | 22 | **<0.001** |
| Melbourne skate | M | TL | Wt | 4.00E-06 | 3.1252 | 0.9951 | 31 | **<0.001** |
| Shovelnose ray | F | TL | Wt | 2.00E-06 | 3.1137 | 0.9914 | 15 | **<0.001** |
| Shovelnose ray | M | TL | Wt | 4.00E-06 | 2.9441 | 0.9953 | 9 | **<0.001** |
| Angel shark | F | TL | Wt | 2.00E-05 | 2.9085 | 0.9775 | 23 | **<0.001** |
| Angel shark | M | TL | Wt | 2.00E-05 | 2.8984 | 0.9879 | 23 | **<0.001** |
| Smooth stingray | F | DW | Wt | 2.00E-05 | 3.0883 | 0.9944 | 9 | **<0.001** |
| Smooth stingray | M | DW | Wt | 6.00E-06 | 3.2521 | 0.9841 | 10 | **<0.001** |
| Eagle ray | F | DW | Wt | 7.00E-08 | 3.8052 | 0.9487 | 10 | **<0.001** |

*Effect of site*

The effect of site on the relationships between length and weight was examined for three species (Table III). Significant site effects were observed for Port Jackson sharks, but not for cobbler wobbegongs and fiddler rays, which were morphometrically similar at all sites where they occurred. There was a significant interaction between site and sex on the length-weight relationship in Port Jackson sharks ($F_{(4, 936)}$=9.592, p<0.001). Females were heavier than males for any given length, and there was a significant decrease in size from north to south (Site 1 through to Site 5), with individuals from Site 1 being lighter for any given length than those at Site 5, which were heavier for any given length.

Table II.  Results from a one-way ANCOVA with weight as the dependent variable, length the covariate, and sex the fixed factor for six common species. P values in bold indicate a significant effect.

| Species | source | SS | df | MS | F | P | partial eta squared | Power |
|---|---|---|---|---|---|---|---|---|
| Angel shark | logTL | 3.564 | 1 | 3.564 | 1519.263 | **<0.001** | 0.974 | 1.000 |
| | sex | 0.002 | 1 | 0.002 | 0.806 | 0.375 | 0.019 | 0.142 |
| | Error | 0.096 | 41 | 0.002 | | | | |
| | Total | 454.157 | 44 | | | | | |
| Elephant shark | logTL | 3.863 | 1 | 3.863 | 1746.698 | **<0.001** | 0.959 | 1.000 |
| | sex | 0.091 | 1 | 0.091 | 41.361 | **<0.001** | 0.355 | 1.000 |
| | Error | 0.166 | 75 | 0.002 | | | | |
| | Total | 734.484 | 78 | | | | | |
| Melbourne skate | logTL | 20.516 | 1 | 20.516 | 5684.962 | **<0.001** | 0.994 | 1.000 |
| | sex | 0.021 | 1 | 0.021 | 5.849 | **0.021** | | 0.653 |
| | Error | 0.130 | 36 | 0.004 | | | | |
| | Total | 340.220 | 39 | | | | | |
| Smooth stingray | logTL | 3.101 | 1 | 3.101 | 764.282 | **<0.001** | 0.979 | 1.000 |
| | sex | 0.004 | 1 | 0.004 | 1.102 | 0.310 | 0.064 | 0.167 |
| | Error | 0.065 | 16 | 0.004 | | | | |
| | Total | 338.223 | 19 | | | | | |
| Stingaree | logTL | 17.823 | 1 | 17.823 | 7727.704 | **<0.001** | 0.966 | 1.000 |
| | sex | 0.013 | 1 | 0.013 | 5.763 | **0.017** | 0.021 | 0.667 |
| | Error | 0.625 | 271 | 0.002 | | | | |
| | Total | 1084.903 | 274 | | | | | |
| Shovelnose ray | logTL | 2.999 | 1 | 2.999 | 1664.799 | **<0.001** | 0.990 | 1.000 |
| | sex | 0.008 | 1 | 0.008 | 4.212 | 0.057 | 0.208 | 0.488 |
| | Error | 0.029 | 16 | 0.002 | | | | |
| | Total | 151.631 | 19 | | | | | |

Table III. Results from a two-way ANCOVA with weight as the dependent variable, length the covariate, and site and sex fixed factors for three species. P values in bold indicate a significant effect.

| Species | source | SS | df | MS | F | P | partial eta squared | Power |
|---|---|---|---|---|---|---|---|---|
| Cobbler wobbegong | logTL | 1.755 | 1 | 1.755 | 1309.744 | **<0.001** | 0.972 | 1.000 |
| | site | 0.002 | 3 | 0.001 | 0.405 | 0.750 | 0.031 | 0.123 |
| | sex | 0.030 | 1 | 0.030 | 22.132 | **<0.001** | 0.368 | 0.996 |
| | site*sex | 0.011 | 3 | 0.004 | 2.765 | 0.055 | 0.179 | 0.622 |
| | Error | 0.051 | 38 | 0.001 | | | | |
| | Total | 457.600 | 47 | | | | | |
| Fiddler ray | logTL | 13.454 | 1 | 13.454 | 5651.683 | **<0.001** | 0.988 | 1.000 |
| | site | 0.011 | 4 | 0.003 | 1.177 | 0.328 | 0.063 | 0.351 |
| | sex | 0.023 | 1 | 0.023 | 9.536 | **0.003** | 0.120 | 0.861 |
| | site*sex | 0.005 | 4 | 0.001 | 0.498 | 0.737 | 0.028 | 0.162 |
| | Error | 0.167 | 70 | 0.002 | | | | |
| | Total | 588.784 | 81 | | | | | |
| Port Jackson shark | logTL | 127.276 | 1 | 127.276 | 77912 | **<0.001** | 0.988 | 1.000 |
| | site | 0.108 | 1 | 0.027 | 16.538 | **<0.001** | 0.067 | 1.000 |
| | sex | 0.282 | 1 | 0.282 | 172.403 | **<0.001** | 0.157 | 1.000 |
| | site*sex | 0.063 | 4 | 0.016 | 9.592 | **<0.001** | 0.040 | 1.000 |
| | Error | 1.511 | 925 | 0.002 | | | | |
| | Total | 8866.011 | 936 | | | | | |

**Size frequency**

The size frequencies of males and females for each species, pooled for site and season are shown in Figure 2. For some species, sexual dimorphism is obvious with females attaining a greater size than males (e.g. Port Jackson sharks, stingarees, elephant shark and fiddler rays). In the remaining species for which there was enough data, males and females appeared to reach similar sizes (e.g. skates, wobbegongs and angel sharks), although the largest individual was always female. The other species were encountered too infrequently to allow comparison between males and females. The mean total length (MTL) for both sexes is presented in Table IV. Significant differences in mean length between the sexes were found for Port Jackson sharks and fiddler rays only. For both species, females had a higher MTL than males.

Table IV. Mean total length and standard error for male and female elasmobranchs. Data are pooled for site and season. Results of the t-test comparing the sexes for each species are also presented. Significance at 5% is shown in bold.

| Species | Sex | MTL (mm) | SE | n | t statistic | P |
|---|---|---|---|---|---|---|
| Port Jackson shark | M | 492.18 | 6.387 | 375 | 7.907 | **<0.001** |
| Port Jackson shark | F | 573.15 | 7.159 | 570 | | |
| Stingaree | M | 219.59 | 2.815 | 193 | 1.106 | 0.269 |
| Stingaree | F | 214.45 | 3.707 | 182 | | |
| Elephant shark | M | 585.67 | 8.365 | 43 | 1.007 | 0.317 |
| Elephant shark | F | 615.45 | 26.935 | 50 | | |
| Fiddler ray | M | 364.63 | 20.577 | 41 | 3.455 | **0.001** |
| Fiddler ray | F | 560.41 | 38.929 | 81 | | |
| Cobbler wobbegong | M | 586.93 | 20.885 | 24 | 0.089 | 0.929 |
| Cobbler wobbegong | F | 589.59 | 21.560 | 28 | | |
| Melbourne skate | M | 476.76 | 43.493 | 34 | 0.408 | 0.685 |
| Melbourne skate | F | 502.15 | 44.162 | 25 | | |
| Angel shark | M | 561.85 | 25.871 | 23 | 0.262 | 0.794 |
| Angel shark | F | 550.75 | 32.130 | 24 | | |

*Effect of site*

The only species that occurred at all sites in high enough numbers to compare size frequency data was the Port Jackson shark. Fiddler rays, while present at all sites, were often less than seven in number. All other species did not occur at multiple sites in high enough numbers to allow comparison. The size frequency of both male and female Port Jackson sharks varied with site (Figures 3 and 4). Site 5 was dominated by small, immature individuals (constituting more than 85% for both sexes), while Site 1 had higher numbers of larger, mature animals (100% and 70% for males and females respectively). Larger, mature males also dominated the catch at Sites 3 and 4 (62-67%). Sites 2 and 3 were found to have higher numbers of small, immature females (68-74%).

Mean total length for male and female Port Jackson sharks at the five sites is shown in Figure 5. At each site, females were larger than males, and mean length decreased from Site 1 through to 5, that is, from north to south.

Figure 2. Size frequencies of male and female sharks, skates and rays in Spencer Gulf. Data are pooled for site and season.

123

Figure 3. Size frequency of male Port Jackson sharks at the five sites. Data are pooled for season. Grey bars = immature males (<550 mm TL), black bars = mature males (> 550 mmTL).

Figure 4. Size frequency of female Port Jackson sharks at the five sites. Data are pooled for season. Grey bars = immature females (<700 mm TL), black bars = mature females (>700 mm TL).



Figure 5. Mean length of male and female Port Jackson sharks at the five sites. Error bars are 95% CI.

A three-way ANOVA found a significant effect of sex ($F_{(1, 945)}$ =10.348, p<0.001), site ($F_{(4, 945)}$ =43.805, p<0.001), and season ($F_{(6, 945)}$ =3.089, p=0.005) on length, with a significant interaction between site and season ($F_{(24, 945)}$ =3.498, p<0.001). 16.7% of the variance was explained by site (partial eta squared = 0.167) while sex and season explained less than 3% of the variance.

One-way ANOVA's show significant differences in TL across all sites for each season in male Port Jackson sharks (October 2003: $F_{(3, 53)}$= 7.720, p<0.001; April 2004: $F_{(4, 61)}$=16.280, p<0.001; October 2004: $F_{(4, 21)}$=4.014, p=0.018; January 2005: $F_{(3, 67)}$=19.283, p<0.001; April 2005: $F_{(4, 39)}$=2.828, p=0.039; July 2005: $F_{(4, 70)}$=8.521, p<0.001; October 2005: $F_{(3,56)}$=18.074, p<0.001). Significant length differences were found in five of the seven seasons for female Port Jackson sharks (April 2004: $F_{(4, 90)}$=15.326, p<0.001; October 2004: $F_{(4, 69)}$=12.941, p<0.001; January 2005: $F_{(4, 55)}$=14.256, p<0.001; July 2005: $F_{(4, 141)}$=13.911, p<0.001; October 2005: $F_{(4, 88)}$=9.761, p<0.001). Total length of females did not vary across the five sites in October 2003 ($F_{(4, 74)}$= 0.896, p=0.471) and April 2005 ($F_{(4, 45)}$= 1.451, p=0.235). Dunnett C post hoc tests reveal several overlapping differences in TL between sites over the seven seasons, however a clear pattern emerged, with individuals at Site 5 being significantly smaller from individuals at all other sites for all seasons. During October 2003, Sites 1, 3 and 5 were significantly different from each other. These results support the data that show that individuals at Site 1 were larger and those at Site 5 smaller compared to those at Sites 2, 3 and 4.

*Effect of season*

Seasonal changes in size frequency data could only be examined for Port Jackson sharks and stingarees. All other species occurred in insufficient numbers to be able to conduct comparisons. Size frequencies of male and female Port Jackson sharks during the different seasons (sites pooled) are shown in Figures 6 and 7. Small individuals (<200 mm TL) were only recorded during October of each year, reflecting the main hatching period for this species. No seasonal patterns were identified.

Mean total length for male and female Port Jackson sharks in each season is presented in Figure 8. Females were larger than the males in each season sampled, with the exception of April 2004 when they were of similar size.

Figure 6. Size frequency of male Port Jackson sharks by season. Data are pooled for site. Grey bars = immature males (<550 mm TL), black bars = mature males (> 550 mm TL).

Figure 7. Size frequency of female Port Jackson sharks by season. Data are pooled for site. Grey bars = immature females (<700 mm TL), black bars = mature females (>700 mm TL).

Figure 8. Mean length of male and female Port Jackson sharks for the seven seasons. Error bars are 95% CI.

The three-way ANOVA performed above on Port Jackson shark length data showed significant seasonal effects on TL and a significant interaction between site and season. One-way ANOVA's showed significant seasonal differences in length at Sites 1, 2 and 5 for males, and at Site 5 for females (Site 1 males: $F_{(4, 16)}= 5.285$, $p= 0.011$; Site 2 males: $F_{(5, 49)}= 5.005$, $p=0.001$; Site 5 males: $F_{(6, 190)}=2.744$, $p=0.014$; Site 5 females: $F_{(6, 213)}= 12.506$, $p<0.001$). Dunnett C post hoc tests show that differences are found between January 2005, July 2005 and October 2005 for male Port Jackson sharks, and females caught in October 2003 and April 2005 were similar in length but statistically longer than females caught in other seasons. The low numbers of males caught at Sites 1 and 2 made conclusions difficult, but larger males were caught at Site 5 during July 2005 compared to January 2005 and October 2005, in concurrence with the larger, mature males aggregating for their annual winter breeding.

Stingarees were predominately found at Site 5, therefore seasonal size frequency data is presented for this site only (Figure 9). Several small individuals (<155 mm TL) were found during October of each year, corresponding to the time of year when this species gives birth (Spring). By January, the stingarees are slightly larger with the smallest being 160 mm TL. Three months later (April), the smallest stingarees have increased in TL by 25-35 mm TL. Large animals (>200 mm TL) were recorded during all seasons.

Figure 9. Size frequency of stingarees at Site 5 for each of the seven seasons. Grey bars = females, black bars = males.

Mean total length for male and female stingarees in each season is presented in Figure 10. There was no sexual dimorphism, with males and females relatively similar in length during most seasons sampled. Females were larger than males in October 2003, but somewhat smaller than males in October 2004. There was no significant effect of sex ($F_{(1, 334)}$ =0.154, p=0.695), but seasonal effects were significant ($F_{(6,334)}$ =7.117, p<0.001) in agreement with observed patterns. The interaction between sex and season was also significant, demonstrating a dependence of the two main effects on length ($F_{(6, 334)}$ =3.038, p=0.007), with season responsible for 11.8% of the variance (partial eta squared 0.118). One-way ANOVA's comparing TL across the seven seasons for both sexes showed a significant effect of season for both sexes (Males: $F_{(6, 179)}$ =11.116, p<0.001; Females: $F_{(6, 153)}$ =2.561, p=0.022). A Dunnett C post hoc test revealed significant differences between October 2003 and most other seasons for males, but there was large overlap between groups making the comparisons difficult. Seasonal patterns were inconsistent. A post hoc test was unable to determine which season(s) were responsible for the significant differences for female stingarees.



Figure 10. Mean total length of male and female stingarees for the seven seasons. Bars are 95% CI.

**Sex ratios**

Overall, sex ratios did not vary significantly from 1:1 except for Port Jackson sharks, fiddler rays and eagle rays where females significantly outnumbered the males (Table V). Site had no effect on sex ratios, with the exception of Port Jackson sharks at Sites 1, 2 and 4, where females were more common ($\chi^2$ =6.1, n=53; $\chi^2$ =22.4; n=161 and $\chi^2$ =28.4, n=188). Seasonal effects on sex ratios were tested for Port Jackson sharks and stingarees only, because all other species did not occur in sufficient numbers to conduct an analysis. Significantly more female Port Jackson sharks were caught in four seasons (April 2004: $\chi^2$ =5.124, n=153; October 2004: $\chi^2$ =24.01, n=92; July 2005: $\chi^2$ =23.01, n=213 and October 2005: $\chi^2$ =6.58, n=146).  For the remaining seasons, sex ratios did not deviate from unity (October 2003: $\chi^2$ =2.78, n=130; January 2005: $\chi^2$ =0.80, n=125 and April 2005: $\chi^2$ =0.41, n=87).

Stingarees were found only at Site 5, with the exception of three individuals, and sex ratios did not deviate from unity for each season ($\chi^2$ =2.76, n=71; $\chi^2$ =2.63, n=46; $\chi^2$ =0.19, n=21; $\chi^2$ =0.00, n=68; $\chi^2$ =0.69, n=23; $\chi^2$ =1.24, n=29 and $\chi^2$ =0.75, n=85).

Table V. Sex ratios for the ten most common species. Data are pooled for site and season. Only those species for which there was enough data are shown. Values in bold represent a significant deviation from a 1:1 distribution.

| Species | M:F | n | Males | Females | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| Port Jackson shark | 0.69:1 | 1107 | 452 | 655 | 36.86 | **<0.001** |
| Sparsely spotted stingaree | 0.91:1 | 281 | 134 | 147 | 0.51 | >0.1 |
| Fiddler ray | 0.48:1 | 122 | 40 | 82 | 13.78 | **<0.001** |
| Melbourne skate | 1.41:1 | 53 | 31 | 22 | 1.21 | >0.1 |
| Elephant shark | 1.05:1 | 85 | 42 | 40 | 0.01 | >0.9 |
| Cobbler wobbegong | 0.86:1 | 52 | 24 | 28 | 0.17 | >0.5 |
| Australian angel shark | 1:1 | 46 | 23 | 23 | 0.02 | >0.5 |
| Smooth stingray | 1.11:1 | 19 | 10 | 9 | 0.00 | >0.98 |
| Western shovelnose ray | 0.6:1 | 24 | 9 | 15 | 1.04 | >0.1 |
| Eagle ray | 0.2:1 | 12 | 2 | 10 | 4.08 | **<0.05** |

## Discussion

### Morphometrics

The power function ($y = a x^b$) is a suitable equation to characterize allometry in elasmobranchs (Filiz and Mater 2002). There were strong allometric relationships between all variables for all species, enabling the estimation of unknown parameters from known ones. The difficulties encountered on board fishing vessels and with live animals in obtaining accurate weight measurements can be overcome by utilising the allometric relationships. The relationships can be described as close to isometric, that is, the weight increased proportionally with the cube of length, resulting in the general shape of the individual being maintained through time. Only in the dorsally flattened fiddler ray, male western shovelnose ray, and angel shark did length increase more rapidly than weight with growth. The remaining species demonstrated positive allometric growth with weight increasing more rapidly then length with growth. Positive allometry is common amongst elasmobranch species (Kohler *et al.* 1995, Filiz and Mater 2002, Martins *et al.* 2005, San Martin *et al.* 2005). Comparative morphological data for each species is limited or absent in the literature. Stingarees in Spencer Gulf appeared to be heavier for any given length than those studied in Victoria by Edwards (1980), although the range of sizes differed markedly from the range in this study. Edwards (1980) had few small individuals and many larger individuals in his study.

Most species demonstrated differences in morphological shape between males and females, and across all sites at which they occurred. Females were generally larger than males for any given length and this is likely to be related to reproductive conditioning for females. Port Jackson sharks were the only species that demonstrated a difference in its morphological relationship between sex and site, with females being heavier for any given length, and significantly heavier at Site 1 and lighter at Site 5.

### Size frequency

For some species (Port Jackson shark, stingarees, elephant shark and fiddler rays) there was a clear sexual dimorphism in maximum size recorded, with females attaining greater size than their male counterparts. For the remaining species, the size

ranges recorded for both species were similar but the largest individual was always female. This phenomenon is commonly observed amongst elasmobranchs (Last and Stevens 1994, Cortés 2000, Jakobsdóttir 2001), where females can be as much as 10% larger than the males. It must be acknowledged that the sampling in this study was likely to underestimate the true size range for individual species, as larger animals may be able to avoid capture by swimming out of the way of oncoming trawl nets.

There was a definite trend for decreasing size of Port Jackson sharks from north (Site 1) to south (Site 5), with few large animals found at the latter site. In contrast, Site 1 (Western Shoal) was comprised of mainly larger, mature animals. Habitat complexity is likely to be the influencing factor for the discrepancy in sizes at Sites 1 and 5. Site 1 is dominated by large populations of the mytelid *Trichoma hirsutu* (bearded mussel) (Svane 2003) that are likely to form an important component of the diet for adult Port Jackson sharks (McLaughlin and O'Gower 1971). This mussel is rare at Site 5. Echinoderms, molluscs, crustaceans and fish appear to be the main prey items for adults, and their dentition is suited to crushing prey. Juveniles, in comparison, were found to prefer soft-bodied invertebrates, as evidenced by stomach contents, and the morphology of their teeth that were more raptorial than that of the adult (McLaughlin and O'Gower 1971). Dietary studies in this project examined only one individual from Site 1, however there was evidence of mussel shell fragments in the stomach content. No shell fragments were found in any of the other individuals examined (n=68) from any other site. The few individuals caught from Site 1 made greater sampling difficult. Clearly, a more detailed dietary study is required to determine if there are any ontogenetic changes in the diet of Port Jackson sharks, as suggested by the changes in the morphology of the dentition.

No distinct seasonal patterns in the size frequency data were identified for the Port Jackson shark, which was not surprising because this species is relatively slow growing and can hatch from its egg capsule any time between August and January. In addition, size at hatching is extremely variable and strongly dependent on the size of the egg capsule (Rodda 2000). Small individuals (<200 mm TL) were only recorded during October of each year, reflecting the main hatching period for this species. During later seasons (January and April), slightly larger individuals were recorded (>250 mm TL), representing sharks several months of age. During July, individuals

were over 320 mm TL, and were likely to be over 6 months old (with one exception that was likely to be an early hatchling). There were no size classes missing during any of the seven seasons for either sex, or any pattern in the percentage of mature animals over time (Section V), suggesting that there was not a strong migration of reproductive animals to or from the sites over the three years. Stingarees also reproduce annually (White and Potter 2005), giving birth in late spring as observed in the appearance of small individuals in October each year. The subsequent growth of the new recruits can be followed in January, April and July. However, the presence of larger individuals year round means that the mean total length is similar for each period.

A commonly used reference point in fisheries management is a decrease in the size of the fish over time, indicating that the large mature animals are being selectively removed from the fishery and the population is not being renewed as quickly as it is being taken. In some shark fisheries where long-term data are available, there is evidence that the median size of fish declines over a period of time (Rago *et al.* 1998, Walker and Hislop 1998, Baum and Myers 2004, Campana *et al.* 2005). A change in the mean total length in Port Jackson sharks and stingarees was not observed over the duration of the project, however longer time series are required to be confident that the populations are not being compromised. In addition, while significant numbers of both these species are caught annually (estimated at 130,000 Port Jackson sharks and 25,000 stingarees in Table III in Chapter 3, Section III), the ability to survive the encounter will reduce the fishery effect. This is almost certainly the case for Port Jackson sharks that have very low mortality rates but probably not for stingarees that are less likely to survive (Chapter 5). Another important consideration that will influence the fishery effects on the populations is the overlap between reproductive seasons and fishing activity that are discussed in Chapter 3, Section V.

**Sex ratios**

For most species similar numbers of males and females were recorded. Port Jackson sharks, fiddler rays and eagle rays were the only species that showed a significant female bias in the population, however the number of eagle rays caught was very low (12) and more data is needed to see if this is a true representation of the sex ratio for this species. While an equal proportion of males and females is the usual, it is not

uncommon among elasmobranchs to see a female bias (Heithaus 2001, Jakobsdóttir 2001), with deviation from unity reflecting a migration of one sex from the area or vice versa (Fitz and Diaber 1967, Vaudo and Lowe 2006). A greater proportion of females can be considered a healthy reproductive strategy because reproductive output from the population will be increased. Males can reproduce with several females, and therefore fewer are necessary to maintain the population.

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# Section V: Reproduction

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# Abstract

Size at maturity was assessed in nine key elasmobranch by-catch species (Port Jackson shark, *Heterodontus portusjacksoni,* sparsely spotted stingaree, *Urolophus paucimaculatus*, Australian angel shark, *Squatina australis*, cobbler wobbegong, *Sutorectus tentaculatus,* southern sawshark, *Pristiophorus nudipinni*, southern fiddler ray, *Trygonorrhina fasciata,* western shovelnose ray, *Aptychotyrema vincentiana*, Melbourne skate, *Dipturus whitleyi* and the elephant shark, *Callorhinchus milii*) from Spencer Gulf, South Australia. Maturity, based on external examination of clasper length and calcification, was determined for 788 males from the nine species, and from internal examination of 134 females from the two most common species, the Port Jackson shark and the sparsely spotted stingaree. The results showed that for the majority of species, both male and female elasmobranchs in Spencer Gulf reach sexual maturity at a smaller size than reported elsewhere. The exception was the female stingaree, where maturity appeared to occur at a larger size than previously reported, because despite large females being caught, none were mature. The commercial prawn fleet that operates within Spencer Gulf has significant interactions with many species of elasmobranch. The number of immature animals caught by trawls ranged from low (male elephant sharks, female fiddler rays) to high (angel sharks, stingarees, female elephant sharks and male fiddler rays), and the latter may have severe consequences for the populations of elasmobranchs in the gulf over time. The onset of earlier maturity may be a response to fishing pressure in conjunction with other environmental and ecological factors. The effect of site on the occurrence of mature animals was tested for the Port Jackson shark only, because this was the only species to occur in adequate numbers across all sites. Mature Port Jackson sharks were found to predominate in the most northerly sites of the gulf, and were significantly less abundant in the south. This pattern is likely to be related to habitat complexity within the gulf. Seasonal effects on the occurrence of mature animals were found for stingarees, but not for Port Jackson sharks, due, in part, to the influx of newborn stingarees into the population in January. The reproductive season of the Port Jackson shark is more protracted and patterns are less apparent.

# Introduction

Among the elasmobranchs there are two basic reproductive patterns: oviparity and viviparity (Price and Daiber 1967, Bone and Marshall 1982, Hamlett 1989, Wourms and Demski 1993). Both reproductive patterns involve a large maternal investment for the production and survival of relatively few young compared to the teleost reproductive pattern of producing thousands of eggs, of which only a few will survive. Oviparity has been demonstrated in three families of sharks (Heterodontidae, Orectolobidae, Scyliorhinidae), the chimeras, and skates (Wourms 1977, Bone and Marshall 1982). The remaining families employ a form of viviparity. In oviparous species, the fertilised egg is contained within a protective outer capsule and expelled from the female's body. Viviparity involves the development of the embryo in utero, where it is protected and provided with nutrients throughout gestation (Wourms 1977). Viviparity offers a more protective environment for the embryo than oviparous development, and several nutritional pathways are available (Fujita 1981, Gilmore 1993, Hamlett and Wourms 1984, Natanson and Cailliet 1986, Hamlett 1989, Capape et al. 1990, Tanaka et al. 1990). Viviparity enhances the survival of the young because predators are absent in the uterine environment (with the exception of those species that practice intra-uterine cannibalism), however both mother and young are exposed to interactions with fisheries for the entire gestation period, which can be up to 12 months long. Oviparous species have an advantage in fished areas because once they have laid their egg capsules, the developing embryo is unlikely to be impacted by commercial fisheries until it has hatched and is free-swimming.

Knowledge of reproductive patterns and seasonality is fundamental to understanding the population dynamics of shark species, and the consequence of any fisheries interactions. The selective removal of the larger, mature animals, can lead to growth overfishing, with the population unable to recover quickly enough over time. Localised depletions have been recorded in several species world wide (Walker and Heesen 1996, Baum et al. 2003, Baum and Myers 2004, Campana et al. 2005). Hence, knowing the size at which maturity occurs is important in understanding the impact a fishery may have on a species. Dimorphism in maximum length and size at maturity within the same species in different geographic regions is not uncommon. Environmental conditions, availability of food, and genetic variability may result in

individuals not reaching similar sizes at all locations within their range. The spiny dogfish, *Squalus acanthias*, has a worldwide distribution, with the exception of the polar and tropical regions (Compagno 1984) and biological parameters can vary across its range (see Table in Saunders and McFarlane 1993). For example, it reaches a smaller maximum length, reaches sexual maturity at a smaller size, and has a lower fecundity, in the eastern Mediterranean Sea than in other parts of the world (Chatzispyrou and Megalofonou 2005). Mediterranean populations of the thorny stingray, *Dasyatis centroura*, also demonstrate smaller maximum size, size at maturity and size at birth than populations of the same species from the southeastern United States (Capapé 1993).

There is little information on the reproduction of the key species of elasmobranch found in Spencer Gulf.  The aim of this study was to identify the reproductive seasons for these species, estimate their size at maturity, and determine the impact of interactions with commercial trawl fisheries in Spencer Gulf.

## Materials and Methods

### Sampling

The length at which sexual maturity occurred was estimated for as many species of elasmobranch as possible.  Males of each species were examined externally and internally to estimate size at sexual maturity. Sexual maturity in females could not be determined easily by external examination unless the female had obvious signs of internal capsules or young.  Internal examination was necessary to determine the size at which sexual maturity was reached, however only two species (Port Jackson shark, *H. portusjacksoni*, and sparsely spotted stingaree, *U. paucimaculata*) occurred in sufficient numbers to conduct full biological examinations.  A total of 111 Port Jackson sharks and 121 stingarees were examined internally. The Port Jackson sharks were collected from tag returns and selective sampling to cover the entire size range for each sex. The stingarees were obtained from stress experiments (see Chapter 5) and from selective sampling to obtain enough animals of the appropriate size and sex. All animals were immediately killed with a sharp trauma to the head and frozen for later examination. Each animal was thawed and dissected. For each individual we recorded: total mass, a variety of morphometric measurements (see Section IV), liver

mass, stomach mass and content, and reproductive measurements specific to the sex as detailed below.  Weights were recorded to the nearest milligram and lengths to the nearest mm.

**Stages of maturity**

Three stages of maturity were defined for each sex in this study.

*Males*

Stage 1: immature, with small, flexible claspers, thin and thread-like epididymis and small, undeveloped testes.

Stage 2: maturing, with claspers that were more extended and partially calcified, signs of coiling of the epididymis and larger testes within the surrounding epigonal tissue.

Stage 3: mature, with fully extended and calcified claspers, tightly coiled epididymis and large testes. In reproductively active mature males, there was seminal fluid in the epididymis and clasper tubules.

*Females*

Stage 1: immature, with small ovaries containing ova less than 1 mm diameter, narrow, undeveloped oviducts, oviducal glands and uterii.

Stage 2: maturing, with slightly enlarged ovaries containing enlarged ova, uterii and oviducal glands starting to develop.

Stage 3: mature with large ovaries containing large ova, greatly expanded uterii and oviducal glands. Reproductively active mature females had either egg capsules or developing embryos in the uterii.

**Size at sexual maturity**

Size at maturity was estimated from the relationships between the size or weight of various reproductive organs and the total length of the individual. Upon reaching sexual maturity, these organs greatly increase in dimension.  Males were classified as mature based on clasper morphology, testes weight, degree of coiling of the epididymis and presence of sperm. Females were classified as mature based on ova diameter, ovary weight, uterus width and presence of capsules and/or embryos.

*Males*

Clasper morphology (length and degree of calcification) was examined in all male sharks, skates and rays that were captured. In total, 788 males were examined from nine species. Externally, sexual maturity was based on two variables: clasper length (measured to the nearest mm using Vernier callipers) (see Figure 1) and degree of calcification of the clasper (non-calcified, semi-calcified, fully calcified). For each species where enough data were collected, the size at sexual maturity in males was determined (Table I).

Additional information on sexual maturity was obtained from 42 male Port Jackson sharks and 66 male stingarees through internal examination. Testes length and weight (left and right), epididymis width (left and right) and degree of coiling and presence of seminal fluid were recorded and plotted against total length. The epigonal gland and testes are very closely associated, and in very young specimens and in stingarees, the two organs cannot be separated and were weighed together.



Figure 1. Clasper measurements in a male Port Jackson shark. For each male shark, skate and ray, the distance from the distal portion of the anal fin to the tip of the clasper (a) was measured. For Port Jackson sharks and stingarees clasper length was also measured from the tip of the clasper to the cloacal opening (b). The degree of calcification of the clasper was also recorded.

*Females*

For a few Port Jackson sharks, it was clear that the female was sexually mature by the presence of an egg capsule or wide cloacal opening suggesting the recent release of a capsule. For the remaining individuals and other species, it was not possible to determine if the female was mature by external examination. The reproductive status of 55 Port Jackson sharks and 69 stingarees was established through internal examination by measuring ovary weight (left and right), the number of ova >1mm, diameter of the largest ova, uterii width and the weight of uterus, epigonal gland and oviducal duct (left and right).

**Statistical Analysis**

A three-way ANOVA with the proportion of mature Port Jackson sharks as the dependent variable was performed to assess the effect of sex and site (fixed factors) and season (random factor). The data were arcsine transformed and tested for normality and homogeneity of variances using Kolmogorov-Smirnov and Levene's tests respectively in SPSS v.14 (SPSS Inc., Chicago). Post hoc tests were conducted to examine where statistical differences lay in the data. Maturity could only be established for male stingarees, which were only found at one site; therefore the effects of sex and site were not assessed. The data were too few to perform an ANOVA, therefore the effect of season on maturity of male stingarees was assessed using a Goodness of Fit test with the null hypothesis that the occurrence of mature male stingarees was independent of season. Observed values were taken from the raw data for each season and expected values calculated as the total number of mature males divided by the number of seasons.

## Results

**Size at maturity**

*Males*

Based on clasper morphology, the size at maturity from 788 males comprising nine species is summarised in Table I. Included in the table are data from current studies and literature reports to allow comparison between Spencer Gulf populations and populations elsewhere in Australia. For the majority of species, maturity in males

appeared to be reached earlier in Spencer Gulf, although the number of males examined was relatively low and the data should be treated with caution.

Table I. The number of males examined externally and the range of sizes at which sexual maturity occurs in male sharks, skates and rays based on clasper calcification. Sources: [1] McLaughlin and O'Gower 1971, [2] A. Jones (pers. comm. Murdoch University), [3] Last and Stevens 1994, [4] F. Trinnie (pers. comm. Melbourne University), [5] J. Bell (pers. comm. Melbourne University). [6] M. Readon (pers. comm. Melbourne University). na = not available.

| Species (common name) | Number examined | Estimated size at sexual maturity (this study)(mm TL) | Estimated size at sexual maturity (other studies)(mm TL) |
|---|---|---|---|
| Port Jackson shark | 484 | 500-575 | 750[1],550[2] |
| Sparsely spotted stingaree | 158 | 200-250 | <250[3], 280[4] |
| Elephant shark | 34 | 520 | 650[3], 550[5] |
| Southern fiddler ray | 34 | 580-730 | 750-800[6] |
| Melbourne skates | 27 | na | na |
| Australian angel shark | 21 | 700 | 750[2] |
| Cobbler wobbegong | 17 | 550-610 | 650[3] |
| Southern saw shark | 8 | 625-800 | 900[3] |
| Western shovelnose ray | 4 | 400-750 | 650[3], 600[2] |

Port Jackson sharks

The claspers gradually increased in length with TL until about 500 mm TL when there was a rapid increase in length and subsequent calcification over a short increase in TL. After 575 mm TL, the clasper growth rate declined and the calcification process was complete (Figure 2a). At approximately 500-575 mm TL, there was a rapid increase in the weight of testes and the size of the epididymis relative to TL (Figures 2 b-d). Based on clasper morphology and reproductive morphology, male Port Jackson sharks matured between 500-575 mm TL, similar to what is being found in Western Australia (Jones pers. comm.), and considerably smaller than the 825 mm TL in New South Wales populations reported by McLaughlin and O'Gower (1971).

Figure 2. Development of claspers (a), testes weight (b), testes length (c) and epididymis (d) in male Port Jackson sharks. Key to 2 a: open circles = non-calcified claspers, asterisks = semi-calcified claspers, black circles = fully calcified claspers.

<u>Stingarees</u>

Reported values for size at maturity in male stingarees ranged from about 240-280 mm TL (Trinnie pers. comm., White *et al.* 2001, Last and Stevens 1994). Our data indicated that male stingarees in Spencer Gulf matured earlier, somewhere between 200-250 mm TL. The relationship between clasper length and TL appeared to increase rapidly after approximately 160 mm TL. However, the calcification of claspers only begins around 200-210 mm TL, with full calcification occurring between 210-250 mm TL (Figure 3a). Maximum testes weight and maximum epididymis width also demonstrated a rapid increase between 210-250 mm TL (Figures 3b & c).

*Females*

Due to the lack of specimens, determination of sexual maturity in females was possible for only two species, the Port Jackson shark and stingaree (Table II). Female Port Jackson sharks matured at a similar length as populations in Western Australia, and considerably earlier than the south-eastern populations. The relationships between the weight of the functional ovary (right), oviducal gland and uterii width and ova diameter with TL all began to increase exponentially between 650-750 mm TL in female Port Jackson sharks (Figures 4 a-d). The left ovary is not functional and did not increase appreciably in mass as the female grew (Figure 4a).  Based on reproductive morphology, sexual maturity was reached between 650-750 mm TL.

Our data suggested that stingarees were born smaller in Spencer Gulf than elsewhere, with at least 26 of the 364 stingarees caught (7%) being less than the 155 mm TL birth length reported by Trinnie (pers. comm.) and White and Potter (2005). Female stingarees appeared to mature later in Spencer Gulf than other areas, although it is difficult to say definitively due to the lack of mature specimens. Despite 10 female stingarees captured over the reported size at maturity, no sign of maturity was observed, with no mature ova (14 mm diameter) or evidence of embryos in this, a species that has a long gestation (10 months). The largest ovum recorded was 10.8 mm in diameter from a 237 mm TL female stingaree.

Figure 3. Development and degree of calcification of the claspers (a), testes (b) and epididymis (c) in male stingarees. Key to 3 a: open circles = non-calcified claspers, asterisks = semi-calcified claspers, black circles = fully calcified.

Figure 4. Development of the ovary (a), ovum (b), uterus (c) and shell gland (d) in female Port Jackson sharks.

Table II. Estimated sizes at which sexual maturity occurs in female sharks, skates and rays. Source: [1] McLaughlin and O'Gower 1971, [2] A. Jones (pers. comm. WA Fisheries), [3] Tovar-Avila (pers. comm. Melbourne University), [4] F. Trinnie (pers. comm. Melbourne University), [5] J. Bell (pers. comm. Melbourne University). [6] M. Readon (pers. comm. Melbourne University) and [7] White *et al.* 2001.

| Species (common name) | Est.size at sexual maturity (this study) (mm TL) | Est. size at sexual maturity (other studies) (mm TL) |
|---|---|---|
| Port Jackson shark | 650-750 | 900[1],700[2],700-1100[3] |
| Sparsely spotted stingaree | >310? | 270[4], 310[7] |
| Elephant shark | | 700[5] |
| Southern fiddler ray | | 750-800[6] |
| Australian angel shark | | 800-1000[3], 850[2] |
| Western shovelnose ray | | 700[2] |
| Eagle ray | | 750[2] |

**Proportion of immature animals caught by trawls**

Data on size at maturity collected in this study and from other studies on the same species in other states of Australia (Victoria, NSW and WA) was used to estimate the percent of immature animals captured by the trawl. This extrapolation was possible only for those species where approximate size at sexual maturity was known (Tables I & II). For those species occurring in Spencer Gulf where we have this information (Table III), we generally observed a high proportion of the females caught were immature, with the exception of southern fiddler rays where only 30% of the captured females were immature. For males, immature individuals were dominant in three species (Port Jackson sharks, angel sharks and fiddler rays), and constituted approximately half the catch of another two species (stingarees and cobbler wobbegongs). Of the 34 male elephant sharks captured, none were immature.

Table III. Percentages of immature animals for each species caught by trawls. na = not available.

| Species | % immature males | % immature females |
|---|---|---|
| Port Jackson shark | 63 | 70 |
| Stingaree | 56 | 100 |
| Angel shark | 87 | 100 |
| Elephant shark | 0 | 75 |
| Southern fiddler ray | 93 | 30 |
| Cobbler wobbegong | 46 | na |

**Effect of site**

The mean proportion of mature Port Jackson sharks for each site, replicated by the seven seasons is shown Figure 5. Individual trawl data were too few to allow further subdivision of the dataset. Site 5 had only a small number of mature individuals (constituting between 10-19%), while Site 1 was dominated by larger mature animals (approximately 70%). Mature males also dominated the catch at Sites 3 and 4 (62-67%). Sites 2 and 3 were found to have lower numbers of mature females (25-36%). Similar analyses were not possible with the other species because of insufficient data across the sites or a lack of maturity data with which to distinguish between juveniles and adults. A three-way ANOVA found a significant effect of site ($F_{(4, 24)}$ =6.992, p=0.001), but not for sex ($F_{(1, 24)}$ =5.331, p=0.060), season ($F_{(6, 24)}$ =1.365, p=0.360) or any significant interactions between any factors. A Dunnett C post hoc test identified Sites 1 and 5 to be dissimilar to each other and all other sites, and Sites 2, 3 and 4 were comparable. The trend was a decrease in mature Port Jackson sharks of both sexes from north (Site 1) to south (Site 5), which is mirrored in the observed decrease in size (Section IV).

**Effect of season**

The mean proportion of mature Port Jackson sharks for each season, replicated by the five sites is shown Figure 6. The highest number of mature male Port Jackson sharks was recorded in April 2004 and April 2005. The three-way ANOVA performed above found no significant effect of season ($F_{(6, 24)}$ =1.365, p=0.360), although the power to detect significant differences was low (0.238).

Figure 5.  Mean proportion of mature male and female Port Jackson sharks at the five sites. Error bars are 95% CI.



Figure 6.  Seasonal variation in the mean proportion of mature male and female Port Jackson sharks. Error bars are 95% CI.

Based on male maturity occurring around 200-250 mm TL for stingarees, and with the exception of October 2003, there was a slight trend for fewer mature males during winter (47%) and an increase in mature males during summer (78%) (Figure 7). Stingarees were only found at one site and therefore the data represent the proportion of mature males for Site 5. Results from the Chi squared Goodness of Fit test showed a significant effect of season ($\chi^2$ =83.085, df=6, P<0.001) on the proportion of mature male stingarees was dependent on season. Similar data for female stingarees was not obtained because no mature females were found during the study.



Figure 7. Proportion of mature male stingarees during the seven seasons. Error bars are absent due to a lack of replicates.

## Discussion

### Size at maturity

There are few studies in the literature describing size at maturity for many of the species found in this study. Based on clasper morphology and development of the reproductive organs, it was generally found that the species in Spencer Gulf mature at a smaller size than in other areas of Australia, although the numbers of individuals recorded were low and more data are required to be confident in this conclusion.

Late maturity is a general characteristic of elasmobranch life histories. Typically, individuals are not able to reproduce until they reach approximately 60-90% of their maximum size (Snelson *et al.* 1988, Sunye and Vooren 1997, Ebert 2005, San Martin *et al.* 2005, Ruocco *et al.* 2006). Previous records of Port Jackson sharks from New South Wales fit within this range (67 and 73% for males and females, respectively, McLaughlin and O'Gower 1971). However, data from this study shows that populations of *H. portusjacksoni* in Spencer Gulf mature much earlier. Maturity is reached between 43-64% of maximum TL (based on a maximum TL of 1167 mm TL Section VI) or between 500-575 and 650-750 mm TL in males and females, respectively. Tovar-Avila (2004) reported a similar occurrence of maturity (55% of TL) in Australian angel sharks, *Squantina australis,* much earlier than the closely related species *S. guggenheim* (82%) and *S. occulta* (84%) (Sunye and Vooren 1997). These comparisons require accurate data on the maximum size for any given species and often the data are conflicting. For example, the maximum size recorded for Port Jackson sharks ranges from 1160 – 1650 mm TL (McLaughlin and O'Gower 1971, Last and Stevens 1994, Izzo 2005), with the largest animal collected in Spencer Gulf during this three-year study being 970 mm TL, well below these values.

Table IV summarises the known reproductive characteristics of stingarees. Australian populations appear to have similar maximum sizes, but Spencer Gulf populations mature at smaller disc widths (142-146 mm DW) than their Victorian (186-192 mm DW) and Western Australian (207-223 mm DW) counterparts. The smallest free-swimming individual recorded in this study (90 mm DW) is within the recorded ranges for birth size for this species (65-126 mm DW).

Early maturity may be a population response to fishing pressure (Law 2000) but this applies to a situation when there is a constant harvesting of larger individuals (Stockwell *et al.* 2003, Williams and Shertzer 2005). In the commercial prawn fishery, elasmobranchs are not retained, however this does not necessarily mean that they are not removed from the population due to post-trawl mortality. In addition, the gear is not size selective and it is possible for all sizes of elasmobranch to be caught. Certainly, populations of Port Jackson shark in Spencer Gulf are exposed to many commercial (prawn, crab and snapper) and recreational fishing pressures and large numbers are caught each year. However, because trawl fishing mortality for this

species is likely to be low, earlier maturity and smaller size recorded in this study are more likely to be the results of different environmental factors such as food availability, salinity and water temperature in Spencer Gulf. Similarly, it is estimated that large numbers of stingarees (in excess of 25,000) are caught by the commercial prawn fleet each year (Table III in Chapter 3, Section III), but for this species, fishing mortality is likely to be high (Chapter 5) and fishing pressure may influence reproductive patterns.

**Proportion of immature animals caught by trawls**

Data on size at maturity collected in this study and from other studies on the same species in other states of Australia (Victoria, NSW and WA) were used to estimate the percent of immature animals captured by the trawl. This extrapolation was possible only for those species where approximate size at sexual maturity was known (Tables I & II). Data on the percentage of mature animals caught is important in estimating the effects of trawling on the population, assuming a low survival rate post capture. Data on size at maturity for female sharks, skates and rays is generally poorly known. For those species occurring in Spencer Gulf where we have this information, we observed that a high proportion of females caught from the species listed in Table III were immature, with the exception of southern fiddler rays where only 30% of the captured females were immature. Sexual maturity in males was easier to determine and more information exists in this regard. For those species occurring in Spencer Gulf where we have this information, immature males predominated in three species (Port Jackson sharks, angel sharks and fiddler rays), and constituted approximately half the catch of a further two species (stingarees and cobbler wobbegongs). Of the 34 male elephant sharks captured, none were immature. In south-western Australian trawl fisheries, a substantial number of captured *U. paucimaculata* were mature (White and Potter 2005), as was the case for the remaining three species of Urolophidae caught. However, more than half of the male and all female stingarees caught in Spencer Gulf were immature. Assuming poor survival associated with interaction with fishing gear, these individuals will never contribute reproductively to the population.

The ability of a species to cope with and survive the trawl process can have severe consequences for the population of the species. With the exception of stingarees, the listed species appeared to be quite resilient and may have high survival rates.

Experiments on post trawl recovery were not possible on all species in this study due to the patchiness of animals caught during each sampling trip, but should be carried out to gain a full understanding of the effects of trawling.

**Effect of site and season**

The occurrence of mature Port Jackson sharks was uneven between the five sites. There was a significant trend for decreasing size and maturity of Port Jackson sharks from north (Site 1) to south (Site 5). Site 1 (Western Shoal) was comprised of mainly mature animals, while there where few mature animals found at The Gutter (Site 5). In addition, while males were fewer in number compared to females, a significantly greater proportion of them were mature.

There was a seasonal dependence on the number of mature male stingarees, reflecting the reproductive cycle of this species. In the Victorian and Western Australian populations of *U. paucimaculatus* and the closely related *U. lobatus*, young are born during October through to December (Table IV, Trinnie pers. comm.) and are immediately recruited to the fisheries as by-catch. In the absence of comparable data, we can assume that the reproductive season is similar in South Australia. The appearance of small individuals around 130 mm TL in October each year (Section IV, Figure 9) supports a spring birthing period in Spencer Gulf.

A similar seasonal effect on the relative number of mature animals was not observed for Port Jackson sharks. The species has a more protracted reproductive season (Rodda 2000). In addition, this species is relatively slow growing (Chapter 3, Section VI), so that the transition from immature to mature is slow. The young hatch from their capsules anytime between July and November, more commonly September to October. Newly hatched sharks are found in similar habitats to juveniles, as observed by the size range caught in our trawls, and we might therefore expect to record a decrease in the percent of mature animals in October and particularly during January when the hatching season has finished. Lower numbers of mature animals for each sex were observed in January 2005 and October 2005, but the pattern was not consistent between years and would require a substantial number of young to enter the fishery to observe much change.

**Overlap of fishing season with reproductive seasons**

The Spencer Gulf prawn fishery is a limited entry fishery with 39 licensed operators. Trawling occurs at night in waters greater than 10m in depth. Effort restrictions include spatial fishing closures and gear restrictions such as vessel size and power, the number and type of nets, maximum headline length and minimum mesh sizes. The fleet operates for approximately 65 days a year in six fishing periods lasting from the last to first quarter moon during November, December, March through to June (Dixon *et al.* 2005). Based on the total annual effort (approximately 20000 hours for 2004/2005) and the area covered in a single trawl with two nets in one hour (0.128 $km^2$), we can estimate that the fleet can cover a total of 2500 $km^2$ of the gulf with their trawls in any one season. In reality, trawling often occurs over the same ground so the actual area affected will be less. Notwithstanding, interactions with the shark, skate and ray populations are inevitable, but the consequences of the interaction can vary and be mitigated by natural patterns in reproductive cycles and fishing strategies. Table V is a summary of the reproductive patterns for some species found in Spencer Gulf. The potential overlap of fishing activities with reproductive seasons can be inferred from this information. For example, by the time the fishing fleet begins the new season (November), the majority of Port Jackson sharks have laid their eggs and some of the stingarees have given birth. Therefore, interactions of the fleet's trawling activities with capsule carrying Port Jackson sharks would be minimal, and interactions with heavily pregnant stingarees reduced. Discussions with commercial fishermen revealed that the area where stingarees are common (The Gutter) is generally not fished until later in the season (April-May) so the likelihood of interaction with heavily pregnant or newborn fish is further reduced. There is potential overlap in the reproductive seasons of elephant sharks and southern fiddler rays with trawling activity, causing potentially serious consequences for the population dynamics of these species. However, elephant sharks were only found in during winter-spring (July-October), and in some surveys during November. Sampling trips and surveys conducted during summer and autumn did not catch any individuals. The elephant shark is known to migrate into large estuaries and inshore bays to breed during spring (Last and Stevens 1994). In Spencer Gulf, this migration takes place in late spring/summer when they presumably move into shallower water to breed and then lay their eggs over a protracted season (February-May) (Bell pers.

comm.). All male elephant sharks caught were mature. It is possible that the young and juveniles remain in a nursery area for some time and hence avoid capture.

Tagging studies (Section III) suggest that the Port Jackson shark is relatively immobile in some areas of the gulf. If this can be extrapolated to the Western Shoal area, where a higher proportion of mature Port Jackson sharks can be found, then it is likely that interactions with the commercial trawl fishery will be minimal because little fishing effort takes place in that area.

**Indirect effects of trawling**

There are indirect effects of fishing that may also impact on the life history of species. Some types of bottom trawling may result in disturbances of the benthic habitat (Tanner 2003), or in the production of a considerable volume of discarded by-catch (Svane 2003, Castro *et al.* 2005), both leading to a greater amount of food available to scavengers. These alterations in physical habitat might ultimately lead to changes in the environmental parameters dictating life histories (Law 2000). Just as fishing can affect one species directly, in terms of reduced biomass, cascading effects through the ecosystem may occur. Removal of one species may ultimately have far reaching consequences for the ecosystem. For example, intensive trawling in Port Philip Bay over a 20-year period has led to the decline of several important commercial and recreational species. The resultant decline in competition may account for the increase in the population of other species, such as stingarees over the same period of time (Hobday *et al.* 1999). Such increases in populations of some species have been observed in Gulf St Vincent, South Australia as well, with a decline in some taxa after trawling and a subsequent increase in recruitment of other taxa, presumably a result of reduced competition between the groups (Tanner 2003).

Table IV. Comparisons of reproductive parameters in four Urolophidae species found in Australian waters. * no mature female was identified, therefore it is likely that this is an underestimate of maximum size for females. Key: DW = disc width, F = female and M = male. Where available, data on DW are mean ± 95% CI.

| Parameter | *U. paucimaculatus* | *U. paucimaculatus* | *U. paucimaculatus* | *U. lobatus* | *T. personata* | *T. mucosa* |
|---|---|---|---|---|---|---|
| Conception period | ? | Dec/Jan | ? | Dec/Jan | Jun/Jul | May/Jun |
| Parturition | ? | Nov/Dec | ? | Oct/Dec | Apr/May | May/Jun |
| Gestation period (month.) | ? | 11 | <6 | 10 | 10 | 12 |
| Litter size | ? | 1-2 | 2-6 | 1-2 | 1-2 | 1-2 |
| DW at birth (mm) | 90 | 126 | 65 | 105 | 113 | 128 |
| DW at maturity (mm) - F | ? | 222.9 (± 2.9) | 186 | 200 | 228 (± 2.8) | 253 (± 3.2) |
| DW at maturity (mm) - M | 142 | 206.9 (± 1.9) | 192 | 160 | 221(± 4.1) | 222 (± 2.6) |
| Maximum DW (mm) - F | 256* | 260.8 (± 5.9) | 323 | 277 | 302.8 (± 12.7) | 308.1 (± 9.2) |
| Maximum DW (mm) - M | 256 | 243.1 (± 5.0) | 253 | 237 | 269.1 (±16.1) | 261.2 (± 4.4) |
| Location | Spencer Gulf, SA | S-W Australia | S-E Australia | S-W Australia | S-W Australia | S-W Australia |
| Source | This study | White and Potter 2005 | Edwards 1980 | White *et al.* 2001 | White *et al.* 2002 | White *et al.* 2002 |

Table V. Reproductive information on some species in Spencer Gulf based on scientific literature and the presence of small individuals, representing newborns, captured during this study.

| Species | Reproductive mode | Reproductive season | Source |
|---|---|---|---|
| Port Jackson shark | oviparous | Breeds July-August, lays eggs August-November. Young hatch July-November. | McLaughin and O'Gower 1971, Rodda 2000. |
| Stingaree | viviparous | Conceives in December-January, 10-12 month gestation with young born November-December. | White and Potter 2005, Trinnie pers. comm. |
| Elephant shark | oviparous | Eggs laid February–May and young hatch August-November. | Bell (pers. comm.) |
| Southern fiddler ray | viviparous | 12 month cycle with young born in April. | Readon (pers. comm.) |
| Smooth stingray | viviparous | born in Summer ? (only 1 individual) | This study |
| Black ray | viviparous | born in Summer ? (only 1 individual) | This study |

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# Section VI. Age–growth Relationships of the Port Jackson shark, *Heterodontus portusjacksoni* (Myer 1793), in Spencer Gulf, South Australia.

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This chapter was submitted by the primary author to the School of Biological Sciences, Flinders University of South Australia, as partial fulfilment for the degree of Bachelor of Science (Marine Biology) Honours.

# Abstract

Port Jackson sharks, *Heterodontus portusjacksoni*, are frequently caught as by-catch during commercial prawn trawling operations in Spencer Gulf, South Australia. To determine the impacts of these operations on populations of *H. portusjacksoni*, information on their life-history is needed. In this study, relationships between the age and growth of *H. portusjacksoni*, in Spencer Gulf, South Australia, were estimated from growth bands in vertebral centra and dorsal fin spines. Specimens were collected from Spencer Gulf prawn fishery and government research vessels between April 2004 and January 2005. Vertebral centra and dorsal fin spines were collected from 14 males and 24 females (total length range 170–877 mm) for determination of age based on counts of growth bands. Growth parameters for combined sexes were obtained by fitting a modified version of the von Bertalanffy growth function to counts of growth bands. The von Bertalanffy growth parameters based on vertebral centra band counts for combined sexes were K = 0.0852; $L\infty$ = 1166.6 mm and $t_0$ = -1 years. Longevity of *H. portusjacksoni* was estimated to be 40.7 years (combined data from both sexes). Age at maturity for males was estimated to be between 6-8 years and 8-11 years for females. This study shows that Port Jackson sharks possess life-history traits that render them potentially susceptible to fisheries pressure.

## Introduction

An understanding of the biology of exploited fish species is an essential component of effective fisheries management (Pauly, 1987). However, many species of sharks are heavily exploited commercially despite a striking lack of knowledge about their life-histories (Bonfil, 1994). Basic life-history parameters, including population sizes and age structures, growth rates, reproductive cycles, fecundity and longevity, remain unknown for most commercially exploited species of sharks (Frisk *et al.* 2001). Consequently, catches of many species of sharks have been maintained at unsustainable levels and the available data (albeit limited) indicate that populations of sharks are declining worldwide (Casey and Myers, 1998; Musick, 1999; Kitchell *et al.* 2002). Population declines resulting from direct exploitation by commercial fisheries are further intensified by high rates of incidental capture of some species of sharks (*i.e.* as components of by-catch) by other fisheries (Pauly *et al.* 1998; Walker, 1998; Baum *et al.* 2003). For example, the combination of direct exploitation and incidental capture of sharks in the northwest Atlantic have resulted in alarming declines in population sizes within the last 10-15 years: populations of blue, oceanic white tip, white, thresher and hammerhead sharks are estimated to have declined by between 60-90% (Baum *et al.* 2003). Population declines as a result of increasing catches of sharks as both target and non-target species are compounded by a general life-history pattern that makes this group of animals susceptible to over-fishing (Hoenig and Gruber, 1990; Musick, 1999; Cortés, 2004). The life-history parameters of sharks differ from those of most finfish, and they are often referred to as being K-selected species (Hoenig and Gruber, 1990). The von Bertalanffy growth coefficient (K) is the rate at which maximum length of an individual is achieved (Bone *et al.* 1995). Generally, finfish have a high K-value, which is associated with rapid growth, early onset of maturity, high reproductive output (i.e. fecundity), a short life span, and a small maximum size (Quinn and Deriso, 1999). In contrast, the life-history patterns of sharks are characterized by late onset of sexual maturity, long gestation periods, low fecundity, and slow growth over a long lifespan (Hoenig and Gruber, 1990; Pratt and Casey, 1990; Frisk *et al.* 2001).

In southern Australia, the Port Jackson shark, *Heterodontus portusjacksoni*, is a common, and often dominant, component of by-catch in commercial trawling

operations targeting prawns (Carrick, 1997; McShane *et al.* 1998). Svane (2003) speculated that these high rates of incidental capture of *H. portusjacksoni* may result from a combination of their benthic, nocturnal feeding habit (*i.e.* a strong overlap in location and activity with the commercial trawlers), and the attraction of these sharks to discarded by-catch from trawling operations. The mortality rates of *H. portusjacksoni* sharks that are incidentally captured and subsequently released during trawling operations are unknown, but may be dependant on the size of the sharks, the duration of the trawl and the size of the total catch. The prevalence of *H. portusjacksoni* in by-catch of prawn trawling, suggests that fishing operations have the potential to impact upon their populations. A major impediment to understanding the impacts of trawling operations on populations of *H. portusjacksoni* is the limited knowledge base regarding their biology and ecology. Data on relationships between the age and the size of these sharks is particularly needed because it can be used to estimate key life-history parameters, including growth rates, the age structures of populations, and reproductive output (Pauly, 1987; Cailliet and Goldman, 2004). These estimates can then be used to monitor populations over time, and to predict the impacts of fishing operations on their populations in the future (Simpfendorfer and Donohue, 1998; Walker, 2004).

In this study, we aimed to: a) determine the growth rates of *H. portusjacksoni* in Spencer Gulf, South Australia, b) determine the relationships between size and age of *H. portusjacksoni* in Spencer Gulf, South Australia, and c) to validate the periodicity of formation of growth bands through the recapture of chemically marked specimens of *H. portusjacksoni*.  The ages of *H. portusjacksoni* were estimated from counts of growth bands in vertebrae and fin spines. In combination with data on the size of each shark, these data are fitted to the von Bertalanffy (1938) growth functions to predict the rate of growth of *H. portusjacksoni*. The von Bertalanffy growth function was chosen because it is widely used and therefore facilitates comparisons with other species. In addition, unlike some other models, the von Bertalanffy growth function accurately predicts the growth rates of a wide range of fish species and therefore has great utility (Quinn and Deriso, 1999).

## Materials and Methods

### Sampling

Specimens of *H. portusjacksoni* were obtained from three sources between April 2004 and January 2005: 1) the RV *Ngerin* ; 2) from commercial prawn trawlers; and 3) hatchlings from eggs obtained from Spencer Gulf that were maintained in a flow-through seawater aquarium at the Lincoln Marine Science Centre (LMSC), Port Lincoln, South Australia.

### Determination of size and reproductive state

Specimens were sexed and weighed (to the nearest gram) using a Marel Series 2000 electronic marine scale. Total body length (TL) and fork length (FL) (Figure 1) were measured (to the nearest mm) using a steel ruler mounted to a wooden board. The clasper lengths (CL) of males, and the degree of calcification of claspers were used to determine whether a specimen was sexually mature (Conrath, 2004) (Table I). In general, calcified claspers greater than 60 mm in length are indicative of a mature male specimen (McLaughlin and O'Gower, 1971; Last and Stevens, 1994). Relationships between the size of specimens (TL) and onset of reproductive maturity were determined (Chapter 3, Section V). These data were also compared to, and combined with, data from the literature (McLaughlin and O'Gower, 1971; Rodda, unpublished data) to determine relationships between size (and hence age) at maturity.



Figure 1. Length measurements taken from Port Jackson sharks.

Table I.  Determining the state of maturity in male Port Jackson sharks, *Heterodontus portusjacksoni,* based on the degree of clasper calcification, adapted from Gallucci 2002.

| Stage of Maturity | Male Maturity | State of Clasper Calcification |
| --- | --- | --- |
| Immature | Juvenile | Small, soft and flexible |
| Maturing | Adolescent | Extended, tips structured but flexible |
| Mature | Adult | Fully formed and stiff, barbs present |

**Tagging**

Between April 2004 and January 2005, a total of 694 specimens of *H. portusjacksoni* were captured and tagged in the first dorsal fin using conventional Rototag's (Dalton ID Systems Ltd. Oxon, U.K.) (see Chapter 3, Section III for methods). In an attempt to validate the periodicity of band formation in the calcified structures of *H. portusjacksoni*, the majority of these sharks (86%) were also chemically tagged with oxytetracycline (referred to as OTC from hereon). Sharks were injected intraperiotoneally with OTC (supplied by Lyppard, South Australia Pty. Ltd. Adelaide, S.A.) at a concentration of 25 mg/kg body weight using established methods (see Gelsleichter *et al.* 1998; Simpfendorfer *et al.* 2002). Tagged specimens were allowed to recover in on-board flow-through seawater tanks for 30–45 min prior to release.

**Preparation of vertebrae and spines for counts of growth bands**

A total of 38 sharks (14 males and 24 females) were euthanised and frozen in order to collect vertebrae and dorsal fin spines.  Sections of 10–12 vertebrae were dissected from each specimen from below the first dorsal fin (*i.e.* the largest vertebrae) at the LMSC. The first and second dorsal fin spines were also removed from these specimens by cutting horizontally above the notochord, thereby ensuring that the base of the spine remained intact (McFarlane and Beamish, 1987). The largest vertebrae were prepared for examination of growth bands because they contain the most conspicuous bands (Cailliet, 1990; Goldman, 2004). Most of the excess tissue, including neural and haemal arches, was carefully removed from the vertebrae with a scalpel. The remaining tissue was removed by soaking the vertebrae in a solution of sodium hypochlorite (3.5% w/v in distilled water) for 15 min and then rinsing them in

distilled water for 45 min. Vertebrae were then dried in an oven for 2 hours at 60° C and stored at -20° C prior to sectioning. Two vertebrae from each specimen were embedded in polyester resin and sectioned (~ 300 µm thick) sagittally at the fish aging laboratory of the South Australian Research and Development Institute (SARDI) Aquatic Sciences, West Beach, using a Gemmasta lapidary saw. Sectioning along the sagittal plane prevents the growth bands clumping together, which can result in underestimations of the number of bands present (Cailliet *et al.* 1983; MacNeil and Campana, 2002). The remaining vertebrae were kept frozen as backup samples. The first and second dorsal fin spines were prepared for counts of growth bands by soaking them in boiling water for 1–2 min, which enabled excess tissue to be peeled away by hand (Idhe and Chittenden, 2002). The number of growth bands on the spines was then counted without further preparation of the spines.

**Counts of growth bands**

Sections of vertebrae were mounted on glass microscope slides with clear setting cyanoacrylate glue. Counts of growth bands on each vertebra were made with the aid of a dissecting microscope (40× magnification). Growth bands on vertebrae were defined as a pair of opaque and translucent bands, with each band representing one growth cycle (Figure 2). Counts of bands commenced after the birth ring (age = 0), which can be identified by a change in the angle of the outer edge of the corpus calcareum (see Wintner and Cliff, 1999; Conrath *et al.* 2002; Wintner *et al.* 2002; Sulikowski *et al.* 2003) (Figure 2). Growth bands on the dorsal fin spines were defined as pairs of ridges and valleys on the anterior edge of the mantle of the fin spine (McFarlane and Beamish, 1987). Counts were made from the first distinct band to the tip of the spine under a dissecting microscope (10× magnification) with a laterally orientated light source (see Francis and Maolagáin, 2000; Oshitani *et al.* 2003) (Figure 3). In order to ensure the accuracy of counts of growth bands of all vertebrae and dorsal fin spines, two readers made multiple, independent, non-consecutive counts. Samples were re-examined without prior knowledge of specimen size, sex or the numbers of bands found in previous counts. Variability and precision of these repeated counts were analyzed using Chang's (1982) coefficient of variation (CV) and index of precision (D), which are defined as:

$$CV = \frac{s}{Y} \times 100$$

$$D = (\frac{s}{Y} \times 100) / \sqrt{R}$$

where: s is the standard deviation of repeated band counts, R is the number of counts per individual and Y is the mean of the repeated band counts.

The reproducibility of counts for each reader, and between readers, was analyzed using the index of average percentage error (IAPE) (Beamish and Fournier, 1981):

$$IAPE = \frac{1}{N} \Sigma \left( (\frac{1}{R} \Sigma \left( \frac{|Xij - Xj|}{Xj} \right)) \times 100 \right)$$

where: N is the number of sharks aged, R is the number of times each shark is aged, $X_{ij}$ is the $i$th age determination of the $j$th shark and $X_j$ is the mean age estimate of the $j$th shark.

The index of precision (D) gives the percent error contributed by each reading to the average of multiple readings of the same calcified structure (Gallagher and Nolan, 1999) and an upper threshold limit of 5% was set for D following Chang (1982). Upper threshold limits of 10% for the CV and the IAPE have been used previously (Campana, 2001) and were also employed here. Counts of the growth bands on vertebrae and dorsal fin spines with acceptable CVs and IAPEs (i.e. ≤ 10%) were used for calculations of growth rates. The average of multiple counts of growth bands were used to estimate the age of each specimen.

Figure 2. Sagitally sectioned vertebral centra from a 645 mm total length male Port Jackson shark. Dashes represent counts of bands. Where: BR = birth ring (= 0 years); Int = intermedialia; CC = corpus calcareum. Scale bar = 1 mm.



Figure 3. Whole, second dorsal fin spine from a 620 mm total length female Port Jackson shark. Dashes represent counts of bands. Scale bar = 0.5 mm.

**Analyses of age and growth**

Holden's modified version of the von Bertalanffy growth function for embryonic growth was used to calculate the value of the growth coefficient for embryonic growth of *H. portusjacksoni* (*Ke*), as described by Francis (1981):

$$L_{0+t} = L\infty \, (1 - e^{-KeG})$$

where: $L_{0+t}$ is length at birth (mm), $L_{\infty}$ is maximum theoretical length (mm), G is gestation period (years) and *Ke* is the growth coefficient for embryonic growth.

The *Ke* values were calculated based on the approximate time taken to rear *H. portusjacksoni* eggs in captivity, within ± 1 week of gestation (Rodda, 2000). In order to determine the theoretical age (*t0*) when *H. portusjacksoni* length is equal to zero, the von Bertalanffy growth function was fitted to embryonic growth data, described by Skomal and Natanson 2003 as:

$$t_0 = t + \frac{1}{K_e} \left( \ln \left( 1 - \frac{L_0}{L\infty} \right) \right)$$

where: $L_0$ is length at birth (mm), $L_{\infty}$ is maximum theoretical length (mm), $t_0$ is theoretical age when length is equal to zero (years) and $K_e$ is the growth coefficient for embryonic growth.

The value from *Ke* is derived from the von Bertalanffy growth function for embryonic growth. Therefore, we assumed that the embryonic growth coefficient of *H. portusjacksoni* was valid in calculating the theoretical age when length is equal to zero. The values of *t0* were calculated based on an average size at birth of 191.5 mm TL, with the age of the hatchling *H. portusjacksoni* (t) equal to zero (Skomal and Natanson, 2003). Counts of bands were fitted to two separate versions of the von Bertalanffy growth function, and the resulting growth curves were compared for best fit of the data (Cailliet and Tanaka, 1990; Wang and Milton, 2000; Cailliet and Goldman, 2004). The first growth function was the von Bertalanffy (1938) growth function defined as:

$$L_t = L\infty\,(1 - e^{-K(t-t0)})$$

The second function used was a modified version of the von Bertalanffy from Simpfendorfer *et al.* 2000, defined as:

$$L_t = L_{0+t} + \left(L\infty - L_{0+t}\right)\left(1 - e^{-kt}\right)$$

where: $L_t$ is length at age (t) (years), $L_\infty$ is maximum theoretical length (mm), $t_0$ is theoretical age when length is equal to zero (years), $L_{0+t}$ is length at birth (mm) and K is the growth coefficient.

**Longevity**

The oldest *H. portusjacksoni* specimen, determined from counts of growth bands on vertebrae and fin spines, provided an initial conservative estimate for longevity. Using Fabens (1965) equation for theoretical longevity ($T_{max}$), age to 99% of the maximum theoretical length ($L\infty$) (Natanson *et al.* 2002; Skomal and Natanson, 2003) is defined as:

$$T_{max} = 5\left(\frac{\ln 2}{K}\right)$$

Where: K is the growth coefficient.

Growth analysis was performed using Microsoft Excel 2000.

## Results

**Morphological measurements**

Samples of vertebrae and dorsal fin spines from 38 *H. portusjacksoni* were used in this study (14 males and 24 females). These specimens ranged in size from 170 mm TL to 877 mm TL, with the majority of specimens ranging in size between 600 and 800 mm TL. The relationships between TL and the diameter of vertebral centrums (Figure 4; $r^2 = 0.97$; n = 38, P<0.001) and TL and spine diameter (Figure 5; $r^2 = 0.93$;

n = 69, P<0.001) were linear, indicating that both structures are suitable structures for aging the shark.



Figure 4. Linear regression of the diameter of vertebral centrums against total lengths of male and female Port Jackson sharks (*Heterodontus portusjacksoni*) from Spencer Gulf. Intercept –0.6128; slope 0.0143; $r^2 = 0.97$; n = 38.



Figure 5. Linear regression of the diameter of dorsal fin spine against total lengths of male and female Port Jackson sharks (*Heterodontus portusjacksoni*) from Spencer Gulf. Intercept 0.4228; slope 0.0052; $r^2 = 0.93$; n = 69.

An ANCOVA with vertebral centrum diameter as the dependent variable, spine diameter as the covariate and sex as the main factor showed no significant effect of sex ($F_{(1, 84)5} = 0.579$, P = 0.449) and therefore data were pooled for sex. Spine diameter was significantly correlated to vertebral centrum diameter ($F_{(1, 84)} = 61.644$,

P<0.001).  Partial eta squared was 0.432 for the covariate, which explained 43.2% of the variance of the dependent variable.  The variance of the dependent variable was found to be homogenous.

**Recaptures**

Of the 694 sharks tagged and released between April 2004 and January 2005, a total of 12 OTC-tagged specimens were recaptured (a 2% recapture rate), with a strong female bias (11:1). The longest time at liberty of a tagged specimen in this study was 180 days. The specimens were not at liberty long enough to validate the periodicity of band formation. Therefore, age estimates presented from hereon are based on counts of growth bands, with the explicit assumption that the periodicity of band formation in *H. portusjacksoni* is annual (*i.e.* 1 band = 1 year).

**Data analysis**

Mean counts of bands in the vertebrae and dorsal fin spines, and IAPE's were similar for both readers (Table II; Table III). Mean IAPE's for vertebrae (8.96%) and fin spines (10.07%) were considered acceptable (i.e. ≤ 10%). Individual and mean CV and D based on counts of bands in vertebrae were considered to be within accepted error thresholds (Table II). However, the mean CV for counts of fin spines (13.35%) was considered unacceptable, whilst the mean D (5.45%) was considered acceptable (Table III).

Table II. Summary of assessment of vertebrae count reproducibility, variance and precision of the Port Jackson shark by two readers making three reads of the structures.  Including the mean reader confidence of the sectioned vertebral centra analysis.

| Reader | Mean count | IAPE | Coefficient of variation (CV) | Precision (D) | Mean readability |
|---|---|---|---|---|---|
| A | 8.79 | 8.18 | 4.1 | 2.37 | 3.3 |
| B | 10.19 | 9.75 | 7.19 | 4.15 | 3.34 |
| Total | 9.48 | 8.96 | 10.87 | 4.44 | 3.32 |

Table III. Summary of assessment of dorsal fin spine count reproducibility, variance and precision of the Port Jackson shark by two readers making three reads of the structures. Including the mean reader confidence of the dorsal fin spine analysis.

| Reader | Mean count | IAPE | Coefficient of variation (CV) | Precision (D) | Mean readability |
|--------|-----------|-------|-------------------------------|---------------|------------------|
| A | 9.21 | 10.88 | 12.83 | 7.41 | 3.41 |
| B | 9.23 | 9.26 | 10.01 | 5.81 | 3.67 |
| Total | 9.22 | 10.07 | 13.35 | 5.45 | 3.55 |

**Age and growth estimates**

The growth coefficient for rate of embryonic growth in *H. portusjacksoni* ($K_e$) was 0.174. Embryonic growth parameters derived from the modified von Bertalanffy growth function are presented in Table IV. Embryonic growth calculations were based on gestation times and data on the size at birth from hatchlings reared in captivity at the LMSC (191.5 ± 15 mm TL; n = 8).

Table IV. Estimates of embryonic growth parameters for combined sexes of the Port Jackson shark from Spencer Gulf based on Francis (1981) modified version of the von Bertalanffy growth function and captive rearing. Key: n = sample size; $K_e$ = von Bertalanffy growth coefficient for embryonic growth; $L_{o+t}$ = length at birth; G = gestation period; t = age at birth; $t_0$ = theoretical age when length equals 0.

| Parameters | Sexes combined |
|------------|----------------|
| n | 8 |
| $K_e$ | 0.174 |
| $L_{0+t}$ (mm TL) | 191.5 |
| G (years) | 1 |
| t (years) | 0 |
| $t_0$ (years) | -1 |

Counts of bands on sections of vertebral centra were fitted to the von Bertalanffy (1938) growth function, and the modified version of the von Bertalanffy growth function given by Simpfendorfer *et al.* (2000). The von Bertalanffy growth curve provided a reasonable fit to data on the counts of bands at length for females (Figure 6b) and combined sexes (Figure 6c) but not for males (Figure 6a). However, unlike the Simpfendorfer *et al.* (2000) function, which takes length at birth into account, the von Bertalanffy underestimated the size and age at birth. Therefore, estimated growth parameters from vertebrae shown from hereon are derived from the Simpfendorfer *et al.* (2000) growth function that took the actual size at hatch/birth into account. Males had a higher growth coefficient (K = 0.0952) than females (K = 0.0794), yet both males and female achieved similar estimated maximum lengths ($L_\infty$ of 1167.2 mm TL and 1166.8mm TL, respectively (Table V). As no significant difference was found between total length and the diameter of vertebral centra between the sexes, data from both sexes was combined to derive new growth parameters for *H. portusjacksoni*: K = 0.0852 and $L_\infty$ = 1166.6 mm TL (Table V). The Simpfendorfer *et al.* (2000) version of the von Bertalanffy growth function also provided a better fit to dorsal fin spine count at length data for both males (Figure 7a) and females (Figure 7b), and when the sexes were combined (Figure 7c). Thus, all growth parameters shown from fin spines were derived from the Simpfendorfer *et al.* (2000) function. Values for K differed between sexes, with males having a higher growth rate (0.1039) than females (0.0894), and a slightly lower $L_\infty$ (1172.1 mm TL and 1180 mm TL respectively; Table VI). However, as no significant difference was identified between the sexes, band counts from fin spines were combined to yield new growth parameters for *H. portusjacksoni*: K = 0.0907 and $L_\infty$ = 1167.9 mm TL (Table VI).

Figure 6. von Bertalanffy growth curves from counts of growth bands on the vertebrae and length for males (a), females (b) and combined sexes (c). von Bertalanffy growth parameters are given in Table V. Dashed lines are the original von Bertalanffy growth curves, solid lines are calculated from the Simpfendorfer *et al*. (2000) derivation.

Figure 7. von Bertalanffy growth curves from counts of growth bands on the dorsal fin spines and length for males (a), females (b) and combined sexes (c). von Bertalanffy growth parameters are given in Table VI. Dashed lines are the original von Bertalanffy growth curves, solid lines are calculated from the Simpfendorfer *et al*. (2000) derivation.

Table V. Estimates of growth parameters of the Port Jackson shark from Spencer Gulf based on vertebral centra growth increment counts using Simpfendorfer *et al.* (2000) modified version of the von Bertalanffy growth function.  Key: n = sample size; K = von Bertalanffy growth coefficient; $L_\infty$ = maximum theoretical length; $T_{max}$ = estimated longevity; $T_{mat}$ = estimated age at maturity.

| Parameter | Male | Female | Combined |
|---|---|---|---|
| n | 14 | 24 | 38 |
| K | 0.0952 | 0.0794 | 0.0852 |
| $L_\infty$ (mm TL) | 1167.2 | 1166.8 | 1166.6 |
| $T_{max}$ (years) | 36.4 | 43.7 | 40.7 |
| $T_{mat}$ (years) | 6 – 8 | 8 – 11 | |

Table VI. Estimates of growth parameters of the Port Jackson shark from Spencer Gulf based on dorsal fin spine growth increment counts using Simpfendorfer *et al.* (2000) modified version of the von Bertalanffy growth function.  Key*:* n = sample size; K = von Bertalanffy growth coefficient; $L_\infty$ = maximum theoretical length; $T_{max}$ = estimated longevity; $T_{mat}$ = estimated age at maturity.

| Parameter | Male | Female | Combined |
|---|---|---|---|
| n | 14 | 24 | 38 |
| K | 0.1039 | 0.0894 | 0.0907 |
| $L_\infty$ (mm TL) | 1172.1 | 1180 | 1167.9 |
| $T_{max}$ (years) | 33.4 | 38.8 | 38.2 |
| $T_{mat}$ (years) | 5 – 7 | 7 – 10 | |

**Longevity**

The maximum age of specimens examined in this study, determined from counts of vertebral bands, was 11 and 17 years for males and females, respectively. Using Fabens (1965) method for estimating longevity from counts of vertebral bands ($T_{max}$), yielded estimates of 36.4 and 43.7 years for males and females respectively, with a combined longevity estimate of 40.7 years (Table V). Longevity estimates were also derived from counts of bands in fin spines: 33.4 and 38.8 years for males and females,

respectively, with a combined estimated longevity of 38.2 years (Table VI). Averaging longevity estimates based on dorsal fin spines and vertebral band counts produced a longevity estimate of 39.45 years for *H. portusjacksoni*.

**Age at maturity**

Length at maturity for male *H. portusjacksoni* was within the range of 500–575 mm TL (Chapter 3, Section V). Therefore, assigning an estimated age ($T_{mat}$) based on the von Bertalanffy age at size plots, males mature between 6-8 years and 5-7 years, for vertebral and dorsal fin spine data, respectively (Table V; Table VI). Length at maturity for female *H. portusjacksoni* was within the range of 650–750 mm TL (Chapter 3, Section V). Therefore, assigning an estimated ($T_{mat}$) age based on the von Bertalanffy age at size plots, females mature between 8-11 years and 7-10 years, for vertebral and dorsal fin spine data, respectively (Table V; Table VI).

## Discussion

*Heterodontus portusjacksoni* is a slow growing, long-lived shark that achieves reproductive maturity relatively early in life, and has a low reproductive output. These findings have important implications for *H. portusjacksoni* because they are indicative of a species that is vulnerable to either direct exploitation or mortalities induced by human activities, including incidental capture in nets during fishing operations. Global catches of sharks as a result of direct and indirect fishery exploitation, have continued to increase despite records of dramatic declines of populations and localised extinctions of some species (Bonfil, 1994; Walker, 1998). High rates of capture are compounded by the life-history attributes of sharks that make their populations slow to recover (*i.e.* slow growth, late age at maturity, low fecundity); thus, populations of some shark species are declining at an unsustainable rate (Bone *et al.* 1995; Musick, 1999). *H. portusjacksoni* shares life-history characteristics with other species of sharks that render it vulnerable to fisheries pressure.

The growth of *H. portusjacksoni* was rapid in the first seven years with individuals increasing from approximately 191.5 mm TL to 600 mm TL. The rapid growth of *H. portusjacksoni* in this juvenile phase is widely considered to be a survival mechanism that reduces predation upon juvenile sharks, and the rates reported here are similar to

those reported for the whiskery shark, *Furgaleus macki* (Simpfendorfer *et al.* 2000), the spiny dogfish, *Squalus acanthiasi* (McFarlane and Beamish, 1987) and the smooth dogfish, *Mustelus canis* (Conrath *et al.* 2002). Growth after 7 years is slower, coinciding with the onset of sexual maturity and gonad development, and is possibly the result of a trade-off between somatic growth and reproductive investment (Bone *et al.* 1995; Simpfendorfer *et al.* 2000).

Growth rates in sharks appear to be extremely variable and show no correlation with size or geographic location. However, different growth rates between sexes are common, with males generally possessing faster growth rates than females (see Cailliet and Goldman, 2004). Estimated growth rates for *H. portusjacksoni* cannot be compared to other species of the Family Heterodontidae because comparable data does not exist (Compagno, 2001). However, the derived growth rate of *H. portusjacksoni* is similar to those reported for other species of dog sharks. The longevity estimates given here (36 and 44 years for males and females respectively) greatly exceed the 12 years maximum age previously attained by *H. portusjacksoni* maintained in captivity (McLaughlin and O'Gower, 1971), and exceeds the estimated ages of males and females captured in this study (11 years and 17 years respectively). Sharks in general are long-lived, although longevity estimates vary between species. The estimates of longevity reported here are similar to those reported for other species of dog sharks and are therefore likely to be valid. Both male and female *H. portusjacksoni* achieve similar maximum lengths: however, due to differing growth rates between males and females, estimates of longevity were different between the sexes. Sexual size dimorphism is common in species of sharks and was expected in *H. portusjacksoni*, as females are generally observed to be approximately 250 mm larger than males of the same age (Compagno, 2001). *H. portusjacksoni* is believed to have a maximum length in excess of 1300 mm TL (see Campagno, 2001), yet during this study, the largest specimen was 877 mm TL. The small size of specimens sampled here may simply reflect a sampling bias. The area of Spencer Gulf that was sampled during this study was restricted to prawn trawling grounds, which have sandy substrata between 20-40 m depth. It is likely that larger individual *H. portusjacksoni*, which are known to migrate to deeper offshore waters (McLaughlin and O'Gower, 1971), were not sampled during this study.

This study estimated that reproductive maturity is reached between 6-8 years for males and 8-11 years for females. In the only previous study examining age at reproductive maturity of *H. portusjacksoni*, males and females were estimated to mature at a later age and a larger size (8-10 years, 700-800 mm FL and 11-14 years, 800-950 mm FL for males and females respectively) (McLaughlin and O'Gower, 1971) (Table VII). Fisheries exploitation can reduce the size and age of maturity and increase the growth rate of species of sharks (Carlson and Baremore, 2003; Baum and Myers, 2004; Williams and Shertzer, 2005). Whether differences in the time to reproductive maturity and size at maturity of *H. portusjacksoni* reported here and by McLaughlin and O'Gower (1971) are a result of fishing pressure in Spencer Gulf, or differences between the populations examined, is unknown because of a lack of baseline data. Growth bands in calcified structures were assumed to represent annual growth increments in this study. However, further investigation is required to validate this assumption.

Table VII. Length comparisons (fork length) in two Port Jackson sharks populations, observed along coastal New South Wales and Spencer Gulf, South Australia, at different life-history stages. Lengths from coastal NSW observed by McLaughlin & O'Gower (1971).

|  | Coastal NSW | Spencer Gulf, South Australia |
| --- | --- | --- |
| Hatchling size (mm) | 230 – 240 (FL) | 152-190 (FL) |
| Male maturity (mm) | 700 – 800 (FL); 8 – 10 | 500-590 (FL); 6 – 8 |
| Female maturity (mm); (years) | 800 – 950 (FL); 11 – 14 | 590-680 (FL); 8 – 11 |
| Maximum length (mm) | 1228 (FL) | 1063 (FL) |

This study attempted to validate the periodicity of growth band formation through the tagging and recapture of chemically marked specimens. This technique is considered the best means of validating the absolute age of wild caught fish specimens, as growth bands are formed in the wild under natural conditions (Campana, 2001). Although this study had a moderate recapture rate of OTC marked specimens within the limited time frame, there were no recaptures of sharks that had been at liberty for more than one year (a minimum requirement for successful validation of the periodicity of band

formation). However, recaptured chemically marked sharks indicate that OTC is a suitable chemical marker for *H. portusjacksoni*, as the rate of OTC incorporation is rapid and it forms a distinct band in calcified structures (Izzo unpublished data). Calcified structures have been used extensively as tools for aging fish, including sharks (Cailliet and Goldman, 2004). This study found that the sectioned vertebrae were the best structures for aging *H. portusjacksoni* as sectioning improved the clarity of bands. However, preparation and sectioning of vertebral centra is both costly and time consuming. The dorsal fin spine was initially considered the best option as an aging structure of *H. portusjacksoni*, because it had a clear banding pattern, required little preparation and could be potentially sampled non-lethally (Beamish and McFarlane, 1983). However, due to the deterioration of spines from mechanical wear, these structures were considered a less reliable structure for aging sharks. Because of the reader variability associated with counts of bands, the requirement for validation of the periodicity of band formation, coupled with the destructive sampling required to collect vertebrae and dorsal fin spines, new methodologies for aging fish need to be developed.

A potential non-destructive, rapid and accurate means of aging fish, including sharks may be provided by new molecular techniques such as telomere restriction fragment length analysis. Telomere length analysis has been successfully been used to age several species of birds and mammals (including humans) (Nagakawa *et al.* 2004), and is based on the natural rate of decline in telomere lengths that occurs with age (Haussmann and Vleck, 2002). As yet, this aging method has not been used in the marine environment but may have great utility in aging studies of marine organisms. Investigations into these techniques are therefore needed.

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# Section VII. Conclusion

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## General Conclusion

Global landings of elasmobranchs are estimated at 1.6 million metric tonnes (Stevens *et al.* 2000), and concerns about declining population sizes have been raised internationally. Elasmobranchs are long-lived, slow growing, late to mature and have a low fecundity making them susceptible to over-exploitation by commercial fisheries. These life history traits mean that populations are often slow to recover if they are overfished. Elasmobranchs play an important role in ecosystem structure and function; they are often the top order predator and their removal, whether it is targeted or incidental, can trigger widespread changes in the overall marine ecosystem. The biology of many of these species is poorly described and highlights the need for more research if we are to understand the impacts of fishing on these populations.

Spencer Gulf is home to a diverse group of marine fauna and flora. The aim of the study was to obtain measurements of the trawling catchability and population parameters of important by-catch and scavenger species, particularly the smaller sharks, skates and rays at five sites in Spencer Gulf. This study was conducted over a limited geographical distribution within Spencer Gulf, covering five representative fishing grounds. Coastal areas up to 15 m in depth were not sampled, nor were large areas within Spencer Gulf. Thus the diversity, richness and distribution data does not describe the entire gulf (to do so is beyond the scope of this project), however it does reflect what species are likely to be encountered by operational prawn trawlers in different areas of the gulf. The sites chosen were characterised by varying sediment types, environmental parameters and history of annual fishing effort. Differences in species richness, distribution, abundance and reproductive patterns, based on habitat and environmental complexity and exposure to varying intensity of fishing, are likely. Variation in seasonal abundance is not uncommon. Most species are distributed heterogeneously both spatially and temporally because of the diversity of habitats and the requirements of individual species lifecycles (Poulard and Léauté 2002). Fishing activity also varies spatially and temporally. Hence, interactions between prawn trawling and by-catch species will not be consistent.

This study found that the species richness and overall diversity of elasmobranchs caught by trawlers was low. Across the five sites, 22 species of elasmobranchs were caught between October 2003 and October 2005, comprising 10 shark, 8 ray, 3 skate and 1 chimera species.

Almost half of these were caught in low numbers (less than five). Port Jackson sharks were the most common species, making up 61.6% of all elasmobranchs caught, followed by the sparsely spotted stingaree, with 17.6%. The number of species per site ranged from eight at Site 3 to 15 at Site 5. Many species were found at several sites, and only three were caught at all sites, but most species demonstrated a clear preference for a single site over all others. In general, the highest species richness, diversity and site preferences were found at Site 5, followed by Sites 2 and 1. Site 3 was found to have the least number of species, lowest diversity and no species displayed a preference for it.

Diversity, species richness and distribution at the five sites were strongly related to habitat type. Sites 1, 2 and 5 were characterised by sandy sediment, while Sites 3 and 4 are characterised by a coarse sand or gravel bottom, with strong tidal flow. Many of the species encountered in trawl fishing operations in Spencer Gulf are generally small (under one metre in length), bottom dwelling and spend considerable time buried or at least partially buried in the sediment. The sandy sediments of Sites 1, 2 and 5 provide a habitat for burrowing below the sand, and demersal species such as angel sharks, stingarees, skates and shovelnose rays, were found to occupy these sites. Other species with greater mobility and a reduced tendency to burrow, such as the Port Jackson shark, cobbler wobbegong, elephant shark, fiddler ray and the large stingrays, tend to have a wider distribution and were found at most sites. Low diversity and species richness at Site 3, where the substrate is hard and tidal currents are strong (Svane 2003), was a reflection of the unsuitable habitat type for many of the species recorded. The strong tidal currents typical at this site are also likely to lead to greater energetic costs for sedentary species trying to maintain position in or near the seafloor.

Patterns of abundance closely followed that for distribution. Site 5 was the preferred site for many species, and was the site with highest overall abundance. The most northerly site (Site 1) had the lowest abundance, closely followed by Site 3. Abundance varied both spatially and temporally, but the pattern of abundance at the five sites over the seven seasons was largely inconsistent between species, reflecting biological characteristics and possible migration patterns related to food and reproduction. Significant differences in abundance at the different sites were observed for most species, reflecting habitat preferences. Seasonal changes in the abundance of elephant sharks represented a possible migration into shallow waters for reproduction during the warmer months, as seen in Victorian populations (Last and Stevens 1994, Bell pers. comm.). In contrast, a lack of seasonal variability in abundance data,

in conjunction with results from the tagging program, lead to the conclusion that Port Jackson sharks demonstrate strong site fidelity. Dispersal of Port Jackson sharks within the gulf was low, with 55% of individuals recaptured within 10 km of the original tag site.

The impact of fishing on the populations of sharks, skates and rays in Spencer Gulf depends on a variety of factors and may differ between species. Some species are able to avoid capture in the first instance by behavioural adaptations such as burying in the sand, swimming away from the net or migration away from the area. Mortality, as a result of capture, can also vary substantially between species.

For the majority of species in Spencer Gulf, sexual maturity was reached at a smaller size than reported elsewhere. Similarly, the age at maturity for Port Jackson sharks was estimated to be much earlier than previously reported (6-8 and 8-11 years for males and females respectively). The onset of earlier maturity may be a response to fishing pressure in conjunction with environmental and ecological factors. The commercial prawn fleet has significant interactions with many species of elasmobranch in their trawl operations. The number of immature animals caught by trawls ranged from low (male elephant sharks, female fiddler rays), to high (angel sharks, stingarees, female elephant sharks and male fiddler rays), and provided post-release mortality is high, this may have a dire consequence for the populations of sharks in the gulf over time. The removal of immature individuals before they have the opportunity to contribute to the population may eventually lead to recruitment over-fishing. Similarly, the removal of large, reproductively active animals can have a negative impact on the status of populations. However, the partial separation of fishing activity with reproductive cycles can reduce the impact the Spencer Gulf prawn industry has on some populations of elasmobranch. For example, stingarees have an annual breeding cycle and give birth to live young in late spring. This species is found predominantly at Site 5 (The Gutter), an area that is generally not fished until late in the season (April/May), hence the heavily pregnant fish are less likely to be caught. Elephant sharks migrate to shallow waters to lay their egg capsules between February to May, and reproductively active females can potentially avoid capture because fishing does not occur in these areas. Port Jackson sharks lay their egg capsules between August and November each year, therefore capsule carrying females are protected because their reproductive season has all but finished before fishing commences.

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# CHAPTER 4: POPULATION PARAMETERS OF SAND TREVALLY, FIVE SPECIES OF LEATHERJACKET AND BLUE CRAB: A COMPARISON BETWEEN FIVE SITES IN SPENCER GULF, SOUTH AUSTRALIA

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# Abstract

During three trawl surveys each covering five sites in Spencer Gulf, the population parameters of the quantitatively important species sand trevally (*Pseudocaranx wrighti*), bridled leatherjacket (*Acanthaluteres spilomelanurus*), rough leatherjacket (*Scorbinichthys granulatus*), toothbrush leatherjacket (*Acanthaluteres vittiger*), Degens leatherjacket (*Thamnaconus degeni*), southern pygmy leatherjacket (*Brachaluteres jacksonianus*) and blue crab (*Portunus pelagicus*) were studied. All of the species studied are important by-catch species, and with the exception of sand trevally, are important scavengers on discarded by-catch. At each site, eight 30 minute trawl transects were conducted, four during the day and four at night, using a standard prawn trawl. During the first survey (Oct 2003) only the studied species were counted, measured and weighed, while on the two later surveys (Oct 2004 and Jan 2005) all material caught was separated into the major groups and weighed. The five selected sites cover an area from 8 nm south of Whyalla (Site 1) to the Gutter off Franklin Harbour in the central Spencer Gulf (Site 5), 66 nm further to the south, and constitute areas of different fishing history of the Spencer Gulf prawn fishery. The purpose of the study was firstly to gain an understanding of population characteristics and the distribution and abundance patterns of the most important smaller by-catch species and secondly to correlate variations in these parameters to fishing intensity and habitat. The results showed a significant variation between sites in size, weight, and biomass of all the studied species. For the 2004/05 surveys, the correlation analyses showed that biomass of rough leatherjackets and Degens leatherjackets were positively correlated to trawl hours and negatively correlated with benthos biomass. Excluding the Oct 2003 survey, sand trevally showed a negative correlation with trawl hours and no correlation with benthos biomass, which can be explained by a pelagic existence. The correlations for blue crabs with trawl hours and benthos biomass were mixed. The observed patterns of abundance correlated to trawl hours and habitat complexity (benthos biomass), suggest that habitat affects abundance but the important correlation is with trawl hours. Trawl hours were negatively correlated to habitat heterogeneity, which explains 23.3% of the variance for the Oct 2004 survey and 28.6% for the Jan 2005 survey.

## Introduction

The ecological impact and long-term consequences of discarding by-catch in trawl fisheries is largely unknown, particularly how discards affect scavenger feeding habits and their populations (Andrew and Pepperell 1992, Hall 1999, Jennings & Kaiser 1998, Ramsay *et al.* 1998). This study focuses on the morphometric parameters of the key by-catch species, sand trevally, (*Pseudocaranx wrighti*), five species of leatherjackets (Degens leatherjacket: *Thamnaconus degeni*; bridled leatherjacket: *Acanthaluteres spilomelanurus*; southern pygmy leatherjacket: *Brachaluteres jacksonianus*; rough leatherjacket: *Scorbinichthys granulatus*, and toothbrush leatherjacket: *Acanthaluteres vittiger*) and blue crabs (*Portunus pelagicus*). Sand trevally and leatherjackets are the most abundant teleosts found in the by-catch of the Spencer Gulf prawn fishery (Carrick 1997). By numbers, sand trevally constitutes up to 31.3%, Degens leatherjacket 16.8%, bridled leatherjacket 3.7%, rough leatherjacket 3.7%, toothbrush leatherjacket 1.4%, and the remaining leatherjackets less than 0.5% of the by-catch, with all caught individuals being relatively small (see Carrick 1997). Blue crabs are the most important crustacean by-catch species contributing 11.0% by numbers. Of the by-catch species studied here, only the blue crab is subjected to a commercial and recreational fishery. Of the above species, leatherjackets, particularly Degens leatherjacket, and blue crabs are important consumers of discarded by-catch.

The aim of this study was firstly to gain an understanding of the morphometric parameters, and population characteristics including distribution and abundance of these species, and secondly, to compare these parameters between five different sites in Spencer Gulf with different trawling histories.

## Material and Methods

### Selection of Study Areas

As described in Chapter 1, five sites were selected representing areas of different fishing intensity (trawl hours) along a North-South gradient in Spencer Gulf (Figure 1 and Table I in Chapter 1). Furthermore, data on fishing effort was obtained using logbook recordings of trawling hours reported from administrative fishing blocks (Figure 3 in Chapter 1).

**By-catch sampling**

In order to determine by-catch composition and quantity, three trawl surveys (October 2003, October 2004 and January 2005) were carried out in Spencer Gulf using the RV *Ngerin*. The method used is described in Chapter 2. During the October 2003 survey, only the dominant by-catch species sand trevally, leatherjackets and blue crab were sampled for counts and individual measurements. During the October 2004 and January 2005 surveys all by-catch were sampled, identified and weighed.

**Sand trevally**

*Sampling*

During the October 2003 survey, up to 40 fish were measured from each trawl, and a randomly selected sub-sample of up to 20 fish were frozen for analysis, including determination of weight-length relationship, age determination, and Gonado-Somatic Index (GSI) measurements.

*Species identification*

Two species of trevally are recorded in Spencer Gulf namely, sand trevally (*P. wrighti*) and silver trevally (*P. dentex*), but only sand trevally were found in the trawls. These two species are very similar in appearance, and identification was according to Hutchings & Swainston (1986) and Gomon *et al.* (1994).

*Morphometric and biological data collection*

For each individual, the total length to the nearest mm, total weight to the nearest 0.001 g, gutted weight to the nearest 0.001 g, gonad weight to the nearest 0.001 g and sex were recorded. Sex determination was done visually and by gonad identification under dissection. When sex could not be determined, individuals were classified as immature, and disregarded when analysing sex ratios. The Gonado-Somatic Index (GSI index) was calculated from the morphometric data as: GSI index = (gutted weight/gonad weight)*100.

*Ageing*

A sand trevally has three pairs of otoliths, located just behind the eyes lateral to the brain. The largest of these pairs (sagittal otoliths) were carefully removed from up to 12 fish from each site. For analysis of the age-length relationship, samples were taken from individuals ranging from small (8.2 cm) to large (14.8 cm). The otoliths were dried, placed in plastic bags and

sent to the Central Aging Facility (CAF, Marine and Freshwater Research Institute, Queenscliff, Victoria) where annual age estimates were carried out.

**Leatherjackets**

*Sampling*

During the October 2003 survey, leatherjackets were sorted by species and, if possible, sex, and then bagged and frozen for further examination. Identification was according to Hutchings & Swainston (1986) and Gomon *et al.* (1994). In the laboratory, leatherjackets were thawed, and weighed to the nearest 0.01 grams, using an Ohaus Precision standard weight TS 400. Standard length (SL) and total length (TL) were measured to the nearest mm using Vernier callipers (see Gomon *et al.* 1994).

*Otolith analysis*

Otoliths were sampled from Degens leatherjacket females and males at each site. When possible, otoliths were taken from five females and five males selected to get the widest size-range at each site. The otoliths where sent to the Central Ageing Facility (CAF) for age determination.

**Blue crab**

*Sampling*

During the October 2003 survey, the carapace width and sex of all *P. pelagicus* caught in the trawls were determined. A subsample was frozen and later weighed in the laboratory for carapace width-weight relationship determination. Carapace width was measured with Vernier callipers across the base of the largest lateral spines.

**Data analysis**

All data were analysed using the statistical software SPSS 14.0 with a significance level of 0.05. Biomass patterns were analysed using three-way ANOVA's with site, survey and time of day as fixed factors. Homogeneity of variances and normality were tested using Levene's test and Kolomogorov-Smirnoff test, respectively, as provided in SPSS 14.0. Post-hoc comparisons were done using the Tukey HSD post-hoc test. Partial eta squared values are provided as an alternative measure for effect size (Pallant 2005). Post-hoc comparisons were done using the Tukey HSD post-hoc test when variances were homogeneous and the Dunnett C test when they were non-homogeneous. Regression analyses were carried out to determine

the significance of the length-weight relationship for each species. Three-way ANCOVA's with time (day/night), sex and site as the fixed factors, length as the dependent variable and weight as covariate were performed to determine if length/weight relationships varied with any of these factors. When necessary, the covariate "weight" was transformed to obtain linearity, and the assumptions of normality and homogeneity of variances were met. For analysis of age, one-way ANOVA's were used. Chi square tests were used to test deviation from a 1:1 sex ratio. Spearman Rank correlations were used to give an overall view of correlation patterns between, on one hand, the biomass of the most dominant smaller by-catch species from replicated trawl samples (Chapter 2), and on the other hand, annual trawl hours and benthos. The abundance of benthos is a measure of habitat heterogeneity/complexity (see McCoy & Bell 1991). For Spearman Rank correlations there is no assumption of linearity and it is insensitive to proportional and additive differences between samples (see Krebs 1999). Because of the significance levels of Spearmans rho are strongly influenced by sample size, the focus is on the amount of shared variance (see Cohn 1988, Pallant 2005). Spearman Rank correlations have been found useful as a similarity measure of trawl samples (see Johnsen 2003).



Figure 1. Biomass (g/100m$^2$) of sand trevally (*Pseudocaranx wrighti*) sampled at three occasions at five sites in Spencer Gulf. Error bars are 95% CI.

Figure 2. Biomass (g/100m$^2$) of sand trevally (*Pseudocaranx wrighti*) sampled at five sites during day and night in Spencer Gulf. Error bars are 95% CI.



$$y = 0.0062x^{3.2214} \quad R^2 = 0.9693$$

Figure 3. Length-weight relationship and regression line for sand trevally (*Pseudocaranx wrighti*). Equation and $R^2$ is shown for the regression line.

# Results

## Sand trevally

### *Abundance and biomass*

The biomass of sand trevally sampled during the three trawl surveys is shown in Figures 1 and 2. A three-way ANOVA with site, time and survey as the main factors on fourth root-transformed data was carried out. Normality was best obtained by a fourth root transformation, but it was not possible to obtain homogeneity of variances by transformation partly because of many zeros in the data. Because ANOVA's are usually robust against heterogeneous variances, the analysis was carried out regardless. The results showed a significant effect of site ($F_{[4, 118]}$=20.291, P=0.001, partial eta squared = 0.480), time of day ($F_{[1, 118]}$=7.441, P=0.008, partial eta squared = 0.078) and survey ($F_{[2, 118]}$= 53.438, P=0.001, partial eta squared = 0.548) with significant interaction for site * survey only ($F_{[8, 118]}$= 3.491, P=0.002, partial eta squared = 0.241). The partial eta squared is an alternative for effect size. Site and survey thus had a large effect while time had a moderate effect (see Cohn 1988). Differences between surveys accounted for a large proportion of the variability, particularly during the Oct 03 survey where few sand trevally were caught (Figure 1). The results showed that the effect of site was inconsistent among surveys, primarily because of the exceptionally low numbers of sand trevally caught during the Oct 03 survey. Therefore, two-way ANOVA's were carried out for the individual surveys excluding the Oct 03 survey.

The results of a two-way ANOVA on data with non-homogeneous variances from the Oct 04 survey showed a significant effect of site ($F_{[4, 40]}$=54.004, P<0.001, partial eta squared = 0.878) but not of time of day  ($F_{[1, 40]}$=0.533, P=0.471, partial eta squared = 0.017) with no significant interaction ($F_{[4, 40]}$=2.375,  P=0.074, partial eta squared = 0.240).

The results of a two-way ANOVA on fourth root transformed data with homogeneous variances from the Jan 05 survey showed a significant effect of site ($F_{[4, 37]}$=3.317, P=0.025, partial eta squared = 0.329) and time of day  ($F_{[1, 37]}$=4.260, P=0.049, partial eta squared = 0.136) with no significant interaction ($F_{[4, 37]}$=0.734, P=0.577, partial eta squared = 0.098). The effect size of the factors site and time were large, but the power 0.769 and 0.512, respectively. These values are lower than the conventionally accepted value of 0.8, giving a relatively high probability of Type 1 error (rejecting the null hypothesis when it is false). However, the power of factor site for the Oct 04 survey was 1.0. Accordingly, the overall

results showed that sand trevally is likely to be caught in greater numbers during day than at night. Differences among sites were not consistent between surveys demonstrated by the significant interactions in the three-way ANOVA. A Tukey HSD post hoc test for the Oct 04 survey separated Site 4 from 1, 2, and 3 while Site 5 was different from 1, 3 and 4. Biomass distribution among sites was 4 → 3 → 1 → 2 → 5 from low to high biomass. A Dunnett C test on the Jan 05 survey separated Site 5 from Site 4 with Sites 1, 2 and 3 in between, thus confirming that more sand trevally were caught at the southern sites. The large variability between and within sites reflects the schooling behaviour of the species.



Figure 4. Length and weight for sand trevally (*Pseudocaranx wrighti*) at five sites in Spencer Gulf. Error bars are 95% CI.

*Length-weight relationships*

The length-weight relationship for sand trevally sampled on the October 2003 survey shows an allometric growth pattern with weight increasing at a greater rate than length (Figure 3). The regression line was significant ($F_{[1, 286]}$=8997.309, P<0.001). Sand trevally does not show external sexual dimorphism and sexes were therefore not separated.

Length and weight as a function of sex and site are shown in Figure 4. The results of a three-way ANCOVA with time (day/night), sex and site as the main factors, length as the dependent variable and log (x+1) transformed weight as covariate showed a significant effect of site ($F_{[4, 287]}$ = 4.680, P=0.001, partial eta squared 0.066) with no significant effect of time ($F_{[1, 287]}$ = 1.244, P=0.290, partial eta squared = 0.009) and sex ($F_{[1, 287]}$=0.010, P=0.919, partial eta squared = 0.000) with no significant interactions. Preliminary analyses showed that the variances and slopes were homogeneous. For the factor site, the effect size was moderate, but the power was 0.948. The results showed that sand trevally were significantly larger and heavier at Site 5 than elsewhere (Figure 4). The length/weight relationship was independent of sex and time (day and night trawls).



Figure 5. Age of sand trevally (*Pseudocaranx wrighti*) at five sites in Spencer Gulf. Error bars are 95% CI.

*Age-length relationships*

The results of the otolith analysis by CAF are shown in Appendix I. The estimated ages of sand trevally from Spencer Gulf ranged between 1 and 5 years. The readability score was found to be 2.94, which indicated reliable results. A linear relationship between length and otolith zone count was found to be y=1.3454x + 6.426, $R^2$ = 0.6393.

The mean age of a sub-sample of sand trevally as a function of site is shown in Figure 5. The mean age was higher at Site 5 and fairly similar at the other sites. A one-way ANOVA on data

with homogeneous variances showed a significant effect of site ($F_{(4, 47)}$=3.493, P=0.015, partial eta squared 0.25). A Tukey HSD test separated Site 5 from the other sites. It is thus evident that the observed differences in length and weight of the trawl samples from the five sites can be explained by age, with sand trevally caught at Site 5 being older, longer and heavier than at the other four sites.



Figure 6. Gonado-Somatic Index for female and male sand trevally (*Pseudocaranx wrighti*) at five sites in Spencer Gulf. Error bars are 95% CI.

*Gonado-Somatic Index*

The Gonado-Somatic Index (GSI) [(gutted weight/gonad weight)*100] as a function of site and sex is shown in Figure 6. A two-way ANOVA with site and sex as the main factors with the dependent variable "GSI" log (x+1) transformed to obtain homogeneity of variances, showed a significant effect of site ($F_{(4, 288)}$=6.612, P<0.001, partial eta squared = 0.087), sex ($F_{(1, 288)}$=14.760, P=0.001, partial eta squared = 0.05) and with significant interactions ($F_{(4, 288)}$=2.485, P=0.044, partial eta squared = 0.053). The significant interaction is caused by interference mainly at Site 3 and less so at Site 1 and 2 (Figure 6). The result largely reflects the differences between mean length and weight with site (Figure 4) showing that the development of GSI is dependent on size and weight, which in turn is dependent on site.

**Sand trevally**

Figure 7. Proportions of sand trevally (*Pseudocaranx wrighti*) females at five sites in Spencer Gulf. Line indicates 1:1 sex ratio (0.5 proportion of females). Error bars are 95% CI.

*Sex ratio*

A one-way ANOVA on arcsine transformed data showed no significant effect of site ($F_{(4, 19)}$=1.771, P=0.191, partial eta squared = 0.336) on the proportion of females (Figure 7). A $\chi^2$-test was conducted to test for departure from a 0.5 proportion (equal proportion of females and males). The results showed that sand trevally populations did not statistically deviate from equal proportions of female and males ($\chi^2 = 14.8324$, $\chi^2_{crit.} = 52.192$, df =37, $\alpha = 0.05$).

**Leatherjackets**

*Abundance and biomass*

Leatherjacket biomass increased from north (Site 1) to south (Site 5) of the gulf (Figures 8 and 9). A three-way ANOVA with site, time and survey as the main factors on log (x+1) transformed data was carried out. It was not possible to obtain homogeneity of variances by transformation partly because of many zeros in the data. Normality was best obtained by a log (x+1) transformation and because ANOVA's are usually robust against heterogeneous variances, the analysis was carried out regardless. The results showed a significant effect of

212

site ($F_{[4, 118]}$=44.067, P=0.001, partial eta squared = 0.667), time of day ($F_{[1, 118]}$=24.194, P=0.001, partial eta squared = 0.216) and survey ($F_{[2, 118]}$=122.315, P=0.001, partial eta squared = 0.735) with significant interactions for all three factors. The results showed that leatherjacket biomass is highly variable between sites and inconsistent between surveys and time of day.



Figure 8. Biomass (g/100m$^2$) of leatherjackets sampled at three occasions at five sites in Spencer Gulf. Error bars are 95% CI.

Figure 9. Biomass (g/100m$^2$) of leatherjacket sampled at five sites during day and night in Spencer Gulf. Error bars are 95% CI.



Figure 10. Biomass (g/100m$^2$) of dominant leatherjackets sampled at five sites in Spencer Gulf. Error bars are 95% CI.

214

Figure 10 shows the distribution of biomass of the most dominant species, Degens and rough leatherjackets. The category of "other" comprises bridled, toothbrush and mosaic leatherjackets. A one-way ANOVA showed a significant effect of site for Degens leatherjacket ($F_{[4, 118]}$=17.596, P=0.001, partial eta squared = 0.384), and for rough leatherjacket ($F_{[4, 118]}$=6.826, P=0.001, partial eta squared = 0.195), but not for the group "others" ($F_{[4, 118]}$=1.137, P=0.355, partial eta squared = 0.115). It was not possible to obtain homogeneous variances by transformation but the analysis was carried out on non-transformed data regardless. Dunnett C tests separated Site 5 from other sites for Degens leatherjacket and Sites 2 and 4 from other sites for rough leatherjacket. The results showed that the largest biomass of Degens leatherjacket was found at Site 5 (The Gutter) and it was rare at the other sites. Rough leatherjackets were more abundant at Sites 2 and 4 than at other sites. A large variation in leatherjacket biomass between trawls was evident because of the schooling behaviour of the dominant species.

*Length-weight relationships*

Bridled leatherjacket

The length-weight relationship and regression lines for both female and male bridled leatherjacket are shown in Figure 11 and the mean length and weight for both sexes as a function of site is shown in Figure 12. Site 5 was not included because few bridled leatherjackets were found there.

A two-way ANCOVA with length as the dependent variable and log weight as covariate showed that it was not possible to obtain homogeneity of regression slopes because of the site * log weight interaction ($F_{[4, 532]}$=9.271, P<0.001, partial eta squared = 0.068), and sex * log weight interaction ($F_{[1, 532]}$=77.682, P<0.001, partial eta squared = 0.132) thus violating the assumptions for an ANCOVA. The results suggest that differences between sexes and variations in the length weight relationships were too large to detect any differences between sites. Bridled leatherjackets showed allometric growth patterns for both sexes with sexual dimorphism where males are larger than females for a given weight. The regression lines were significant for females ($F_{[1, 332]}$=2600.025, P<0.001) and for males ($F_{[1, 501]}$=3772.802, P<0.001).

**Bridled leatherjacket**

Figure 11. Length-weight relationships for female and male bridled leatherjacket (*Acanthaluteres spilomelanurus*) and their regression lines. Equation and $R^2$ is shown for each regression line.



**Bridled leatherjacket**

Figure 12. Length and weight for bridled leatherjacket (*Acanthaluteres spilomelanurus*) at four sites in Spencer Gulf. Error bars are 95% CI.

216

Rough leatherjacket

There was no sexual dimorphism with respect to length-weight relationships for rough leatherjacket, which showed an allometric growth pattern (Figure 13). The regression line was significant ($F_{[1, 739]}$=24522.385, P<0.001).

The mean length and weight as a function of site is shown in Figure 14. An ANCOVA with length as the dependent variable showed that it was not possible to obtain homogeneity of regression slopes because of site * log weight interaction ($F_{[5, 740]}$=2237.671, P<0.001, partial eta squared = 0.938), thus violating the assumptions for an ANCOVA. The results suggest that variations in the length-weight relationships were too large to detect any differences between sites. This result is likely to be because only a few rough leatherjackets were caught at Site 5. However, these individuals were much larger than elsewhere.

Toothbrush leatherjacket

In the field, the sexes of toothbrush leatherjacket can be morphologically easily separated where males tend to be larger than females. Figure 15 shows length-weight relationships for both females and males, and Figure 16 shows the mean length and weight for the two sexes as a function of site.

An ANCOVA with length as the dependent variable showed that it was not possible to obtain homogeneity of regression slopes because of sex * log weight interaction ($F_{[1, 532]}$=77.682, P<0.001, partial eta squared 0.132) and site * log weight interactions ($F_{[4, 175]}$=9.271, P<0.001, partial eta squared 0.068) thus violating the assumptions for an ANCOVA. The results suggest that variations in the length-weight relationships as a function of sex and site were too large to detect any differences. Toothbrush leatherjacket showed allometric growth patterns for both sexes. The regression line was significant for females ($F_{[1, 310]}$ = 5269.10, P<0.001) and for males ($F_{[1, 220]}$ = 5055.906, P<0.001).

## Rough leatherjacket



$$y = 0.0001x^{2.7468,} \; R^2 = 0.9708$$

Figure 13. Length-weight relationships for female and male rough leatherjacket (*Scorbinichthys granulatus*) and their regression lines. Equation and $R^2$ is shown for each regression line.

## Rough leatherjacket



Figure 14. Length and weight for rough leatherjacket (*Scorbinichthys granulatus*) at five sites in Spencer Gulf. Error bars are 95% CI.

## Toothbrush leatherjacket



Figure 15. Length-weight relationships for female and male toothbrush leatherjacket (*Acanthaluteres vittiger*) and their regression lines. Equation and $R^2$ is shown for each regression line.

## Toothbrush leatherjacket



Figure 16. Length and weight for toothbrush leatherjacket (*Acanthaluteres vittiger*) at five sites in Spencer Gulf. Error bars are 95% CI.

219

Degens leatherjacket

The length-weight relationship and regression lines for both female and male Degens leatherjacket are shown in Figure 17 and the mean length and weight for the two sexes as a function of site is shown in Figure 18. Degens leatherjacket shows sexual dimorphism where males are longer than females for a given weight.

A two-way ANCOVA with sex and site as the main factors and the dependent variable length and with the covariate log weight showed a significant effect of site ($F_{[3, 175]}=8.901$, P<0.001, partial eta squared = 0.139) and sex ($F_{[1, 175]}=93.457$, P<0.001, partial eta squared = 0.360) with no significant interactions ($F_{[3, 175]}=0.905$, P=0.440, partial eta squared = 0.016). Homogeneity of regression slopes was obtained by square root transformation but not for the variances. There was a strong relationship between the dependent variable length and the covariate log weight ($F_{[1, 175]}=792.539$, P<0.001, partial eta squared = 0.827). These results show that length and weight relationships are different between sexes and different between sites for both sexes. No Degens leatherjackets were found at Site 1. The mean length was larger at Site 5 than elsewhere. Degens leatherjackets showed allometric growth pattern. The regression line was significant for females ($F_{[1, 144]} = 707.339$, P<0.001) and for males ($F_{[1, 29]} = 148.032$, P<0.001).

Pygmy leatherjacket

Pygmy leatherjackets were only found in small numbers at all sites with the exception of Site 5, where none were found. Length-weight relationships for females and males are shown in Figure 19 and the mean length and weight as a function of site is shown in Figure 20.

An ANCOVA with length as the dependent variable showed that it was not possible to obtain homogeneity of regression slopes because of site * log weight interactions ($F_{[4, 77]} = 231.015$, P<0.001, partial eta squared 0.928) thus violating the assumptions for an ANCOVA. The results suggest that variations in the length-weight relationships as a function of site were too large to detect any differences (Figure 20). Pygmy leatherjacket showed allometric growth pattern. The regression line was significant ($F_{[1, 76]} = 1172.923$, P< 0.001).

## Degens leatherjacket



Figure 17. Length-weight relationships for female and male Degens leatherjacket (*Thamnaconus degeni*) and their regression lines. Equation and $R^2$ is shown for each regression line.

## Degens leatherjacket



Figure 18. Length and weight for Degens leatherjacket (*Thamnaconus degeni*) at four sites in Spencer Gulf. Error bars are 95% CI.

221

## Pygmy leatherjacket



$$y = 0.0002x^{2.778}, R^2 = 0.9421$$

Figure 19. Length-weight relationships for female and male pygmy leatherjacket (*Brachaluteres jacksonianu*) and their regression lines. Equation and $R^2$ is shown for each regression line.

## Pygmy leatherjacket



Figure 20. Length and weight for pygmy leatherjacket (*Brachaluteres jacksonianu*) at four sites in Spencer Gulf. Error bars are 95% CI.

*Age determination*

Age determination by examination of sagittal otolith pairs was only undertaken for Degens leatherjacket. The results of the CAF examination of 34 otholits are presented in Appendix II. Despite an initial examination suggesting the possibility of estimating the age from saggital otolith pairs, CAF found that age estimates were essentially indeterminate and that the relationships between increment count and fish length /otolith weight were poorly correlated. Further investigation using tag recapture or otolith marking (oxytetracycline or strontium chloride) is needed to resolve the issues.

**Leatherjacket sex ratio**



Figure 21. Proportions of females of four species of leatherjackets at five sites in Spencer Gulf. Line indicates 1:1 sex ratio. Error bars are 95% CI.

*Sex Ratio*

All species of leatherjackets showed sexual dimorphism with the male larger than the female with the exception of rough leatherjacket where the sexes could not be separated by size. Figure 21 shows the proportion of females for bridled, Degens, pygmy and toothbrush

leatherjackets sampled during the October 2003 survey. A one-way ANOVA with site as the main factor conducted for the individual species on arcsine transformed data showed no effect of site (bridled: $F_{[3, 25]}=0.692$, P=0.566, partial eta squared = 0.077; Degens: $F_{[3, 25]}=0.106$, P=0.955, partial eta squared = 0.015; toothbrush: $F_{[4, 34]}=0.819$, P=0.522, partial eta squared = 0.088; pygmy: $F_{[3, 13]}=2.908$, P=0.094, partial eta squared = 0.492). The results showed that the proportion of females in the studied populations was independent of site of occurrence. For each species, $\chi^2$-tests were performed to test for deviation from a 1:1 female-male ratio. The results showed that the populations of bridled, pygmy and toothbrush leatherjackets did not deviate from a 1:1 ratio (bridled: $\chi^2 = 58.7836$, $\chi^2_{Crit.}=7\,7.931$, df = 5 7, P>0.05; pygmy: $\chi^2 = 19.4825$, $\chi^2_{Crit.}= 37.652$, df = 25, P>0.05; toothbrush: $\chi^2 = 62.8206$, $\chi^2_{Crit.}= 9\,8.484$, df = 77, P>0.05) while Degens leatherjacket did ($\chi^2 = 93.2544$, $\chi^2_{Crit.}= 66.339$, df = 49, P<0.001). The results showed that females consistently dominate in the populations of Degens leatherjacket across the sites of occurrence, while the sex ratios did not differ from a 1:1 ratio in the other three species.

**Blue crabs**

*Biomass and Abundance*

Figures 22 and 23 show the distribution of blue crab biomass as a function of site for the three trawl surveys (Oct 03, Oct 04 and Jan 05), and for day and night. A three-way ANOVA on square root transformed data with site, time of day (day/night) and survey as the main factors was performed. It was not possible to obtain homogeneity of variances by transformation partly because of many zeros in the data. Normality was best obtained by a square root transformation and because ANOVA's are usually robust against heterogeneous variances, the analysis was carried out regardless. A significant effect of site ($F_{[4, 118]}=27.692$, P<0.001, partial eta squared = 0.557), time of day $F_{[1, 118]}=84.399$, P<0.001, partial eta squared = 0.490), and survey ($F_{[2, 118]}=17.432$, P<0.001, partial eta squared = 0.284) was found. Significant interactions for all factors were evident. The significant interactions are caused by the large variations between surveys (Figure 22). In particular, the Oct 03 survey was different from Oct 04 and Jan 05 because of low biomass and no crabs were caught at Site 5. Therefore, two-way ANOVA's were carried out for the individual surveys excluding the Oct 03 survey.

The results of a two-way ANOVA on data with non-homogeneous variances from the Oct 04 survey showed a significant effect of site ($F_{[4, 40]}= 0.048$, P<0.001, partial eta squared = 0.573)

and time of day $(F_{[1, 40]}=3\ 2.608, P<0.001$, partial eta squared = 0.521) with no significant interactions $(F_{[4, 40]}= 1.680, P = 0.181$, partial eta squared = 0.183). A Dunnett C test separated Site 4 from Sites 3 and 5. The results of a two-way ANOVA on data with non-homogeneous variances from the Jan 05 survey showed a significant effect of site $(F_{[4, 38]}= 14.834, P<0.001$, partial eta squared = 0.679) and time of day $(F_{[1, 38]}= 89.425, P<0.001$, partial eta squared = 0.762) with no significant interactions $(F_{[4, 38]}= 1.466, P = 0.239$, partial eta squared = 0.173). A Dunnett C test separated Site 3 from Site 4. The effect size of site and time of day for the Oct 04 and Jan 05 survey, respectively, were large giving a low probability for type I error. Accordingly, the overall results showed that blue crabs are less abundant at Site 3 than elsewhere. Blue crabs are likely to be found more abundant at night than during day, and that these effects were consistent among sites.

*Length-weight relationships*

Blue crabs show sexual dimorphism and with respect to carapace width-weight relationships both sexes showed an isometric growth pattern (Figure 24). The regression lines were significant for both females $(F_{[1, 58]}=146.513, P<0.001)$ and males $(F_{[1, 138]}=981.570, P<0.001)$.

The mean carapace width for both sexes as a function of site is shown in Figure 25 (only carapace width was measured in these samples). It was not possible to obtain homogeneity of variances by transformation thus violating the assumption for an ANOVA. However, because the data set is large and that ANOVA's are usually robust against this violation, a two-way ANOVA with site and sex as the main factors was nevertheless performed on untransformed data. The results showed a significant effect of site $(F_{[3, 932]}= 116.408, P<0.001$, partial eta squared = 0.274) with no effect of sex $(F_{[1, 932]}= 0.088, P=0.767$, partial eta squared = 0.000) and no significant interactions $(F_{[3, 932]}= 0.116, P = 0.951$, partial eta squared = 0.000). The effect size for site was large, giving a low probability of type I error. A Dunnett C test showed no significant difference between Sites 1 and 2 but these two sites were significantly different from Sites 3 and 4, which were significantly different from each other. The results showed that the mean size of crabs (CW) is dependent on site. Crabs are larger at Site 4 than any other site. Crabs at Site 3 are much smaller than elsewhere. This pattern was independent of sex. No crabs were caught at Site 5 during the October 2003 survey (8 trawls for 30 min each).

Figure 22. Biomass (g/100m$^2$) of blue crab (*Portunus pelagicus*) sampled at three occasions at five sites in Spencer Gulf. Error bars are 95% CI.



Figure 23. Biomass (g/100m$^2$) of blue crab (*Portunus pelagicus*) sampled at five sites during day and night in Spencer Gulf. Error bars are 95% CI.

**Blue crab**



Male: $y = 4.4993x - 278.44$, $R^2 = 0.8775$

Female: $y = 4.0138x - 247.17$, $R^2 = 0.7194$

◆ Male
□ Female

Figure 24. Carapace width-weight relationships for female and male blue crab (*Portunus pelagicus*) and their regression lines. Equation and $R^2$ is shown for each regression line.

**Blue crab**



● Female
○ Male

Figure 25. Carapace width of female and male blue crab (*Portunus pelagicus*) at four sites in Spencer Gulf. Error bars are 95% CI.

Figure 26. Proportions of females of blue crab (*Portunus pelagicus*) at four sites in Spencer Gulf. Line indicates 1:1 sex ratio (0.5 proportion of females). Error bars are 95% CI.

*Sex Ratio*

Blue crabs show sexual dimorphism in morphology and colour. Figure 26 shows the proportion of females sampled during the October 2003 survey at the four sites of occurrence. A one-way ANOVA with site as the main factor on arcsine transformed data showed no effect of site ($F_{[3, 32]}$=0.129, P=0.942). The results showed that the proportion of females in the studied populations was found to be independent of site. A $\chi^2$-test was performed to test for deviation from a 1:1 female-male ratio. The results showed that the populations of blue crab did not deviate from a 1:1 ratio ($\chi^2$ = 58.7836, $\chi^2_{Crit.}$= 82.529, df = 63, P<0.05).

**Trawl hours, habitat and biomass relationships**

Figures 27, 28 and 29 show the relationships between the relative proportion of biomass (%) of sand trevally, two dominat species of leatherjackets, blue crabs and trawl hours of the prawn fishery at five sites in Spencer Gulf during 2003 and 2004/05, respectively. The relative abundance of benthos as a measure of habitat complexity is included for the Oct 2004 and Jan 2005 samples only because benthos was not sampled in 2003. Sampling took place in October in 2003 and 2004 about four months after fishing stopped and

Figure 27. Relationships between annual trawl hours in the Spencer Gulf prawn fishery and the relative biomass (%) of sand trevally, two species of leatherjackets and blue crabs at five sites in Spencer Gulf in Oct 2003.



Figure 28. Relationships between annual trawl hours in the Spencer Gulf prawn fishery and benthos, and the relative biomass (%) of sand trevally, two species of leatherjackets and blue crabs at five sites in Spencer Gulf in Oct 2004.

**Jan 2005**



Figure 29. Relationships between annual trawl hours in the Spencer Gulf prawn fishery and benthos, and the relative biomass (%) of sand trevally, two species of leatherjackets and blue crabs at five sites in Spencer Gulf in Jan 2005.

Table II. Spearman's rank correlation (rho) between biomass of four quantitatively dominating by-catch species against trawl hours and benthos abundance at five sites in Spencer Gulf. Rho-values indicate the strength of the correlation ($\pm$0.10-0.29 = small, $\pm$0.30-0.49 = medium, and $\pm$0.50-1.0 = large). Values are highlighted for medium and large strength.  SAT = Sand trevally, RLJ = rough leatherjacket, DLJ = Degens leatherjacket and BC = blue crab.

|  | SAT | RLJ | DLJ | BC |
|---|---|---|---|---|
| Trawl hours | 0.064 | 0.248 | **-0.603** | 0.038 |
| Oct 2003 (N = 40) | P = 0.639 | P = 0.123 | P = 0.000 | P = 0.712 |
| Trawl hours | **-0.561** | **0.453** | **0.561** | -0.085 |
| Oct 2004 (N = 40) | P = 0.000 | P = 0.003 | P = 0.000 | P = 0.602 |
| Trawl hours | **-0.359** | -0.077 | **0.534** | **0.313** |
| Jan 2005 (N = 38) | P=0.027 | P = 0.644 | P = 0.001 | P = 0.056 |
| Benthos | 0.087 | 0.066 | -0.241 | **0.382** |
| Oct 2004 (N = 40) | P = 0.593 | P = 0.688 | P = 0.134 | P = 0.015 |
| Benthos | 0.093 | -0.296 | -0.271 | 0.015 |
| Jan 2005 (N = 38) | P = 0.577 | P = 0.071 | P = 0.100 | P = 0.929 |

| Benthos abundance and trawl hours Oct 2004 | rho = **-0.483**, P = 0.002 |
|---|---|
| Benthos abundance and trawl hours Jan 2005 | rho = **-0.535**, P = 0.001 |

230

furthermore in January 2005 after a pre-Christmas fishing period of 10 days. The results of Spearman Rank correlation analyses are shown in Table II. The Spearman's rho values provide a measure of similarity irrespective of significance level (see Pallant 2005) and have been found useful as a similarity measure of trawl samples (see Johnsen 2003). For the two surveys, Oct 2004 and Jan 2005 there was a moderate to large negative correlation between trawl hours and benthos abundance (Table II) where 23.3% and 28.6% of the variance, respectively, can be explained by the correlation. The results from the Oct 2003 samples showed a small to moderate correlation between trawl hours and the biomass of rough leatherjackets and a large negative correlation between the biomass of Degens leatherjacket (Table II, Figure 27). During this survey few leatherjackets were caught compared to the two other surveys (Figure 7) and no blue crabs were caught at Site 5. For the two surveys Oct 2004 and Jan 2005, sand trevally generally showed a moderate to large negative correlation to trawl hours and no correlation to the biomass of benthos. In contrast, leatherjacket biomass showed a moderate to large positive correlation to trawl hours and a small negative correlation to biomass of benthos. Blue crab biomass, however, were in Oct 2004 medium positively correlated to biomass of benthos and to trawl hours in Jan 2005 (Table II, Figures 28 and 29). The overall patterns of correlation showed that trawl hours explain the abundance patterns of sand trevally (non discard scavenger, negatively correlated), rough leatherjacket (discard scavenger, positively correlated), Degens leatherjacket (discard scavenger, positively correlated) and partly blue crab (discard scavenger, positively correlated). Biomass of benthos, as a measure of habitat complexity (see McCoy & Bell 1991), explains the abundance patterns of leatherjackets (negatively correlated  and habitat restricted) and partly blue crab (migratory, discard scavenger, positively correlated).

## Discussion

### Study sites

The five studied sites are subjected historically to different fishing effort and also constitute different environments (see Svane 2003). The bottom of Spencer Gulf is variable with sediment characteristics ranging from fine sand at Sites 1 and 2 (Western Shoal, Plank Point), hard coarse sand and gravel at Site 3 (Middle Bank), mixed sand and patches of gravel at Site 4 (Wallaroo) and sandy plains at Site 5 (the Gutter). At Sites 3 and 4 the coarse sediment grains are usually covered by so-called rhodoliths, which are calcium carbonate fragments of biological origin covered with coralline algae. The physical characteristics of the environment

are different in salinity, temperature and tidal currents. During the summer months, the two northern sites (1 & 2) experience slightly higher temperatures and salinity than further south (Bye 1981, Smith & Veeh 1989). Tidal currents are relatively stronger at Middle Bank and Wallaroo than elsewhere. Differences in habitats are therefore to be expected. There is a lack of baseline data on the habitats of Spencer Gulf, but the cumulative long-term effects of trawling are likely to be pronounced. The effects are likely to be on the ecosystem as a whole, particularly on the benthic habitat and by-catch species (Sparks-McConkey & Watling 2001).

**Sand trevally**

Sand trevally is a quantitatively dominant element of the by-catch of the Spencer Gulf prawn fishery, but little is known of the biology of the species. The species is not known to be a scavenger on discarded by-catch and is widely distributed throughout the gulf but more abundantly at Site 5 in "The Gutter" (Svane 2003). Abundance and distribution show temporal variation (Figure 1) but an effect of small sample sizes in combination with schooling behaviour cannot be ruled out. The results seem to indicate that sand trevally is more abundant during the day than at night at all five sites with the exception of Site 5. This pattern, although not statistically significant, may reflect differences in schooling behaviour between day and night, indicating that sand trevally disperse at night and school during the day. The biomass of sand trevally was highest at Site 5 but with large variability and indicates a habitat preference of plain sandy bottoms and the large variability is likely to be affected by schooling, which may be influenced by habitat complexity.

The mean weight and length of sand trevally was found to be different between sites and the mean size and weight were greater at Site 5, while small individuals were found at Site 3 (Figure 3). This pattern indicates that older schooling individuals were more common at Site 5 than elsewhere. Site 3, where the tidal currents are relatively strong, appears to favour smaller individuals.

This study shows that the prawn fishery is catching individuals ranging from 1 to 5 years of age, indicating that the effects of trawling are concentrated on a younger section of the population, which may affect the age structure (Rowling & Raines 2000, McShane *et al.* 1998). The von Bertalanffy fit, provided in Appendix I, shows that the $L_\infty$ (theoretically maximum length) of sand trevally is 110.5 cm. The low K value indicates a large size at age, a

large size at maturity and a low reproductive output (Jennings *et al.* 2001). The smallest mature individual in this study was 7.3 cm, and as sand trevally reach a maximum length of 22 cm (Hutchins & Swainston 1986) this is not consistent with the K value derived from the von Bertalanfy fit, probably because of the lack of larger individuals in the samples in this study. Rowling & Raines (2000) found that Silver trevally matured progressively, and can mature at very small sizes, as was observed in sand trevally. However, the sample analysed reflects the by-catch size range, and not the actual population size range.

The differences in GSI between sexes and site (Figure 6) largely reflect the differences between mean length and weight and site. However, males appeared to have larger GSI values than females, which may indicate competition between males because relatively less energy is allocated to reproduction compared to somatic tissue with no implication for the sex ratio (see Rowling & Raines 2000).

**Leatherjackets**

*Distribution and abundance*

Leatherjackets are a quantitatively important element in the by-catch of the Spencer Gulf prawn fishery, but little is known of the biology of the species. Rough leatherjackets, and particularly Degens leatherjackets, are known to be voracious scavengers on discarded by-catch (Svane 2003). Although the studied species can be found at all five sites, there were large differences in their relative abundance. The biomass of leatherjackets generally increased from Site 1 towards Site 5 (North to South of central Spencer Gulf). Degens leatherjackets were found abundantly at Site 5 (the Gutter) and only sparsely at other sites. Rough leatherjacket, however, is common at all sites with the exception of Site 5 where only a few individuals were found. The difference in distribution and abundance between these two scavenging species may be a result of competition for food. Variation in leatherjackets between trawls was pronounced, and is likely to be an effect of their schooling behaviour (Masuda & Tsukamoto 1999). Bridled leatherjacket, toothbrush leatherjacket and pygmy leatherjacket are also scavengers on discarded by-catch but not found abundantly (Figure 10).

Leatherjacket biomass was found to be larger at night than during the day at all five sites with the exception of Site 3 where the biomass was low. This pattern is likely to be a result of differences in schooling density between day and night affecting sampling rather than reflecting true differences in abundance. Svane (2003) has shown in so-called cafeteria

experiments that leatherjackets are primarily daytime feeders when schooling. The occurrence and abundance of these species at discarded by-catch during the night was found to be considerably less than during the day.

*Length and weight as a function of site*

Degens leatherjackets were found to increase in mean weight and length from Site 2 to Site 4 and 5 while bridled leatherjackets showed the opposite pattern. The length and weight of rough leatherjackets were not different between Site 1 to 4 but the few individuals caught at Site 5 were large. Site had no effect on size and weight of toothbrush leatherjackets and pygmy leatherjackets. This pattern may be because of differences in habitat preference, with leatherjackets moving from shallow to deeper waters of the gulf as they grow older. However, the pattern may also reflect food availability in combination with competition. Degens leatherjackets were dominating at Site 5 and rough leatherjacket at Site 4, while the other species were evenly distributed among sites 1 to 4 (Figure 10). This pattern of occurrence may indicate competition where heterogeneous, or complex, habitats will favour coexistence of more species than homogeneous habitats. The five selected sites are different in habitat heterogeneity and complexity. The two northern sites, Site 1 and 2, show high habitat heterogeneity and complexity, and consequently high diversity of leatherjackets but low in abundance. The habitat of Site 3 is relatively homogeneous with a hard coarse sandy bottom (high tidal currents) forming large ripples with rhodoliths (coralline algae) in between. This site has certain habitat heterogeneity where sponges and rocks found between the ripples provide refuge for mobile epifauna and dermersal fish species during periods of high tidal currents. At Site 3, which is intensively fished, trawls are likely to pass over the areas in between the ripples thus allowing some erect fauna to develop. Site 4 is sandy with elements of the habitats of Site 3 but without the characteristic ripples found at Site 3 and this site is also heavily trawled. In contrast, the habitat at Site 5 is relatively monotonous sandy bottoms where erect fauna is restricted to depressions in the sand, usually anchored to rocks and other hard substrata (see Svane 2003).

*Sex ratios and sexual dimorphism*

All the studied species of leatherjackets showed sexual dimorphism with the male larger than the female, with the exception of rough leatherjackets where the sexes could not be separated. The proportion of females in the studied populations was found to be independent of site of

occurrence. Females dominate consistently in the populations of Degens leatherjacket across all sites, while the sex ratios did not differ from a 1:1 ratio in the other three species.

In the species showing sexual dimorphism where the males are larger than the females, the pattern can be explained by protogynous sex change, where a change in sex from female to male occurs with age (sequential hermaphroditism). This change occurs because of a reproductive advantage, either because the males are exhibiting parental care and therefore could be more capable of defending a nest, or that males gain a higher fecundity over time (Jennings *et al.* 2001). In prawn trawling, by-catch mortality decreases with increasing individual size (see Chapter 5). Through the sorting process of the "Hopper", where larger species are discarded rapidly while smaller species are retained for longer time, the BRD or the mesh size and type of the cod end, size-dependent mortality is likely to occur. This process may impact on the population structure affecting the relative gender abundance and reproductive output.

*Age determination*

Age determination is important in most stock assessments in order to obtain knowledge of the age structure of an exploited population (Jennings *et al.* 2001). The age of teleosts is most commonly determined through rings laid down in the otoliths. In this study, Degens leatherjacket was used as a pilot species because Degens males were significantly larger than females, and there were fewer males than females, suggesting the occurrence of sequential hermaphroditism. However, it was not possible to determine age through an analysis of the otoliths (Appendix II). A future study on age determination would be necessary to understand the impact of trawling on the population dynamics of leatherjackets by applying alternative ageing techniques (see Dwyer *et al.* 2003, Jennings *et al.* 2001).

**Blue crabs**

*Distribution and abundance*

In addition to being an important component of the by-catch in the Spencer Gulf prawn fishery, blue crabs are also subjected to both a commercial and recreational fishery using pots and drop nets. Commercial and recreational fishers are not allowed to take ovigerous females and crabs smaller than 110 mm carapace width. The commercial annual estimated catch for Spencer Gulf is about 465 t. The magnitude of these fisheries is likely to affect population abundances and temporal variability (see Svane & Cheshire 2005, Svane & Hooper 2004).

Pot surveys conducted by Svane and Hooper (2004) and commercial trawl by-catch analyses (McShane *et al.* 1998), showed a gradual decline in biomass from north to south (Site 1 towards Site 5). The results of this study, using a single prawn trawl, support this finding showing that blue crab biomass varied with site with a decline in biomass from Site 1 to Site 3 and 5 but with high biomass at Site 4. However, this pattern was inconsistent between surveys. The variation between replicate trawls was high, reflecting a patchy distribution of crabs. Blue crabs are known to be highly mobile (Edgar 1990), and while usually benthic, they can swim in the water column using their 'swimming paddles' located on the fifth pair of legs. Indeed, Potter *et al.* (1991) reported a specimen that travelled 20 km in one day. There is also anecdotal evidence that adults can undergo mass migrations (e.g. Smith, 1978). Within South Australia there is a distinct seasonal pattern of adult crab migration, with animals moving into shallow inshore waters during the warmer months of September through to April and then retreating to deeper offshore waters during the colder months of May to August (Smith, 1982; Bryars & Havenhand 2006). Ovigerous female crabs move into the deeper offshore waters prior to the release of larvae (Smith, 1982).

The statistically significant pattern of day-night occurrence where more crabs were caught at night than during the day may indicate that crabs migrate during the night. Svane (2003) showed that crabs occurred at bait more often during the day than at night, but this was statistically significant only at Sites 3 and 4, the sites with the strongest tidal currents. The observations suggest that patterns of feeding and resting, where crabs bury in the sediment, are complex and likely to be affected by temperature, moulting, and reproduction, all factors that were not accounted for in this study.

*Length-weight as a function of site*
Few of the crabs caught during the October 2003 survey were above the legal limit of 110 mm (Figure 25). The samples at Site 3 contained smaller crabs than elsewhere, which can be explained by the habitat characteristics. On the other hand crabs were significantly larger at Site 4 than elsewhere. This pattern was independent of sex. The pattern was observed during the October 2003 survey, where all crabs were individually measured and the abundances were so low that in eight trawl transects, no crabs were caught at Site 5 (Figure 22). During the two other surveys only total weight was recorded. It is unclear how the differences in abundance have affected the mean size at the five sites.

**Trawl hours and biomass relationships**

The effects of trawling on a population or ecosystem level are dependent on the distribution and intensity of trawl effort (Thrush *et al.* 1995, Rijnsdorp *et al.* 1998). However, correlations with distribution and abundance may be intercorrelated with other variables such as habitat heterogeneity, complexity and hydrographical characteristics. Spearman Rank correlations can be used to give an overall view of correlation patterns because there is no assumption of linearity and the technique is insensitive to proportional and additive differences between samples (Krebs 1999). These correlations are not conclusive but tentative and require further studies to be substantiated. The Spearman rho values should be interpreted as a measure of the strength and direction of the relationship, as indicated in Table III, and the significance levels should be treated cautiously due to the strong influence of sample size (see Cohen 1988, Pallant 2005). Spearman Rank correlations have been found to be useful as a similarity measure of trawl samples (see Johnsen 2003). The Spearman Rank correlation analyses showed that biomass of rough leatherjacket and Degens leatherjacket were consistently positive correlated to trawl hours with up to 31.5% of the variance explained by trawl hours (Table II). If discarded by-catch constitutes a major food source of these scavengers, a positive correlation could be expected, but affected negatively by the risk of being caught as by-catch. The negative correlation of Degens leatherjacket with trawl hours for the Oct 2003 survey can be explained by the small numbers caught and the preference for sandy habitats. Indeed, a negative correlation with habitat heterogeneity was found with only 6-7% of the variance explained by habitat complexity (Table II). Excluding the Oct 2003 survey, sand trevally showed a negative correlation with trawl hours with 13 to 31.5% of the variance explained by trawl hours. This result would be expected because this species is not a scavenger on discarded by-catch but a quantitatively important component of the by-catch with high to total mortality when discarded (Chapter 5). Sand trevally showed no correlation with habitat complexity, which can be explained by a pelagic existence. The correlation strength for blue crabs with trawl hours was medium for the Jan 2005 survey, where 9.8% of the variance could be explained by trawl hours. In contrast, the correlation strength with benthos (habitat complexity) for the Oct 2004 survey was also medium, with 14.6% of the variance explained by this variable. Considering that blue crabs are likely to be subjected to substantial by-catch mortality, but at the same time benefit from discards, a mixed correlation pattern would be expected.

The observed patterns may not show direct casual relationships because other factors can plausibly be attributed to the correlations. A major problem is that the five sites are very different environments, with different physical and biological characteristics, making it difficult to separate effects of trawling from natural variability, because studies of the ecosystem before fishing commenced have not been undertaken (Pitcher *et al.* 2000, Thrush *et al.* 1995). However, the observed patterns of abundance correlated to trawl hours and habitat complexity, represented by biomass of benthos, suggests that habitat affects abundance but the important correlation is with trawl hours. Trawl hours are in turn negatively correlated to habitat complexity (benthos biomass), which explains 23.3% of the variance for the Oct 2004 survey and 28.6% for the Jan 2005 survey.

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# Appendix I: Preliminary Age investigation of sand trevally (*Pseudocaranax wrighti*)

Final Report to the
South Australian Research and Development Institute

Simon Robertson

**November 2003**

| Preliminary age estimation of sand trevally (*Pseudocaranax wrighti)* |
|---|

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**Summary**

Kate Rodda from the South Australian Research and Development Institute (SARDI) approached the Central Ageing Facility (CAF), Marine and Freshwater Systems, to provide annual age estimates of sand trevally *(Pseudocaranax wrighti)*. A total of 47 sagittal otoliths collected from sand trevally were sent for age estimation. Age estimates were determined by counting increments structure on the distal face of the otolith from the primordium to the posterior margin. Estimates revealed that, based on the sample size, sand trevally were relatively short lived. Ages ranged from 1 to 5 years. Repeated readings of otoliths from this species indicate a relatively high level of precision.

**Methods**

*Samples*

A total of 47 sand trevally sagittal otolith pairs were forwarded to the CAF for age estimation. Otoliths were stored dry in numbered envelopes with accompanying station and fish numbers. Biological details, including length and sex data were provided. The samples arrived at the CAF and were registered in October 2003. Samples were allocated a unique identification code and registered according to Morison *et al* (1998). Either the left or right otolith was weighed to the nearest 0.0001 grams, on the assumption that there was no significant difference between left and right otoliths.

*Otolith preparation*

An initial examination of the sand trevally otoliths indicated that age estimates were possible when the otoliths were viewed using reflected light under a dissecting microscope. One otolith was embedded in crystal bond and transversely ground to determine whether estimates from whole estimates approximated those obtained from sectioned otoliths.

*Reading Protocol*

Sections were viewed using a dissecting microscope at a magnification of 15.75x (25x primary and 0.63x secondary objective) illuminated with reflected light. Before attempting to assign age estimates the reader first became familiar with otolith structure by making a preliminary examination of all the samples. Age was estimated by counting the number of complete zones (translucent – opaque sequence) on a transect from the biological center (primordium) of the otolith to the posterior edge of the otolith on the distal surface. A customised image analysis system (Morison *et al.* 1998) was used to mark and count increments along the ageing transect. The image analysis system was also used to measure the distance from the primordium to each of the increments. As no date of capture data or spawning date information was available, no age adjustment to zone count was undertaken. The otolith margin was classified as wide in all cases. Other information recorded in the Excel spreadsheet was a readability score. This is a subjective measure of the sample's readability based on the clarity of the increments (Table 1). To avoid potential bias, all counts were made without knowledge of otolith weight or fish length.

Table 1. Interpretation of readability scores

| Score | Interpretation |
|-------|----------------|
| 1 | Sample has excellent readability, increments exceptionally clear |
| 2 | Sample is unambiguous but not as clear as 1 |
| 3 | Sample may be subject to two interpretations |
| 4 | Sample is subject to multiple interpretations |
| 5 | Sample is unreadable |

*Comparison of age estimates*

Repeated readings of the same otoliths provide a measure of intra-reader variability. They do not validate the assigned ages but provide an indication of the size of the error to be expected with a set of age estimates, due to variation in otolith interpretation. Beamish and Fournier (1981) have developed an index of average percent error (IAPE), which has become a common method for quantifying this variation. The IAPE is calculated as:

$$ IAPE = \frac{100}{N} \sum_{j=1}^{N} \left[ \frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j} \right] $$

Where $N$ is the number of fish aged, $R$ is the number of times fish are aged, $X_{ij}$ is the $i$th determination for the $j$th fish, and $X_j$ is the average estimated age of the $j$th fish. The index has the property that differences in age estimates for younger fish will contribute more to the final value than will the same absolute error for older fish (Anderson *et al.* 1992).

To establish confidence intervals to these estimates of precision, a bootstrap technique was employed on the individual error estimates following methods described by Efron and Tibshirani (1993). Five hundred samples of error estimates (each the same size as the original) were randomly taken with replacement from the repeat readings, and a new IAPE calculated for each. The mean of these replicate IAPE's is the mean bootstrap IAPE. The bootstrap procedure exaggerates any bias present in the original estimate, so it is necessary to correct for this by adding the difference between the original statistic and the bootstrap mean, to the original estimate. The bias-corrected bootstrapped IAPE is thus calculated as:

Bias-corrected IAPE = Original IAPE + (Original IAPE- Mean Bootstrap IAPE)


The 95% confidence interval was calculated as:

95 % C.I. = Bias-corrected IAPE ± (1.96* Standard deviation of Mean Bootstrap IAPE)

According to CAF protocol, a minimum of 25% of samples was re-read by the same reader. Precision estimates were compared with the acceptable level of agreement between readings (Morison *et al.* 1998). The distribution of differences between repeat readings was also inspected as another indicator of ageing error, and bias between readings.

**Results**

Whole otoliths from sand trevally displayed relatively clear increments from the primordium to the edge, these were visible on both the posterior and anterior planes on the distal face (Figure 1). The average readability score was 2.94.



Figure 1. Whole otolith from a sand trevally viewed with reflected light. White circles indicate position of presumed annual increments. D = Dorsal, V = Ventral, A = Anterior, P= Posterior and R = rostrum. Sample from 13.7 cm female, Station 5.

Age estimates were attained for all samples. Age classes from 1+ to 5+ years were present in the sample. The modal age class was 3+ with the next most dominant age class being 4 years (Figure 2).

Figure 2. Age frequency distribution of sand trevally.

The distribution between otolith weight and age is depicted in Figure 3. The figure indicates that otolith weights are lineally related to zone count ($R^2$=0.696, y=0.0008 * zone count + 0.0009).



Figure 3. Otolith weight zone count relationship for sand trevally (n=47)

A total of 13 samples were re-aged to determine the level of precision surrounding the estimates. The bias corrected bootstrap mean IAPE was 4.76% (SD 2.74%). The modal difference between the first and second age was zero, while the maximum difference between first and second estimates was one year (Table 2).

Table 2.  Frequency differences between first and second age estimate

| Difference | Frequency |
|---|---|
| -1 | 1 |
| 0 | 10 |
| 1 | 2 |

Von Bertalanffy growth curves were fitted to the combined fish length zone count relationships using Microsoft Excel.  Numbers from male and females separately were considered insufficient to determine growth curve parameters. These results are shown in Table 3.  The relationship between fish length and zone count is shown in Figure 4.

Table 3.  Von Bertalanffy growth curve parameters.

| Sex | $L_{infinity}$ | k | $T_{zero}$ |
|---|---|---|---|
| Combined | 110.5 | 0.001 | 0.10 |



Figure 4.  Fish length zone count relationship (n=47)

Data collected through this study is presented in Tables 4 and 5.

**Discussion**

This report constitutes a preliminary study on the age and growth of sand trevally. Initial results indicate that the samples provided are representative of a short-lived species. It should be noted that these results are invalidated for this species. Incremental structure and morphological characteristics were similar to species previously aged at the CAF. The distribution between otolith weight and age was relatively linear for this species. This suggests that unlike fish length, otoliths continue to grow as the fish age. The index of average percent error (IAPE) was 4.76%, which indicated relatively good precision between repeated estimates. A thin transverse section of a larger sample produced results, which were consistent with that obtained from the whole otolith.

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Table 4.

| CAF Species Code | CAF Batch | Fish Num. | Fish Length | Ot. Weight | Sex | Station | SARDI num. | Specimen | Zone_1 | Zone_2 | Zone_3 | Zone_4 | Zone_5 | Edge | Readability | Age |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 403 | 1 | 1 | 11.6 | 0.0039 | 2 | 1 | 1 | 1 | 0.610377 | 0.814281 | 1.01819 | 1.33218 | | 1.44226 | 4 | 4 |
| 403 | 1 | 2 | 12.2 | 0.0033 | 1 | 1 | 2 | 2 | 0.726732 | 1.13272 | 1.29289 | | | 1.41184 | 3 | 3 |
| 403 | 1 | 3 | 12 | 0.0044 | 1 | 1 | 3 | 3 | 0.53632 | 0.823032 | 1.11649 | 1.31972 | | 1.43862 | 3 | 4 |
| 403 | 1 | 4 | 10 | 0.0031 | 2 | 1 | 4 | 4 | 0.630735 | 1.08506 | | | | 1.34153 | 3 | 2 |
| 403 | 1 | 5 | 9.4 | 0.0025 | 2 | 1 | 5 | 5 | 0.559654 | 0.966109 | 1.22039 | | | 1.38137 | 3 | 3 |
| 403 | 1 | 6 | 9.7 | 0.0027 | 2 | 1 | 6 | 6 | 0.834503 | 1.13491 | 1.40455 | | | 1.51102 | 2 | 3 |
| 403 | 1 | 7 | 8.3 | 0.0022 | 2 | 1 | 7 | 7 | 0.686443 | | | | | 1.00853 | 3 | 1 |
| 403 | 1 | 8 | 8.4 | 0.0022 | 2 | 1 | 8 | 8 | 0.560632 | 0.823835 | 1.03587 | | | 1.19602 | 3 | 3 |
| 403 | 1 | 9 | 8.6 | 0.0025 | 1 | 1 | 9 | 9 | 0.702061 | 0.99971 | | | | 1.20969 | 3 | 2 |
| 403 | 2 | 1 | 8.9 | 0.0021 | 2 | 2 | 1 | 1 | 0.717098 | 0.970153 | | | | 1.1798 | 3 | 2 |
| 403 | 2 | 2 | 8.3 | 0.0017 | 2 | 2 | 2 | 2 | 0.692249 | | | | | 1.07169 | 3 | 1 |
| 403 | 2 | 3 | 8.7 | 0.0023 | 2 | 2 | 3 | 3 | 0.599792 | 0.874581 | | | | 1.08374 | 3 | 2 |
| 403 | 2 | 4 | 10.3 | 0.0027 | 1 | 2 | 4 | 4 | 0.632839 | 0.968108 | 1.29501 | | | 1.43166 | 3 | 3 |
| 403 | 2 | 5 | 10.4 | 0.0038 | 2 | 2 | 5 | 5 | 0.666155 | 0.924287 | 1.1115 | 1.35691 | | 1.49601 | 3 | 4 |
| 403 | 2 | 6 | 10.8 | 0.0037 | 1 | 2 | 6 | 6 | 0.643823 | 1.02564 | 1.49171 | | | 1.64429 | 1 | 3 |
| 403 | 2 | 7 | 12.1 | 0.0048 | 1 | 2 | 7 | 7 | 0.553449 | 0.993893 | 1.33618 | 1.47903 | | 1.58877 | 2 | 4 |
| 403 | 2 | 8 | 12 | 0.0037 | 1 | 2 | 8 | 8 | 0.627597 | 1.11014 | 1.40724 | | | 1.53435 | 3 | 3 |
| 403 | 2 | 9 | 12.1 | 0.0044 | 2 | 2 | 9 | 9 | 0.711993 | 0.948877 | 1.25443 | 1.4829 | 1.59327 | 1.67815 | 3 | 5 |
| 403 | 3 | 1 | 7.6 | 0.0018 | 1 | 3 | 1 | 1 | 0.723866 | 1.00353 | | | | 1.1476 | 3 | 2 |
| 403 | 3 | 2 | 8.2 | 0.0022 | 1 | 3 | 2 | 2 | 0.564032 | 0.96297 | | | | 1.11484 | 4 | 2 |
| 403 | 3 | 3 | 8.2 | 0.002 | 2 | 3 | 3 | 3 | 0.708385 | 1.09122 | | | | 1.25313 | 4 | 2 |
| 403 | 3 | 4 | 10.1 | 0.0034 | 2 | 3 | 4 | 4 | 0.598469 | 0.928395 | 1.3174 | | | 1.44784 | 1 | 3 |
| 403 | 3 | 5 | 10 | 0.0028 | 1 | 3 | 5 | 5 | 0.636384 | 1.02758 | 1.36828 | | | 1.50022 | 3 | 3 |
| 403 | 3 | 6 | 10.1 | 0.0036 | 1 | 3 | 6 | 6 | 0.73245 | 1.08806 | 1.39111 | 1.60241 | | 1.66405 | 3 | 4 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 403 | 3 | 7 | 12.5 | 0.0045 | 2 | 3 | 7 | 7 | 0.802878 | 1.19317 | 1.49259 | 1.61745 | | 1.68487 | 3 | 4 |
| 403 | 3 | 8 | 13.3 | 0.0048 | 2 | 3 | 8 | 8 | 0.693264 | 1.0489 | 1.41623 | 1.58411 | 1.64891 | 1.70209 | 4 | 5 |
| 403 | 3 | 9 | 12.8 | 0.0047 | 2 | 3 | 9 | 9 | 0.696569 | 1.10591 | 1.46847 | | | 1.62311 | 3 | 3 |
| 403 | 4 | 1 | 9.3 | 0.003 | 2 | 4 | 1 | 1 | 0.565905 | 0.957876 | 1.19743 | | | 1.32445 | 3 | 3 |
| 403 | 4 | 2 | 9.6 | 0.0029 | 1 | 4 | 2 | 2 | 0.453235 | 0.825972 | 1.12533 | | | 1.20542 | 3 | 3 |
| 403 | 4 | 3 | 9.4 | 0.0031 | 2 | 4 | 3 | 3 | 0.702328 | 1.11102 | | | | 1.26203 | 4 | 2 |
| 403 | 4 | 4 | 12.4 | 0.0044 | 1 | 4 | 4 | 4 | 0.627723 | 0.962019 | 1.20523 | 1.35153 | 1.47814 | 1.5774 | 2 | 5 |
| 403 | 4 | 5 | 11.5 | 0.0045 | 1 | 4 | 5 | 5 | 0.5922 | 0.931276 | 1.26314 | 1.46088 | | 1.56276 | 3 | 4 |
| 403 | 4 | 6 | 9.9 | 0.003 | 1 | 4 | 6 | 6 | 0.652449 | 1.05291 | | | | 1.28099 | 3 | 2 |
| 403 | 4 | 7 | 10.4 | 0.0035 | 1 | 4 | 7 | 7 | 0.660647 | 1.05964 | 1.30513 | | | 1.48318 | 4 | 3 |
| 403 | 4 | 8 | 10.4 | 0.0038 | 1 | 4 | 8 | 8 | 0.695 | 1.05101 | 1.34774 | 1.49174 | | 1.61034 | 3 | 4 |
| 403 | 4 | 9 | 10.4 | 0.0034 | 1 | 4 | 9 | 9 | 0.460449 | 0.888087 | 1.1764 | | | 1.32989 | 3 | 3 |
| 403 | 5 | 1 | 11.1 | 0.0038 | 2 | 5 | 1 | 1 | 0.739984 | 1.10027 | 1.3967 | | | 1.57897 | 2 | 3 |
| 403 | 5 | 2 | 11.4 | 0.0029 | 2 | 5 | 2 | 2 | 0.679685 | 0.96137 | 1.26151 | 1.45803 | | 1.60155 | 3 | 4 |
| 403 | 5 | 3 | 11 | 0.0035 | 1 | 5 | 3 | 3 | 0.561401 | 1.07151 | 1.295 | 1.48986 | | 1.62859 | 3 | 4 |
| 403 | 5 | 4 | 12.7 | 0.0043 | 1 | 5 | 4 | 4 | 0.647804 | 1.16786 | 1.51341 | | | 1.70331 | 4 | 3 |
| 403 | 5 | 5 | 12.6 | 0.0047 | 2 | 5 | 5 | 5 | 0.535438 | 0.896132 | 1.18517 | 1.39131 | 1.60145 | 1.68921 | 2 | 5 |
| 403 | 5 | 6 | 12.6 | 0.0045 | 2 | 5 | 6 | 6 | 0.577476 | 0.896965 | 1.11803 | 1.36095 | 1.5436 | 1.6098 | 3 | 5 |
| 403 | 5 | 7 | 13.7 | 0.006 | 1 | 5 | 7 | 7 | 0.703778 | 1.08635 | 1.37065 | 1.56165 | 1.76126 | 1.85457 | 3 | 5 |
| 403 | 5 | 8 | 13.8 | 0.0055 | 1 | 5 | 8 | 8 | 0.601038 | 0.864673 | 1.17428 | 1.45141 | 1.57995 | 1.6891 | 3 | 5 |
| 403 | 5 | 9 | 13.7 | 0.0048 | 2 | 5 | 9 | 9 | 0.993221 | 1.39223 | 1.69093 | 1.89265 | | 2.02522 | 3 | 4 |
| 403 | 5 | 10 | 14.8 | 0.005 | 2 | 5 | | 10 | 0.825787 | 1.22659 | 1.53612 | 1.76852 | | 1.85413 | 3 | 4 |
| 403 | 5 | 11 | 14.6 | 0.0051 | 1 | 5 | | 11 | 0.63974 | 1.01604 | 1.32829 | 1.49018 | 1.61652 | 1.78329 | 3 | 5 |

Table 5.

| Age | weight | Fish Length | Fish Wt | Readability | Comments |
|---|---|---|---|---|---|
| 8 | 0.0003 | 84 | 15.55 | 4 | Diffuse |
| 12 | 0.0003 | 110 | 33.18 | 3 | Lost first otolith in grind, second shows clear increments in sulcus |
| 16 | 0.0007 | 116 | 41.23 | 4 | Diffuse, used sulcus at 100 x |
| 13 | 0.0003 | 79 | 16.58 | 4 | |
| 13 | 0.0002 | 83 | 17.45 | 4 | Not ground, |
| 9 | 0.0004 | 74 | 14.81 | 4 | Very dark otolith, through sulcus |
| 0 | 0.0005 | 80 | 16.46 | 5 | Calcium resorption otolith, lots of lines, no annual information |
| 0 | 0.0003 | 81 | 19.57 | 5 | Failed prep |
| 11 | 0.0007 | 108 | 29.19 | | Surface ground - diffuse outer |
| 0 | 0.0006 | 104 | 26.34 | 5 | Failed prep |
| 12 | 0.0004 | 115 | 32.32 | | Surface groound |
| 15 | 0.0007 | 112 | 31.04 | 5 | Failed prep |
| 7 | 0.0006 | 82 | 17.38 | 4 | Surface ground, aged through sulcus (100x) |
| 11 | 0.0011 | 86 | 26.57 | 4 | SG- diffuse, maybe older, dailies?? |
| 7 | 0.0005 | 80 | 19.61 | 3 | Good grind |
| 10 | 0.0005 | 91 | 28.83 | 4 | |
| 15 | 0.0003 | 74 | 16.15 | 3 | Good grind, hard otolith |
| 11 | 0.0006 | 113 | 34.42 | 4 | |
| 7 | 0.0004 | 86 | 20.2 | 4 | Reground and new crystal bond |
| 13 | 0.0003 | 86 | 19.35 | 4 | |
| 8 | 0.0005 | 84 | 18.95 | 4 | Multiple grinds |
| 6 | 0.0003 | 73 | 13.57 | 4 | Very translucent |
| 8 | 0.0004 | 83 | 19.19 | 4 | Maybe older |
| 8 | 0.0007 | 91 | 26.07 | 4 | |
| 9 | 0.0006 | 90 | 26.39 | 4 | Left the otolith unground, check it out |
| 0 | 0.0011 | 103 | 37.08 | 5 | Could not even guess! |
| 14 | 0.0007 | 148 | 79.81 | 4 | Light ground, |
| 7 | 0.0007 | 105 | 22.4 | 4 | Not ground, nearly as clear as ground sample |
| 8 | 0.0006 | 97 | 23.01 | 4 | |
| 14 | 0.0009 | 121 | 39.15 | 4 | Diffuse edge, not ground |
| 12 | 0.0003 | 116 | 34.15 | 3 | Probably the clearest of the samples |
| 13 | 0.0003 | 84 | 23.95 | 3 | Clear inner, edge diffuse |
| 11 | 0.0003 | 80 | 15.82 | 3 | Clear sample (not ground) |
| 0 | 0.0006 | 106 | 28.31 | 5 | Tip too diffuse to guess |
| 7 | 0.0004 | 82 | 20.53 | 4 | Maybe older |
| 11 | 0.0004 | 91 | 27.99 | 4 | |

# Appendix II: Preliminary Age investigation of Degens leatherjacket (*Thamnaconus degeni*)

Final Report to the

South Australian Research and Development Institute

Simon Robertson

November  2003

| Preliminary age estimation of Degens leatherjackets (*Thamnaconus degeni)* |

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**Summary**

Kate Rodda from the South Australian Research and Development Institute (SARDI) approached the Central Ageing Facility (CAF), Marine and Freshwater Systems, to provide annual age estimates of Degens leatherjackets. A total of 36 sagittal otoliths collected from Degens leatherjackets were sent for age estimation. Despite an initial examination suggesting the possibility of estimating the age from an initial preparation and viewing of a transverse section and whole sample, age estimates were essentially indeterminate.  Samples were viewed whole, then the distal face was surface ground to increase the clarity of the structure and re-examined.  Counts were made on the face of the ground otolith.  The relationship between increment count and fish length / otolith weight were poorly correlated. The otoliths had relatively clear increments in the inner area of the otolith, the outer third of the otoliths however is very translucent making identification of zones difficult.  Further, the pattern of increments is open to multiple interpretations.  The samples submitted to the CAF maybe 2-3 years, 7-16 years or maybe even 0+ samples.  Further investigation using tag recapture or otolith marking (oxytetracycline or strontium chloride) is needed to resolve these issues.

**Methods**

*Samples*

A total of 36 Degens leather jacket sagittal otolith pairs were forwarded to the CAF for age estimation. Otoliths were stored dry in numbered envelopes with accompanying station and fish numbers. Biological details, including length and sex data were provided. The samples arrived at the CAF and were registered in October 2003. Samples were allocated a unique identification code and registered according to Morison *et al* (1998). Either the left or right otolith was weighed to the nearest 0.0001 grams, on the assumption that there was no significant difference between left and right otoliths.

*Otolith preparation*

An initial examination of the Degens leatherjacket otoliths indicated that age estimates may be possible when the otoliths were viewed whole using reflected light under a dissecting microscope. Due to relatively small size of the otolith, the otolith was embedded in crystal bond on a glass slide. The samples were also viewed using transmitted light under a dissecting microscope, and transmitted light under a compound microscope at magnifications up to 100x. On examination of the otoliths, the primordial area of the otolith was ground using 1200 wet and dry sanding medium (wet) to reduce the thickness and increase the clarity of the inner zones.

*Reading Protocol*

Age was estimated by counting the number of complete zones (translucent – opaque sequence), on a transect from the biological center (primordium) of the otolith to the posterior edge of the otolith on the distal surface. Due to the difficult nature of the increments, it was not possible to use image analysis zone marking. The zones could only be viewed my adjusting fine focus through the depth of the sample and identifying the zones at different areas of the ground preparation. As no date of capture data or spawning date information was available, no age adjustment to zone count was undertaken. The estimated age, along with sample identification data was recorded in Excel. Comments regarding the estimate were also recorded in the spreadsheet. Other information recorded in the Excel spreadsheet was a readability score. This is a subjective measure of the sample's readability based on the clarity of the increments (Table 1). To avoid potential bias, all counts were made without knowledge of otolith weight or fish length.

Table 2.  Interpretation of readability scores

| Score | Interpretation |
|-------|----------------|
| 1 | Sample has excellent readability, increments exceptionally clear |
| 2 | Sample is unambiguous but not as clear as 1 |
| 3 | Sample may be subject to two interpretations |
| 4 | Sample is subject to multiple interpretations |
| 5 | Sample is unreadable |

*Comparison of age estimates*

No repeated age estimates were made on these samples to determine precision due to the ambiguity of the samples.

**Results**

Surface ground otoliths from Degens leatherjackets displayed ambiguous increments from the primordium to the edge.  Increments are visible throughout the sample and are visible on both the posterior and anterior planes on the distal face (Figure 1).  The translucent outer margins of the otolith are visible in Figure 1.   The average readability score was 4.0.



Figure 1. Whole otolith from a Degens leatherjacket viewed with transmitted light. Sample from 91 mm (SL) cm female, Station Wallaroo.

Age estimates were attained for all samples. Age classes from 1+ to 5+ years were present in the sample.  The modal age class was 3+ with the next most dominant age class being 4 years (Figure 2).



Figure 2. Zone count distribution of samples (n=36).  Note: zero represents unageable samples

The distribution between otolith weight and age is depicted in Figure 3. The figure indicates that otolith weights are poorly related to zone count ($R^2$=0.003, y = zone count + 0.0005).



Figure 3. Otolith weight zone count relationship for Degens leatherjackets

Von Bertalanffy growth curves were not fitted to the estimated age zone count relationship parameters. These results are shown in Table 3.  The relationship between fish length and zone count is shown in Figure 4.

Figure 4.  Fish length zone count relationship (n=31)

Data collected through this study is presented in Table 3.

**Discussion**

This report constitutes a preliminary study on the age and growth of Degens leatherjackets. Initial results indicate that the samples provided are extremely difficult to interpret. The estimates presented here are one of many possible interpretations.  Otolith morphology is similar to dory otoliths, from which estimates have been made.  These species may be able to be used to refine interpretation of the structure in the otoliths of Degens leatherjackets. Further investigation is needed into the longevity of the Degens leatherjackets and this species needs further study using otolith marking or tag recapture to determine the validity of estimates within the rage of possibilities provided in this report.  Further, fine increments, which resemble daily increments, are visible on the otoliths at magnifications of 100 or 500x. Examples of these structures are shown in the Figures below.

Image of sample Batch 002 fish 007. 15.75 x magnification. Following image shows increased magnification (400x). Boxes represents approximate field of view of the images below.

**References**

Morrison, A.K., Robertson, S.G., and Smith, D.C. (1998). An integrated production fish ageing system: quality assurance and image analysis. *North American Journal of Fisheries Management* 18: 587-5.

Table 3.

| Species | Batch | Fish num | Age | weight | Fish Length | Fish Wt | Trawl | Readability | Comments |
|---------|-------|----------|-----|--------|-------------|---------|-------|-------------|----------|
| 715 | 1 | 1 | 8 | 0.0003 | 84 | 15.55 | 6 | 4 | Diffuse |
| 715 | 1 | 2 | 12 | 0.0003 | 110 | 33.18 | 6 | 3 | Lost first otolith in grind, second shows clear increments in sulcus |
| 715 | 1 | 3 | 16 | 0.0007 | 116 | 41.23 | 6 | 4 | Diffuse, used sulcus at 100 x |
| 715 | 1 | 4 | 13 | 0.0003 | 79 | 16.58 | 6 | 4 | |
| 715 | 1 | 5 | 13 | 0.0002 | 83 | 17.45 | 3 | 4 | Not ground, |
| 715 | 1 | 6 | 9 | 0.0004 | 74 | 14.81 | 3 | 4 | Very dark otolith, through sulcus |
| 715 | 1 | 7 | 0 | 0.0005 | 80 | 16.46 | 3 | 5 | Calcium resorption otolith, lots of lines, no annual information |
| 715 | 1 | 8 | 0 | 0.0003 | 81 | 19.57 | 3 | 5 | Failed prep |
| 715 | 1 | 9 | 11 | 0.0007 | 108 | 29.19 | 3 | | Surface ground - diffuse outer |
| 715 | 1 | 10 | 0 | 0.0006 | 104 | 26.34 | 3 | 5 | Failed prep |
| 715 | 2 | 1 | 12 | 0.0004 | 115 | 32.32 | 2 | | Surface ground |
| 715 | 2 | 2 | 15 | 0.0007 | 112 | 31.04 | 2 | 5 | Failed prep |
| 715 | 2 | 3 | 7 | 0.0006 | 82 | 17.38 | 1 | 4 | Surface ground, aged through sulcus (100x) |
| 715 | 2 | 4 | 11 | 0.0011 | 86 | 26.57 | 1 | 4 | SG- diffuse, maybe older, dailies?? |
| 715 | 2 | 5 | 7 | 0.0005 | 80 | 19.61 | 1 | 3 | Good grind |
| 715 | 2 | 6 | 10 | 0.0005 | 91 | 28.83 | 1 | 4 | |
| 715 | 2 | 7 | 15 | 0.0003 | 74 | 16.15 | 1 | 3 | Good grind, hard otolith |
| 715 | 2 | 8 | 11 | 0.0006 | 113 | 34.42 | 8 | 4 | |
| 715 | 2 | 9 | 7 | 0.0004 | 86 | 20.2 | 4 | 4 | Reground and new crystal bond |
| 715 | 2 | 10 | 13 | 0.0003 | 86 | 19.35 | 4 | 4 | |
| 715 | 2 | 11 | 8 | 0.0005 | 84 | 18.95 | 4 | 4 | Multiple grinds |
| 715 | 3 | 1 | 6 | 0.0003 | 73 | 13.57 | 3 | 4 | Very translucent |
| 715 | 3 | 2 | 8 | 0.0004 | 83 | 19.19 | 3 | 4 | Maybe older |
| 715 | 3 | 3 | 8 | 0.0007 | 91 | 26.07 | 3 | 4 | |
| 715 | 3 | 4 | 9 | 0.0006 | 90 | 26.39 | 4 | 4 | Left the otolith unground, check it out |
| 715 | 3 | 5 | 0 | 0.0011 | 103 | 37.08 | 4 | 5 | Could not even guess! |
| 715 | 3 | 6 | 14 | 0.0007 | 148 | 79.81 | 4 | 4 | Light ground, |
| 715 | 3 | 7 | 7 | 0.0007 | 105 | 22.4 | 4 | 4 | Not ground, nearly as clear as ground sample |

| 715 | 3 | 8 | 8 | 0.0006 | 97 | 23.01 | 4 | 4 | |
|---|---|---|---|---|---|---|---|---|---|
| 715 | 3 | 9 | 14 | 0.0009 | 121 | 39.15 | 6 | 4 | Diffuse edge, not ground |
| 715 | 3 | 10 | 12 | 0.0003 | 116 | 34.15 | 7 | 3 | Probably the clearest of the samples |
| 715 | 4 | 1 | 13 | 0.0003 | 84 | 23.95 | 6 | 3 | Clear inner, edge diffuse |
| 715 | 4 | 2 | 11 | 0.0003 | 80 | 15.82 | 8 | 3 | Clear sample (not ground) |
| 715 | 4 | 3 | 0 | 0.0006 | 106 | 28.31 | 7 | 5 | Tip too diffuse to guess |
| 715 | 4 | 4 | 7 | 0.0004 | 82 | 20.53 | 7 | 4 | Maybe older |
| 715 | 4 | 5 | 11 | 0.0004 | 91 | 27.99 | 7 | 4 | |

# CHAPTER 5.  PHYSIOLOGICAL STRESS AND POST-DISCARD SURVIVAL OF QUANTITATIVELY IMPORTANT BY-CATCH SPECIES

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# Abstract

The current research examined the stress level of trawled species in order to establish if there is a relationship between trawl time and fish stress and where possible, survival. Stress is known to cause major physiological changes in fish and crustaceans, which may result in death during capture and increased predation when discarded. Examined here were the blue swimmer crab *Portunus pelagicus*, sparsely spotted stingaree *Urolophus paucimaculatus*, Port Jackson shark *Heterodontus portusjacksoni*, and Degens leatherjacket *Thamnaconus degeni,* which constitute a prominent part of the by-catch of the Spencer Gulf prawn fishery.

Fish and crab species were collected by trawling in Spencer Gulf of South Australia in 2003, 2004 and 2005. Trawls of 15, 30 and 45 minute duration were carried out and the condition of captured fish and crabs were compared to a base-line group, that had been line or drop-net captured, specifically in order to minimise capture stress prior to sampling. Blood and muscle samples of all capture species were analysed for stress indicators. Our results showed that, although the magnitude of the response was species specific, irrespective of time, trawl capture resulted in a response that is consistent with that recognised in animals subject to stress.

For the blue swimmer crab, sparsely spotted stingaree and Degens leatherjacket generally showed a clear blood and muscle stress response to trawl capture including energy mobilisation, increased metabolites and disrupted blood ion concentrations.  Generally, muscle energy balance was also disrupted in these trawl-caught species. In contrast the Port Jackson shark showed little physiological response to trawl capture.

Blue swimmer crab post-capture damage and death increased as post-capture time increased. Post-capture crab death was due almost totally to holes in the carapace or retained limbs and subsequent sea lice attack. For the sparsely spotted stingaree, post-trawl survival is probably low as following trawl capture only one stingaree out of eighteen survived the 3 day sea-cage survival test. For trawl captured Degens leatherjacket we estimated that approximately 46% subject to 15 minute trawls and 78% subject to 45 minute trawls do not survive post release.  In contrast, the Port Jackson shark showed little physiological response to trawl capture and no shark death occurred during trawls or in tanks during post-capture observations and experiments.

The results of this study show that, with the exception of the Port Jackson shark, the species examined suffer stress following trawl capture but that the severity of the response is species specific.  This study has established that trawl capture contributes to mortality in released blue swimmer crabs, sparsely spotted stingaree and the Degens leatherjacket.  There was little evidence to suggest that the Port Jackson shark post release survival was adversely affected by trawl capture.

## Introduction

Restrictions on by-catch in prawn or shrimp fisheries have led to increased efforts in developing By-catch Reducing Devices (BRD's); reviewed by Broadhurst (2000). In addition, semi-automatic sorting devices ("hopper-conveyor") that reduce sorting time and increase survival of by-catch and the quality of the catch, have been installed on many fishing vessels. It is generally assumed that at least a part of the discarded by-catch will survive but it is likely the survival will vary greatly with species (Andrew and Pepperell, 1992, Broadhurst et al., 1999, Robins et al., 1999, Salini et al., 2000).

Studies on survival of discarded by-catch in trawl fisheries usually involve monitoring of captured individuals for several days in containers on research vessels, while recording injuries and survival (Suuronen et al., 1996, Wassenberg et al., 2001). These studies provide an estimation of survival but suffer from lack of a control, making it difficult to separate confinement effects from that caused by capture. Furthermore, a number of factors; long-term health, physical constitution and homeostasis affect post-trawl mortality of the organism and are rarely considered (Broadhurst et al., 2002). In addition, severe exercise and associated stress in fish may result in increased predation long after their capture and release.

Stress is known to cause major physiological changes in fish, which can result in death. High levels of exercise, activity and stress increase metabolism and during strenuous activity fish begin to metabolise anaerobically (Jobling, 1995, Thomas et al., 1999). The adenylate concentrations (ATP, ADP, AMP and IMP) in the muscle tissues expressed as the Adenylate Energy Charge (AEC) ratio or IMP Load ratio are closely correlated to the different biochemical pathways (Vetter and Hodson, 1982). These ratios quantify the available energy within the adenylate system and are powerful measures of physiological stress (Chapman and Atkinson, 1973, Vetter and Hodson, 1982, Van der Boon et al., 1992).

The aim of this study was to test the physiological effects of trawling duration on quantitatively important by-catch species in the South Australian prawn fishery. Physiological stress and post-discard survival were investigated in four common by-catch species of the South Australian prawn fishery representing three taxa; a crustacean: the blue swimmer crab *Portunus pelagicus*, two elasmobranchs: the sparsely spotted stingaree *Urolophus paucimaculatus* and the Port Jackson shark *Heterodontus portusjacksoni*, and a

bony fish: Degens leatherjacket *Thamnaconus degeni*. Other species targeted opportunistically throughout the project were rough leatherjacket *Scobinichthys granulatus*, sand trevally *Pseudocaranx wrighti*, snapper *Pagrus auratus*, eagle rays *Myliobatis australis*, smooth stingrays *Dasyatis brevicaudata* and southern fiddler rays *Trygonorrhina fasciata*. The results of the opportunistically targeted species appear in Appendix 1.

## Materials and Methods

### Collection of animals

Animals were collected from trawl operations, which were carried out to simulate commercial practice, using the South Australian Research and Development Institute (SARDI) research vessel RV *Ngerin*. The vessel was equipped with a single prawn trawl, and travelled at a trawl speed of approximately 3 knots. Trawls were carried out in the waters of Spencer Gulf in South Australia in five commercial prawn fishing sites: Site 1, Western Shoal (33° 11.1' S, 137° 36.0' E); Site 2, Plank Point (33° 25.8' S, 137° 29.6' E); Site 3, Middle Bank (33° 36.3' S, 137° 29.0' E); Site 4, Wallaroo (33° 53.0' S, 137° 28.8' E) and Site 5, The Gutter (34° 06.5' S, 136° 56.3' E). Trawl duration was defined as the time from when the winches stopped paying out wire to the time when they were restarted to retrieve the net. Sites were chosen where the investigated species were known to be found in order to ensure that the greatest numbers of species of interest were caught.

### Measurement of physiological stress indicators

Following the trawl, targeted species were separated from the rest of the catch on the sorting table as soon as the net was emptied (Figure 1), and blood and muscle samples were collected from animals immediately (refer to individual species sections below for specific collection methods). Blood and muscle samples for all species were prepared and analysed as described below, unless otherwise stated.

Figure 1. Prawn trawl sorting table on the RV *Ngerin* illustrating typical by-catch of the Spencer Gulf prawn fishery.

*Blood parameters*

Blood samples were collected from all species as described below. Collected samples were centrifuged at 6,000 rpm for two minutes, and the plasma or hemolymph was transferred to cryovials, then stored in liquid nitrogen to be later analysed for lactate, glucose, pH and electrolytes (sodium, potassium, and chloride).

Plasma lactate, measured as L-lactic acid, was assayed enzymatically using a *Roche* L-Lactic acid UV kit (*Roche Chemicals*, Ryde, NSW, Australia). Blood glucose was determined using a *Randox* Glucose GOD kit (*Randox*, Crumlin, Co. Antrim, U.K.). Following approximately 1 h standing in uncapped vials to allow release of respiratory $CO_2$, plasma pH was determined using a TPS WP-80 pH-mV-temperature meter (TPS Pty Ltd, Brisbane, Australia). Due to a probe malfunction plasma pH was not measured on the sharks sampled in experiment 2.

Laboratory determination of electrolytes sodium, chloride and potassium, were assayed using an Ion Specific Electrode (ISE) within an Olympus AU400 Auto-analyser, by IDEXX Laboratories, Thebarton, South Australia.

*Muscle parameters*

Animals to be sampled were immediately killed following removal from the sorting table. A tissue sample was then excised from the white muscle of fish and crabs, placed in a plastic bag, wrapped in aluminium foil, frozen in liquid nitrogen and then held in a -80 $^{o}$C storage facility prior to analysis. Prior to analysis, all frozen muscle samples were crushed on dry ice and extracted in 0.06M perchloric acid (PCA).

Muscle glucose and lactate determination were carried out using enzymatic methods on a Dupont Dimension® XL Clinical Chemistry system Auto-analyser (Dade Behring Diagnostics, QLD) by the Meat Science Department at the University of New England, Armidale, NSW.

Nucleotides; Adenosine 5'-Triphosphate (ATP) and its catabolites, Adenosine 5'-Diphosphate (ADP), denosine 5'-Monophosphate (AMP), and Inosine Monophosphate (IMP), were measured following the method of Ryder (1985) using a Waters, Alliance 2695, high performance liquid chromatography (HPLC) system. An Alltech column (Alphabond C18 125A (5µm) 300 x 3.9 mm) was used with an isocratic mobile phase of 0.04 M $KH_2PO_4$ + 0.06 M $K_2HPO_4$ at a flow rate of 1 ml min$^{-1}$ with an injection volume of 10 µl. Peak area and retention times were monitored using a Waters 486 UV VIS detector at 254 nm.

The Adenylate Energy Charge ratio (AEC) was calculated following Atkinson, (1968) as:

$$AEC = \left[ \frac{ATP + 0.5ADP}{(ATP + ADP + AMP)} \right]$$

IMP load was calculated following Van der Boon *et al.*, (1992) as:

$$IL = \left[ \frac{IMP}{ATP + ADP + AMP} \right]$$

**Trawl stress and post-discard survival**

Stress effects of commercial trawl operations on prawn trawl by-catch were investigated in the four species, specifically, the effects of trawl durations of 15 and 45 minutes on physiological stress levels. Base-line levels of physiological stress indicators for all of the species were determined to enable comparison to post-trawl levels. An additional experiment was undertaken on Port Jackson sharks to measure the change in these levels up to four hours post-capture, as elevated levels of physiological stress may affect the survival potential of by-catch, either through increased predation or death several hours post-discard (Ryer, 2004). Additionally, trawl stress and handling was correlated to post-discard survival of two of the species; the blue swimmer crab and sparsely spotted stingaree, by conducting a series of field experiments outlined below.

*Blue swimmer crab; Base-line*

Blood and muscle samples were collected from blue swimmer crabs in October 2004, January 2005 and April 2005.

October 2004

In October 2004, six crabs were caught at Site 4 (Wallaroo) in crab traps deployed from the RV *Ngerin.* Using a small tender boat, crabs were retrieved from the traps and placed in a large container of seawater, then brought back to the *Ngerin*. The crabs were placed in individual buckets of seawater to prevent them damaging each other, and weight, length and sex recorded. Approximately 30 minutes after capture, blood was collected from the articulation of the swimmeret joints using a 3 ml heparinised syringe fitted with an 18 gauge needle. Muscle was collected from the posterior dorsal quarter of the carapace. Blood and muscle samples were prepared as described in previously. Crabs were also assessed for visible damage, described as either carapace damage or limb loss. Carapace damage was defined as a penetrating wound through the shell. An arbitrary code, adapted from Wassenberg and Hill, (1989), Table I, was assigned to each crab relevant to the degree of damage sustained as a result of the capture regime, either due to handling or aggressive interaction between crabs while being held in the traps. Old wounds were identified from recent damage by brown scar tissue.

Table I. Arbitrary ratings assigned to the degree of physical damage sustained by blue swimmer crabs (adapted from Wassenberg and Hill, 1989).

| Rating | Description |
|--------|-------------|
| *0* | No damage |
| *1* | 1 leg missing |
| *2* | 2 or more legs missing |
| *3* | 1 claw missing |
| *4* | 1 claw and legs missing |
| *5* | 2 claws missing |
| *6* | 2 claws and legs missing |
| *7* | Hole in carapace (pinched/crushed) |

January 2005- RV Ngerin and LMSC

The sampling method described for crabs collected in October 2004 was repeated in January 2005, for 29 crabs. Eight additional crabs from this group were brought back to the laboratory at the Lincoln Marine Science Centre. At the Science Centre the crabs were transferred to a plastic rectangular tank (40 x 75 x 100 cm) that was aerated and continuously exchanged from a flow-through seawater system, and held in the laboratory in order to determine base-line stress indicators. This determination proved to be difficult, as there was a water supply problem in the laboratory on the ninth day, resulting in the death of one crab, then two more crabs over the following two days. On the 12$^{th}$ day, the remaining five had become increasingly inactive, and some were moribund. Consequently, the decision was made to sample the remaining to avoid further losses.

April 2005

Nineteen crabs were caught in crab traps, near the Cowell public wharf in the upper-middle Spencer Gulf in April 2005. One crab was sampled each time the trap was retrieved to ensure blood and muscle samples were collected immediately (i.e. within 30 seconds) after capture. Physical damage was also recorded, as described above. The greatest numbers of crabs caught and sampled in the shortest individual time periods were those from the April 2005

group. Physiological stress indicator levels from this group were used for comparison with post-trawl stress in blue swimmer crab (experiments 1 and 2, below).

*Blue swimmer crab- Experiment 1*
Effects of 15 and 45 minute trawls, April 2004.

Experimental sampling

Trawls were carried out in April 2004 at Site 1 (Western Shoal) and Site 3 (Middle Bank) as described previously. Trawls were conducted for periods of 15 and 45 minutes and were repeated four times. Nine crabs were collected from each trawl. Immediately after the net was emptied, crabs were sorted from the rest of the catch. Crabs were also opportunistically collected from 30 minute trawls in October 2003 and blood was collected as described above. Muscle was not collected. Physiological stress levels from this trawl group were compared to a base-line group sampled in April 2005, as described above.

*Blue swimmer crab- Experiment 2*
Effects of 30 minute trawl stress response and post-discard survival, October 2004

Experimental sampling

The effect of trawl stress and post-discard survival in blue swimmer crabs was investigated in an experiment conducted at Site 4 (Wallaroo) in October 2004. Fourteen crabs were collected from each of three 30 minute day trawls. Seven crabs from each trawl were removed from the sorting table and immediately placed into individual 10L buckets, half filled with seawater. Each crab was weighed, and carapace width, limb loss (damage) and sex were recorded. Blood and muscle samples were collected as per the methods described previously. These crabs were used to provide an initial measurement of stress as a result of the trawl.

An additional 7 crabs from each trawl were removed from the sorting table and immediately placed in a tube of 1.5 mm oyster mesh, divided into seven sections (300 x 300 x 200mm – approximate length, width and height) to prevent them damaging each other (Figure 2). The tube was then placed in a fibreglass tank with flow-through seawater, and covered with shade cloth to block out the sunlight. This procedure was repeated for each trawl, and crabs were held in the tubes until the trawls were completed.

When three trawls had been completed, a tube of crabs was removed from the tank, and each crab was placed into a bucket, half filled with seawater. Each crab was weighed, and carapace width, limb loss and sex were recorded. The crabs were tagged with a small surface tag, which was attached to the carapace with glue, returned to the tube and placed back into the tank. This procedure was repeated for the remaining 2 tubes.



Figure 2. Mesh tubes divided into seven sections used to contain blue swimmer crabs to prevent them damaging each other prior to placement in sea-cages in survival experiment 1.



Figure 3. Sea-cages composed of oyster mesh and stainless steel used for blue swimmer crab and sparsely spotted stingaree trawl stress and survival experiments.

Prior to collection of the crabs, three sea-cages (1500 x 1500 x 500 mm) composed of a stainless steel frame with 25mm netting across the bottom surface, and 16 mm oyster mesh

on the sides (Figure 3) were moored to the sea floor by divers at a depth of 21 m. The cages were weighted at each corner and pushed into the substrate to provide a layer of sand on the bottom of the cage to a depth of approximately 10 cm. Crabs from each of the three tubes were released into a corresponding sea-cage after sampling. Crabs were kept in the cages for three days following Wassenberg and Hill (1993). Divers checked sea-cages for crab mortalities daily. After three days, one of the sea-cages was brought to the surface. The crabs were retrieved from the cage and transferred into individual buckets half filled with seawater. Survival and limb loss (damage) were recorded, and blood and flesh samples were collected as described previously. This sampling procedure was repeated for the remaining two sea-cages.

*Blue swimmer crab- Experiment 3*

The effect of physical interaction at capture on the post-discard survival of the blue swimmer crab, July 2005

Experimental sampling

The effect of physical interaction at capture on the post-discard survival of blue swimmer crabs was investigated in an experiment conducted at Site 3 (Middle Bank) in July 2005. Crabs were collected from four trawls, carried out as described previously, each conducted over a period of 45 minutes during the day. A trawling duration of 45 minutes was chosen to simulate commercial trawl practice. Immediately after each trawl, crabs were separated from the rest of the catch on the sorting table and those identified with no fresh limb loss (as a result of the trawl), not having a soft shell (not recently moulted) and not suffering recent physical damage (to eliminate damage as a result of the trawl) were chosen for the experiment. Old wounds were identified from trawl damage by brown scar tissue. Crabs were placed into modules composed of 16 mm oyster mesh, 600 x 200 x 100 mm, consisting of three compartments per module. There were a total of nine modules making up 36 compartments, and one crab assigned per compartment (Figure 4).

Figure 4. Mesh modules used to hold blue swimmer crabs after timed physical interaction, post capture, following a 45 minute trawl. Diagrammatic illustration not to scale

Crabs collected from earlier trawls were held in their compartments in an on-board tank with flow-through seawater until the completion of all the trawls. When 36 crabs were caught and all compartments filled, nine crabs for each treatment were removed from their compartments and placed in a plastic container, 400 x 420 x 650 mm, in a depth of seawater of approximately 300 mm, and treated as follows:

Treatment 1- crabs allowed to interact for 10 minutes.
Treatment 2- crabs allowed to interact for 25 minutes.
Treatment 3- crabs allowed to interact for 40 minutes.

The final group of nine crabs (Control) remained physically isolated in their assigned compartments throughout the duration of the treatment application. To ensure there was maximum interaction between crabs, all crabs were agitated at 6 to 8 minutes, crabs from treatment groups 2 and 3 were agitated at 21 to 23 minutes, and crabs from treatment group 3 were agitated at 36 to 38 minutes.

At the end of each treatment, all crabs were assessed for visible damage (as described previously) and returned to their pre-assigned compartment. Control group crabs were also removed from their compartments and inspected for visible damage. Once all crabs had been returned to their compartments, the modules were placed in a sea-cage as described in *Experiment 2*. The cage was then lowered to the sea floor by divers to a depth of 18.3m for three days. The sea surface temperature during the experiment was 14ºC. After three days the sea-cage was brought to the surface. Survival and visible damage for each crab were noted. Crab weight and carapace width were also recorded. Blood and muscle samples were collected from crabs that had survived after three days as described previously.

*Sparsely spotted stingaree- Base-line*

Blood and muscle samples used for base-line measurements of physiological stress in stingarees were collected from animals caught in trawls conducted from the RV *Ngerin* at Site 5 (The Gutter) in April 2005. Immediately after the net was emptied, stingarees were removed from the sorting table and kept alive in a fibre-glass tank with flow-through seawater on the vessel, before being brought back to the laboratory. Stingarees were transferred to a plastic rectangular tank (40 x 75 x 100 cm) that was aerated and continuously exchanged from a flow-through seawater system. Sand was placed in the bottom of the tank to a depth of approximately 10 cm to allow the stingarees to hide, and to prevent abrasion to their undersides from the bottom of the tank. Stingarees were fed small pieces of prawn tail (*Penaeus latisulcatus*) and monitored for mortalities each day for four weeks. Six stingarees died in the first three weeks after being placed in the laboratory, and mortalities were removed daily. After four weeks, the remaining eight stingarees were individually caught with a hand net and killed using a small metal spike through the brain. Immediately after death, blood was collected from the heart cavity using a 3 ml heparinised syringe fitted with a 22 gauge needle, and a small piece of muscle was excised from the wing of each stingaree. Blood and muscle samples were prepared for analysis as described previously.

*Sparsely spotted stingaree- Experiment 1*

Physiological stress effects of 15 and 45 minute trawls in the sparsely spotted stingaree, *Urolophus paucimaculatus*, July 2005.

Experimental sampling

Trawls were carried out using the research vessel RV *Ngerin* in July 2005 at Site 5 (The Gutter) for both 15 and 45 minutes and repeated until a sufficient number of stingarees were caught. Seven stingarees were sampled from a total of six 15 minute trawls, and 11 were sampled from a total of four 45 minute trawls. After the net was emptied, stingarees were immediately sorted from the rest of the catch and blood and muscle samples were collected and prepared as discussed previously.

Most of the stingarees sustained various forms of damage as a result of the trawl; consequently a visual damage assessment was made for each animal. Damage was defined as bruising, cuts or lacerations, and this was observed in four regions of the body; On the ventral surface; the leading edge/snout, wings, belly/gills and general damage on the dorsal surface (Figure 5). Damage was then expressed as a percentage of the total body. Ratings were assigned to each region out of 4 points:

1: damage to a quarter or less of the area,

2: damage between a quarter to half of the area,

3: damage between a half to three quarters of the area, or

4: damage to between three quarters and the entire area.

Figure 5. Body regions of stingarees assessed for damage. Physical damage (bruising, cuts or lacerations) was assessed in four regions; Ventral leading edge/snout, wings, belly/gills and general dorsal surface damage. Ratings were assigned to each region out of 4 points: 1: damage to a quarter or less of the area, 2: damage between a quarter to half of the area, 3: damage between a half to three quarters of the area, or 4: damage to between three quarters and the entire area. The ratings were then expressed as damage as a percentage of the total body.

*Sparsely spotted stingaree- Experiment 2*

The effects of trawl capture on post-trawl stress and survival in the sparsely spotted stingaree, January 2005.

Experimental sampling

The effect of trawl stress and post-discard survival in the sparsely spotted stingaree was investigated in an experiment conducted at Site 5 (The Gutter) in January 2005, using the research vessel RV *Ngerin*, as described previously. A total of 36 stingarees were collected from three trawls, each conducted over a period of 30 minutes during the night. Six stingarees from each trawl were removed from the sorting table immediately after the net was

emptied and placed into a commercial prawn tray (Figure 6) that was then placed into a fibreglass tank on-deck with flow-through seawater, and kept overnight. Six additional stingarees were removed from the sorting table at each trawl immediately after the net was emptied and placed into individual 10L buckets half filled with seawater. Each stingaree was weighed, and sex and visible damage was recorded. Blood and muscle samples were collected as per methods described previously to provide an initial measurement of stress as a result of the trawl.



Figure 6. Commercial prawn trays used to hold sparsely spotted stingarees in the trawl stress and survival experiment (Experiment 2).

The following morning, stingarees that had been held overnight were weighed, and sex and visible damage were recorded. The stingarees were released from the trays by divers into a sea-cage (Figure 3), which was moored to the sea-floor at a depth of 20 m, weighted at each corner and left for three days. After three days, the sea-cage was brought to the surface, and survival was recorded. No blood or muscle samples were collected from stingaree within this experiment.

*Port Jackson shark- Base-line*
Physiological stress parameters for Port Jackson sharks were determined from 5 sharks caught on hand lines from the RV *Ngerin* at Site 3 (Middle Bank).  In October 2003, 5 sharks were caught in 45 minute trawls from the RV *Ngerin* at Site 3 (Middle Bank) and brought back the Lincoln Marine Science Centre (LMSC) in on-deck, flow –through tanks. At LMSC the

sharks were held in a plastic rectangular tank (40 x 75 x 220 cm) with a flow through seawater system. Following 2 weeks acclimation, blood samples were collected to determine physiological stress indicators levels.  In addition, four sharks were line caught from the RV *Ngerin* at Site 3 (Middle Bank) in July 2005 and blood and muscle samples were collected immediately after capture.

Blood was collected using a 3ml heparinised syringe fitted with an 18 gauge needle.  The needle was inserted into the caudal artery at an angle of 60º towards the head, via the ventral surface, at a point under the second dorsal fin. For the July 2005 group, a small muscle biopsy was collected from the ventral surface of the upper portion of the tail using a 3mm Ø stainless steel coring tool. Blood and muscle samples were prepared and analysed as described previously.

*Port Jackson shark- Experiment 1*

Physiological stress effects of 15 and 45 minute trawls, July 2005.

Experimental sampling

Trawls were carried out for 15 and 45 minutes, as described previously, and were repeated twice, in July 2005 at Site 4 (Wallaroo). Sharks were sampled opportunistically at each trawl. Immediately after the net was emptied, sharks were sorted from the rest of the catch and blood and muscle samples were collected and prepared as described above. Sharks were also collected from 30 minute trawls in October 2003 and blood was collected as described above. Muscle was not collected.

*Port Jackson shark- Experiment 2*

Measures of physiological stress at 0, 2 and 4 hrs after capture from 15 and 45 minute trawls, April 2004.

Experimental sampling

In April 2004, an experiment was carried out investigating physiological stress levels in Port Jackson sharks following 15 and 45 minute trawls, as compared to a Base-line group.  Sharks were collected from trawls conducted at Site 3 (Middle Bank) as described for those carried out previously. Base-line group stress indicator levels were determined as described earlier. Four sharks were collected from 15 minute trawls, and seven sharks were collected from 45 minute trawls. Sharks were sampled immediately after capture (0 hours), and sampled again

at two and four hours after capture. Blood samples were collected as described previously. Muscle was not collected. Sharks were tagged and held in fibreglass tanks with flow-through seawater between sample times.

Due to an equipment failure shark plasma pH was not measured for this experiment.

*Degens leatherjacket- Base-line*

Physiological stress parameters for Degens leatherjacket were determined from fish collected in Boston Bay in lower Spencer Gulf (34º 43.5' S, 135º 56' E) in April 2005. Fish were caught in small drop nets deployed from the RV Breakwater Bay, at a depth of approximately 15 m. Blood and muscle were collected immediately after the net was landed in the boat, and one fish was sampled per catch to ensure samples were collected as soon as possible after capture. Due to the small size of the fish (approximately 150 mm and <60g), following a blow to the head, a small incision was made immediately behind the anal and dorsal fins (Figure 7) to enable sufficient blood collection. Blood was then collected from the bleed cut using a 3ml heparinised syringe fitted with a 22 gauge needle. A single fillet from each fish was collected as a muscle sample. Blood and muscle were prepared for analysis as described earlier. Electrolytes were not measured.



Figure 7. Position of tail-cut for blood collection of Degens leatherjacket, sand trevally and rough leather jacket.

*Degens leatherjacket- Trawl stress*

Physiological stress effects of 15 and 45 minute trawls in Degens leatherjacket, April 2004.

Experimental sampling

Trawls were carried out in April 2004 at Site 5 (The Gutter) as previously described. Trawls were conducted for periods of 15 and 45 minutes and were repeated three times. After the net was emptied, fish were immediately sorted from the rest of the catch and placed into containers of ambient sea-water and their general mobility and physical damage was assessed. All fish that were obviously dead, fatally wounded or floating were recorded as dead; fish that were immobile but submerged were recorded as moribund; all fish that were swimming were recorded to be alive (Figure 8). Blood and muscle was collected from nine fish, observed to be alive, for each trawl replicate, as per sampling methods described for base-line determination, above. Fish that were observed to be moribund or dead were not selected for sampling.



Figure 8. Post-trawl survival of Degens leatherjacket from trawls conducted for 15 and 45 minutes duration. All fish that were obviously dead, fatally wounded or floating were categorised as dead, fish that were immobile but submerged, were recorded as moribund, fish that were swimming were recorded to be alive. Fish were collected from three trawls at each trawl duration.

Fish were also collected from 15 and 45 minute trawls in October 2003 and April 2004, and sampled as described above. Sample collection was still being developed at this stage and blood and muscle for these fish were pooled in groups of three fish. Physiological stress levels determined for these trawl groups are presented in Appendix 1.

**Statistical analysis**

Unless otherwise stated, for all trawl experiments, the effect of trawl duration on physiological stress and comparison of Base-line groups were compared using analysis of variance (ANOVA) and where necessary pairwise multiple comparison was carried out using the Holm-Sidak method. In an attempt to satisfy the assumptions of normality and homogeneity of variance, where necessary, the data were transformed. However as ANOVA is robust against violations of assumptions (Underwood 1997, Zar, 1999) and where transformations were unsuccessful an analysis was performed on the data regardless. A significance level of $\alpha < 0.05$ was used for statistical tests. Blue swimmer crab - Experiment 3: Trawl treatment effects were compared using a one-way analysis of variance (ANOVA). Survival data were analysed using the chi-square test. Physical damage ratings were analysed using the Kruskal-Wallis ranks test followed by the Mann-Whitney rank sum test for pair-wise comparisons. Sparsely spotted stingaree - Experiment 2: Physical damage ratings for different trawl times were compared using the Mann-Whitney rank sum test. Port Jackson shark- Experiment 2: Repeated measures of physiological stress levels were statistically analysed using a two-way repeated measures ANOVA with between-subjects factors, followed by the Holm-Sidak test for pair-wise comparisons.

## Results

**Trawl stress and post-discard survival**

*Blue swimmer crab- Base-line*

Obtaining base-line blood and muscle parameters for blue swimmer crabs proved to be difficult, due to water supply problems in the laboratory resulting in crab death and conditions during capture sampling being unavoidably different at each sampling opportunity. Subsequently, most of the blood and muscle physiological stress indicators were significantly different between the four base-line groups (Table II).

Base-line parameters determined from the group of crabs sampled in the laboratory in January 2005 were not considered to be representative for healthy crabs, as they were moribund at the time of sampling, and were not used for statistical comparison between levels measured in crabs collected in trawl stress experiments. The greatest numbers of crabs caught, and sampled in the shortest individual time periods, were those from the April 2005 base-line group. As this sampling regime was considered the most sound, physiological stress levels from this group were used for statistical comparison to post-trawl stress levels in this species throughout the project.

*Blue swimmer crab- Experiment 1*

Physiological stress effects of 15 and 45 minute trawls, April 2004, and 30 minute trawls, October 2003.

15 and 45 minute trawls- Blood parameters

Lactate levels in the 45 minute trawl group were similar to those measured in the 15 minute trawl group, and these were both significantly higher than the Base-line group (Table III). Base-line group pH was significantly lower than both the 15 and 45 minute trawl groups; however there was no significant difference between the 15 and 45 minute trawl groups. Potassium levels in the 15 minute trawl group ($15.8 \pm 0.2$ mmol.l$^{-1}$) was significantly lower than Base-line group ($17.2 \pm 0.7$ mmol.l$^{-1}$); however levels in the 45 minute trawl group ($16.8 \pm 0.4$ mmol.l$^{-1}$) were not significantly different to those in the 15 minute trawl group or to Base-line group. There was no significant change in glucose, sodium or chloride levels.

15 and 45 minute trawls- Muscle parameters

The IMP Load for the 15 and 45 minute trawl groups were significantly higher than the Base-line group ($0.78 \pm 0.03$, $0.78 \pm 0.03$ and $0.09 \pm 0.01$, mean $\pm$ SE, respectively), but there was no significant difference between the 15 and 45 minute trawl groups (Table III). The AEC for the 45 minute trawl group was lower than the 15 minute trawl and Base-line group which were similar to one another. There was no significant difference in muscle glucose or lactate levels between groups.

Damage

The physical damage ratings assigned to the 15 and 45 minute trawl group crabs were significantly higher than those assigned to the Base-line crab group; however the 15 and 45 minute trawl groups were not significantly different to each other (Table IV).

30 minute trawls- Blood parameters

Plasma lactate, pH, sodium and chloride levels for the 30 minute trawl group were all significantly higher than Base-line group (Table V). Plasma glucose and potassium values in the 30 minute trawl group were not significantly different to the base-line group.

Table II. Physiological stress levels measured in blue swimmer crabs collected in October 2004, January 2005 *RV Ngerin*, January 2005 *LMSC* (n=5) and April 2005. Groups (Mean ± SE) sharing the same superscript are not significantly different.

| Parameter | Oct. 2004 | Jan. 2005 [1] | | Apr. 2005 [2] |
|---|---|---|---|---|
| | | *RV Ngerin* | *LMSC* | |
| *Blood* | | | | |
| Lactate mmol.l$^{-1}$ | 7.35 ± 0.96 [a] | 8.71 ± 0.54 [a] | 2.36 ± 0.26 [b] | 3.14 ± 0.3 [b] |
| pH | 7.64 ± 0.04 [a] | 7.36 ± 0.04 [b] | 7.66 ± 0.05 [ac] | 7.20 ± 0.03 [d] |
| Glucose mmol.l$^{-1}$ | 0.49 ± 0.11 [a] | 0.74 ± 0.06 [a] | 0.68 ± 0.10 [a] | 0.52 ± 0.05 [a] |
| Sodium mmol.l$^{-1}$ | 558 ± 6.20 [a] | 546 ± 3.19 [a] | 516 ± 5.67 [b] | 529 ± 5.71 [b] |
| Potassium mmol.l$^{-1}$ | 16.1 ± 0.37 [ac] | 14.5 ± 0.22 [ab] | 13.1 ± 0.35 [b] | 17.2 ± 0.72 [c] |
| Chloride mmol.l$^{-1}$ | 539 ± 8.88 [a] | 546 ± 3.79 [a] | 524 ± 6.30 [a] | 545 ± 4.89 [a] |
| *Muscle* | | | | |
| IMP Load | 0.92 ± 0.05 [a] | 0.60 ± 0.03 [b] | 0.07 ± 0.02 [c] | 0.09 ± 0.01 [c] |
| AEC | 0.95 ± 0.002 [a] | 0.95 ± 0.004 [a] | 0.96 ± 0.003 [a] | 0.94 ± 0.003 [a] |
| Glucose mmol.kg$^{-1}$ | 27.0 ± 3.74 [ab] | 19.1 ± 2.02 [a] | 43.5 ± 10.49 [b] | 17.6 ± 4.20 [ac] |
| Lactate mmol.kg$^{-1}$ | 14.6 ± 5.19 [a] | 16.9 ± 1.92 [a] | 5.79 ± 1.94 [a] | 13.2 ± 1.80 [a] |

[1] Crabs collected in January 2005 were sampled on the RV *Ngerin*, and in the laboratory at the Lincoln Marine Science Centre (LMSC).

[2] The greatest numbers of crabs caught, and sampled in the shortest individual time periods, were those from the April 2005 Base-line group. Consequently, base physiological stress indicator levels from this group have been used for comparison to post-trawl stress levels.

October 2004 hemolymph and muscle (n= 6); Jan 2005 RV *Ngerin* hemolymph lactate, pH and glucose (n= 28), blood sodium, potassium and chloride (n= 27), muscle IMP and AEC (n= 19) and muscle glucose and lactate (n= 28); Jan 2005 LMSC hemolymph and muscle (n= 5), April 2005 blood and muscle (n= 13).

Table III. Physiological stress parameters measured in blood and muscle of blue swimmer crabs after 15 and 45 minute trawls in April 2004, as compared to the Base-line group measured in April, 2005. Mean ± SE, treatments sharing the same superscript are not significantly different.

| | | Trawl | |
|---|---|---|---|
| Parameter | Base-line | 15 min. | 45 min. |
| *Blood* | | | |
| Lactate mmol.l$^{-1}$ | 3.14 ± 0.3 [a] | 8.18 ± 0.49 [b] | 9.69 ± 0.60 [b] |
| pH | 7.20 ± 0.03 [a] | 7.56 ± 0.02 [b] | 7.56 ± 0.02 [b] |
| Glucose mmol.l$^{-1}$ | 0.52 ± 0.05 [a] | 0.60 ± 0.05 [a] | 0.76 ± 0.07 [a] |
| Sodium mmol.l$^{-1}$ | 529 ± 5.71 [a] | 527 ± 3.29 [a] | 536 ± 4.85 [a] |
| Potassium mmol.l$^{-1}$ | 17.2 ± 0.72 [a] | 15.8 ± 0.18 [b] | 16.8 ± 0.36 [ab] |
| Chloride mmol.l$^{-1}$ | 545 ± 4.89 [a] | 533 ± 3.82 [a] | 535 ± 6.07 [a] |
| *Muscle* | | | |
| IMP Load | 0.09 ± 0.01 [a] | 0.78 ± 0.03 [b] | 0.78 ± 0.03 [b] |
| AEC | 0.94 ± 0.00 [a] | 0.90 ± 0.01 [a] | 0.81 ± 0.02 [b] |
| Glucose mmol.kg$^{-1}$ | 17.6 ± 4.20 [a] | 22.2 ± 1.89 [a] | 20.7 ± 3.19 [a] |
| Lactate mmol.kg$^{-1}$ | 13.2 ± 1.80 [a] | 19.1 ± 1.80 [a] | 18.5 ± 1.87 [a] |

Base-line hemolymph and muscle (n=13); 15 minute trawl hemolymph lactate, pH and glucose (n=36), hemolymph sodium, potassium and chloride (n=33) and muscle (n=36); 45 minute trawl hemolymph lactate, pH and glucose (n=36), hemolymph sodium, potassium and chloride (n=9), and muscle (n=30).

Table IV. The extent of damage sustained by blue swimmer crabs after 15 and 45 minute trawls in April 2004, as compared to the Base-line group collected in April 2005. Total damage for each treatment is expressed as the sum of the damage category rating (from 0 to 7) multiplied by the number of crabs assigned that category. Treatments sharing the same superscript are not significantly different.

| Damage category | Rating | Base-line | 15 min. | 45 min. |
|---|---|---|---|---|
| No damage | 0 | 0 | 0 | 0 |
| 1 leg missing | 1 | 0 | 4 | 5 |
| 2 or more legs missing | 2 | 0 | 4 | 22 |
| 1 claw missing | 3 | 3 | 21 | 21 |
| 1 claw and legs missing | 4 | 0 | 12 | 4 |
| 2 claws missing | 5 | 0 | 0 | 0 |
| 2 claws and legs missing | 6 | 0 | 0 | 6 |
| Hole in carapace | 7 | 0 | 0 | 0 |
| *Total damage* | | *3 [a]* | *41 [b]* | *58 [b]* |

Base-line (n= 13); 15 and 45 minutes trawls (n= 36).

Table V. Physiological stress parameters measured in hemolymph of blue swimmer crabs immediately after 30 minute trawls in October 2004, as compared to the Base-line group measured in April 2005. Mean ± SE, treatments sharing the same superscript are not significantly different.

| Parameter | Base-line | 30 min. trawl |
|---|---|---|
| Lactate mmol.l$^{-1}$ | 3.14 ± 0.32 [a] | 7.29 ± 0.72 [b] |
| pH | 7.20 ± 0.03 [a] | 7.58 ± 0.04 [b] |
| Glucose mmol.l$^{-1}$ | 0.52 ± 0.05 [a] | 0.38 ± 0.12 [a] |
| Sodium mmol.l$^{-1}$ | 529 ± 5.71 [a] | 570 ± 4.09 [b] |
| Potassium mmol.l$^{-1}$ | 17.2 ± 0.72 [a] | 16.3 ± 0.33 [a] |
| Chloride mmol.l$^{-1}$ | 545 ± 4.89 [a] | 565 ± 3.37 [b] |

Base-line (n= 13); 30 minute trawl (n= 10).

*Blue swimmer crab- Experiment 2*

Post-trawl physiological stress response and post-discard survival in the blue swimmer crab, *Portunus pelagicus*, October 2004

Survival

There were no crab deaths in any of the replicate sea-cages three days after the trawl.

Damage

There was no difference in the damage scores for the crabs examined immediately following the trawl that were killed and sampled and those that were deployed in the sea-cages for 3 days (Table VI). The damage score of the crabs that were recovered after 3 days in cages on the sea floor was higher than that recorded in the same crabs prior to deployment but the before and after scores were not significantly different.

Blood parameters

Plasma lactate and potassium levels had dropped significantly after three days, while pH level increased significantly after three days (Table VII). There was no significant change in glucose, sodium or chloride levels.

Muscle parameters

Muscle lactate levels dropped significantly, from $8.15 \pm 1.51$ mmol.kg$^{-1}$ to $1.84 \pm 0.67$ mmol.kg$^{-1}$, (Mean $\pm$ SE) after three days, while the AEC increased significantly after three days (Table VII). There was no significant change in muscle glucose level or the IMP Load in the crabs after three days.

Table VI. Damage score for blue swimmer crabs after 30 minute trawls (n = 21), in October 2004, as compared to the Post-trawl group (n = 21). Total damage for each group is expressed as the sum of the damage rating (from 0 to 7) multiplied by the number of crabs assigned that category. Treatments sharing the same superscript are not significantly different.

| Damage category | Rating | Post-Trawl | Day 0 | Day 3 |
|---|---|---|---|---|
| No damage | 0 | 0 | 0 | 0 |
| 1 leg missing | 1 | 1 | 1 | 3 |
| 2 or more legs missing | 2 | 2 | 2 | 2 |
| 1 claw missing | 3 | 3 | 3 | 9 |
| 1 claw and legs missing | 4 | 12 | 12 | 12 |
| 2 claws missing | 5 | 0 | 0 | 5 |
| 2 claws and legs missing | 6 | 6 | 6 | 6 |
| Hole in carapace | 7 | 0 | 0 | 0 |
| *Total damage* | | *24[a]* | *24[a]* | *31[a]* |

Post Trawl, Day 0 and Day 3, (n= 21).

Table VII. Physiological stress parameters measured in hemolymph and muscle of blue swimmer crabs immediately after 30 minute trawls, and three days post-trawl in October 2004, as compared to the Base-line group measured in April 2005. Mean ± SE, treatments sharing the same superscript are not significantly different, the Base-line group was not included in statistical analysis.

| Parameter | Base level | Immediately post-trawl | 3 days post-trawl |
|---|---|---|---|
| *Hemolymph* | | | |
| Lactate mmol.l$^{-1}$ | 3.14 ± 0.32 | 6.96 ± 0.42 [a] | 4.84 ± 0.34 [b] |
| pH | 7.20 ± 0.03 | 7.70 ± 0.03 [a] | 7.91 ± 0.05 [b] |
| Glucose mmol.l$^{-1}$ | 0.52 ± 0.05 | 0.36 ± 0.04 [a] | 0.41 ± 0.04 [a] |
| Sodium mmol.l$^{-1}$ | 529 ± 5.71 | 566 ± 2.79 [a] | 566 ± 2.92 [a] |
| Potassium mmol.l$^{-1}$ | 17.2 ± 0.72 | 15.7 ± 0.36 [a] | 14.3 ± 0.29 [b] |
| Chloride mmol.l$^{-1}$ | 545 ± 4.89 | 549 ± 2.99 [a] | 554 ± 2.82 [a] |
| | | | |
| *Muscle* | | | |
| IMP Load | 0.09 ± 0.01 | 0.86 ± 0.03 [a] | 0.86 ± 0.03 [a] |
| AEC | 0.94 ± 0.00 | 0.94 ± 0.00 [a] | 0.95 ± 0.00 [b] |
| Glucose mmol.kg$^{-1}$ | 17.6 ± 4.20 | 25.7 ± 2.81 [a] | 20.7 ± 1.46 [a] |
| Lactate mmol.kg$^{-1}$ | 13.2 ± 1.80 | 8.15 ± 1.51 [a] | 1.84 ± 0.67 [b] |

Base-line (n= 13); Immediately post-trawl and 3 days post-trawl (n= 21).

*Blue swimmer crab- Experiment 3*

The effect of physical interaction at capture and post-capture on the post-discard survival of the blue swimmer crab, *Portunus pelagicus*, July 2005.

Survival

There were more deaths in crab groups that had been allowed to interact with each other as compared to the control crab groups, ranging from 88.9% survival in the control group, to 77.8% for crabs that had been allowed to interact for 10 minutes, and 55.6% in crabs that had been allowed to interact with each other for 25 and 40 minutes; however this decrease was not significant (Figure 9). All of the crabs that did not survive had been eaten by sea lice after three days.



Figure 9. Survival rate of blue swimmer crabs three days after timed physical interaction following a 45 minute trawl (n = 9).

Damage

Crab groups that had been allowed to interact with each other were significantly more damaged than the control group (Table VIII). However, the degree of damage sustained by crabs between each of the treatment groups was not significantly different.

Table VIII. The extent of damage sustained by blue swimmer crabs after timed physical interaction post capture, following a 45 minute trawl in July 2005. Total damage for each treatment is expressed as the sum of the damage category rating (from 0 to 7) multiplied by the number of crabs assigned that category. Treatments sharing the same superscript are not significantly different.

| Damage category | Rating | No interaction (Control) | 10 min. (T1) | 25 min. (T2) | 40 min. (T3) |
|---|---|---|---|---|---|
| No damage | 0 | 0 | 0 | 0 | 0 |
| 1 leg missing | 1 | 3 | 1 | 0 | 3 |
| 2 or more legs missing | 2 | 0 | 2 | 2 | 2 |
| 1 claw missing | 3 | 0 | 0 | 3 | 0 |
| 1 claw and legs missing | 4 | 0 | 12 | 8 | 8 |
| 2 claws missing | 5 | 0 | 5 | 0 | 0 |
| 2 claws and legs missing | 6 | 0 | 0 | 12 | 0 |
| Hole in carapace | 7 | 0 | 7 | 7 | 21 |
| | | | | | |
| *Total damage* | | *3 [a]* | *27 [b]* | *32 [b]* | *34 [b]* |

Control, (T1), (T2) and (T3), (n= 9).

## Hemolymph parameters

Hemolymph pH in crabs that had been allowed to interact for 40 minutes was significantly lower than that for the control group, but not significantly different to crabs allowed to interact for 10 or 25 minutes (Table IX). There was no significant effect of physical interaction on any other biochemical stress indicator measured in the hemolymph three days post-discard.

## Muscle parameters

There was no significant effect of physical interaction on any of the biochemical stress indicators measured in the muscle of crabs three days post-discard (Table IX).

Table IX. Physiological stress parameters measured in hemolymph and muscle of trawl-caught blue swimmer crabs held in sea-cages for 3 days after timed physical interaction post-capture. Mean ± SE, treatments sharing the same superscript are not significantly different.

| Parameter | Control | Treatment 1 | Treatment 2 | Treatment 3 |
|---|---|---|---|---|
| *Hemolymph* | | | | |
| Lactate mmol.l$^{-1}$ | 2.33 ± 0.20 [a] | 1.85 ± 0.14 [a] | 2.35 ± 0.45 [a] | 1.80 ± 0.53 [a] |
| pH | 7.29 ± 0.11[a] | 7.07 ± 0.06 [ab] | 6.94 ± 0.14 [ab] | 6.81 ± 0.12 [b] |
| Glucose mmol.l$^{-1}$ | 0.43 ± 0.04 [a] | 0.38 ± 0.10 [a] | 0.43 ± 0.12 [a] | 0.38 ± 0.07 [a] |
| Sodium mmol.l$^{-1}$ | 482 ± 5.40 [a] | 475 ± 11.45 [a] | 487 ± 7.19 [a] | 487 ± 7.29 [a] |
| Potassium mmol.l$^{-1}$ | 12.7 ± 0.56 [a] | 13.2 ± 0.46 [a] | 13.3 ± 0.40 [a] | 13.9 ± 0.30 [a] |
| Chloride mmol.l$^{-1}$ | 475 ± 5.64 [a] | 472 ± 11.86 [a] | 482 ± 6.06 [a] | 481 ± 8.43 [a] |
| *Muscle* | | | | |
| IMP Load | 0.07 ± 0.01 [a] | 0.09 ± 0.02 [a] | 0.08 ± 0.01 [a] | 0.08 ± 0.02 [a] |
| AEC | 0.92 ± 0.00 [a] | 0.91 ± 0.01 [a] | 0.92 ± 0.01 [a] | 0.93 ± 0.01 [a] |
| Glucose mmol.kg$^{-1}$ | 30.1 ± 4.51 [a] | 21.4 ± 3.50 [a] | 22.4 ± 4.54 [a] | 36.7 ± 7.12 [a] |
| Lactate mmol.kg$^{-1}$ | 4.74 ± 2.17 [a] | 6.48 ± 5.39 [a] | 3.51 ± 1.36 [a] | 4.02 ± 2.26 [a] |

Control, (n= 8); Treatment 1, (n=7); and Treatment 2 and 3, (n=5).

*Sparsely spotted stingaree- Experiment 1*

15 and 45 minute trawls, July 2005

Blood parameters

Plasma lactate and glucose levels for the 15 and 45 minute trawl groups were significantly higher than the Base-line group, but the difference between the 15 and 45 minute trawl groups was not significant (Table X). Plasma pH values for the 15 and 45 minute trawl groups were significantly lower than the Base-line group, but there was no significant difference between the 15 and 45 minute trawl groups. There was no significant change in sodium, potassium or chloride levels as a result of the trawl.

Muscle parameters

There was no significant change in muscle IMP Load due to trawl; however there was a small but significant increase in the AEC values in the 15 and 45 minute trawl group, compared to the Base-line group. Muscle glucose level was significantly higher in the 45 minute trawl group than the other groups which had similar levels to each other (Table X). Although there appeared to be a trend of increased muscle lactate with longer trawl time there was no significant difference between groups.

Damage

Damage score for stingarees in the 15 minute trawl was 36.6% (n=7), as compared to 38.1% to stingarees in the 45 minute trawl group (n=11), however this difference was not significant. It was not possible to assess a Base-line group damage rating for comparison.

*Sparsely spotted stingaree- Experiment 2*

Post-trawl physiological stress response and survival in the sparsely spotted stingaree, January 2005.

Survival

Of the stingaree placed in seacages only one of the eighteen survived three days post-trawl; it appeared that the rest had been eaten by sea lice, as only cartlidge remnants remained in the cage. Therefore it was not possible to measure post-discard physiological stress levels.

Table X. Physiological stress parameters measured in blood and muscle of sparsely spotted stingarees after 15 and 45 minute trawls in July 2005, as compared to a Base-line group, measured in April 2005. Mean ± SE, treatments sharing the same superscript are not significantly different.

| Parameter | Base-line | Trawl | |
| | | 15 min. | 45 min. |
| --- | --- | --- | --- |
| *Blood* | | | |
| Lactate mmol.l$^{-1}$ | 0.41 ± 0.06 [a] | 1.14 ± 0.10 [b] | 1.66 ± 0.25 [b] |
| pH | 7.32 ± 0.06 [a] | 6.84 ± 0.15 [b] | 6.67 ± 0.08 [b] |
| Glucose mmol.l$^{-1}$ | 0.65 ± 0.05 [a] | 1.07 ± 0.20 [a] | 1.78 ± 0.23 [b] |
| Sodium mmol.l$^{-1}$ | 293 ± 4.61 [a] | 296 ± 7.16 [a] | 292 ± 3.69 [a] |
| Potassium mmol.l$^{-1}$ | 7.88 ± 0.51 [a] | 7.31 ± 0.21 [a] | 8.11 ± 0.68 [a] |
| Chloride mmol.l$^{-1}$ | 288 ± 4.36 [a] | 292 ± 7.23 [a] | 293 ± 2.87 [a] |
| *Muscle* | | | |
| IMP Load | 0.54 ± 0.16 [a] | 0.14 ± 0.03 [a] | 0.37 ± 0.10 [a] |
| AEC | 0.93 ± 0.008 [a] | 0.95 ± 0.009 [b] | 0.96 ± 0.07 [b] |
| Glucose mmol.kg$^{-1}$ | 12.9 ± 1.72 [a] | 15.9 ± 1.69 [ab] | 18.7 ± 1.33 [b] |
| Lactate mmol.kg$^{-1}$ | 7.88 ± 0.97 [a] | 15.5 ± 6.36 [a] | 13.9 ± 2.62 [a] |

Base-line blood lactate and glucose (n= 7), blood pH, sodium, potassium and chloride (n= 6) and muscle parameters (n= 8); 15 minute trawl blood and muscle (n=7); 45 minute trawl blood and muscle (n=11).

Blood parameters

Physiological stress parameters measured in stingarees immediately after the trawl were compared to a Base-line group measured in May 2005 (Table XI). In the 30 minute trawl group plasma lactate and glucose and potassium levels were significantly higher than the Base-line group. Conversely, in the 30 minute trawl group blood pH was significantly lower than the Base-line group. There were no significant changes in sodium or chloride.

Muscle parameters

IMP Load was significantly higher in the trawl group than the Base-line group. AEC for the trawl group was significantly lower than Base-line group. Similarly, the muscle glucose level was significantly lower in the trawl group than Base-line group (Table XI). Muscle lactate concentration was significantly higher in the trawl group than the Base-line group.

Table XI. Physiological stress parameters measured in blood and muscle of sparsely spotted stingarees immediately after 30 minute trawls in January 2005, as compared to the Base-line group measured in May 2005. Mean ± SE, treatments sharing the same superscript are not significantly different.

| Parameter | Base-line | 30 min. trawl |
|---|---|---|
| *Blood* | | |
| Lactate mmol.l$^{-1}$ | 0.41 ± 0.06 [a] | 3.34 ± 0.41 [b] |
| pH | 7.32 ± 0.06 [a] | 7.13 ± 0.05 [b] |
| Glucose mmol.l$^{-1}$ | 0.65 ± 0.05 [a] | 1.57 ± 0.16 [b] |
| Sodium mmol.l$^{-1}$ | 292.83 ± 4.61 [a] | 289.65 ± 3.53 [a] |
| Potassium mmol.l$^{-1}$ | 7.88 ± 0.51 [a] | 9.34 ± 0.32 [b] |
| Chloride mmol.l$^{-1}$ | 288.33 ± 4.36 [a] | 289.41 ± 4.26 [a] |
| *Muscle* | | |
| IMP Load | 0.54 ± 0.16 [a] | 1.25 ± 0.15 [b] |
| AEC | 0.93 ± 0.008 [a] | 0.87 ± 0.01 [b] |
| Glucose mmol.kg$^{-1}$ | 12.91 ± 1.72 [a] | 8.55 ± 0.52 [b] |
| Lactate mmol.kg$^{-1}$ | 7.88 ± 0.97 [a] | 19.24 ± 2.20 [b] |

Base-line blood lactate and glucose (n= 7), blood pH, sodium, potassium and chloride (n= 6) and muscle parameters (n= 8); 30 min. trawl blood lactate, pH and glucose and all muscle (n=18), blood sodium, potassium and chloride (n= 17).

*Port Jackson shark- Base-lines*

Base-line group physiological stress indicators measured in the blood of Port Jackson sharks are presented in Table XII. Lactate, glucose and potassium levels in the blood were not significantly different between any of the three Base-line groups. Plasma sodium and chloride values for the July 2005 Base-line group and the Base-line group RV *Ngerin* October 2003 were significantly different, however sodium and chloride values the LMSC October 2003 group were similar to the RV *Ngerin* October 2003 and the July 2005 group. Plasma pH values of all three Base-line groups were significantly different. The July 2005 group muscle IMP Load was $0.25 \pm 0.07$ (mean $\pm$ SE) and the AEC was $0.87 \pm 0.02$ (mean $\pm$ SE). Muscle glucose and lactate concentration of the July 2005 group were $11.13 \pm 2.84$ and $28.55 \pm 3.54$ (mean $\pm$ SE) respectively.

*Port Jackson shark- Experiment 1*

15 and 45 minute trawls, July 2005

Blood parameters

Plasma lactate level in the 45 minute trawl group were significantly higher than those measured in the 15 minute trawl group and Base-line group (Table XIII); however there was no significant difference between the base-line group and the 15 minute trawl group. There were no significant changes in plasma glucose, pH, sodium or chloride levels as a result of the trawls. Although plasma potassium appeared to decrease with trawl length, there was also no significant change in this parameter.

Muscle parameters

There were no significant changes in muscle IMP Load or the AEC as a result of the trawls (Table XIII). There was no significant difference in muscle lactate level between capture groups. Muscle glucose level was significantly higher in the base-line group sharks than in the 15 and 45 minute trawl groups; however there was no significant difference between trawl groups.

30 minute trawls, October 2003

Blood parameters

Plasma pH, sodium and chloride values for the 30 minute trawl group were all significantly higher than Base-line group (Table XIV), while the plasma potassium level was significantly

lower than the Base-line group. Plasma lactate and glucose levels and the in the 30 minute trawl group were not significantly different to the Base-line group level.

Table XII. Base-line group physiological stress indicators in blood of Port Jackson sharks collected in; October 2003, measured immediately after line capture on the RV *Ngerin* (n=5); after being held in the laboratory at the Lincoln Marine Science Centre (LMSC), (n=5); and following line capture on RV *Ngerin,* in July 2005 (n=4). Mean ± SE of groups sharing the same superscript are not significantly different.

| Parameter | RVN Oct 03 | LMSC Oct 03 | RVN-Jul-05 |
|---|---|---|---|
| Lactate mmol.l$^{-1}$ | 1.25 ± 0.37 [a] | 0.86 ± 0.20 [a] | 1.33 ± 0.27 [a] |
| pH | 7.60 ± 0.03 [a] | 7.91 ± 0.07 [b] | 7.01 ± 0.18 [c] |
| Glucose mmol.l$^{-1}$ | 2.15 ± 0.23 [a] | 2.54 ± 0.55 [a] | 2.07 ± 0.49 [a] |
| Sodium mmol.l$^{-1}$ | 341 ± 3.79 [a] | 323 ± 2.33 [ab] | 320 ± 2.96 [b] |
| Potassium mmol.l$^{-1}$ | 5.96 ± 0.20 [a] | 7.30 ± 0.76 [a] | 7.55 ± 0.38 [a] |
| Chloride mmol.l$^{-1}$ | 339 ± 3.27 [a] | 323 ± 4.96 [ab] | 313 ± 1.03 [b] |

*RV N Oct 03* (n= 5), except glucose (n=4); *LMSC Oct 03* (n= 5); *RVN-Jul 05* (n=4).

Table XIII. Physiological stress parameters measured in blood and muscle of Port Jackson sharks after 15 and 45 minute trawls, as compared to the Base-line group, measured in July 2005. Mean ± SE, treatments sharing the same superscript are not significantly different.

| Parameter | Base-line | Trawl | |
| | | 15 min. | 45 min. |
| --- | --- | --- | --- |
| *Blood* | | | |
| Lactate mmol.l$^{-1}$ | 1.33 ± 0.27 $^a$ | 1.58 ± 0.09 $^a$ | 2.20 ± 0.23 $^b$ |
| pH | 7.01 ± 0.18 $^a$ | 6.78 ± 0.06 $^a$ | 6.67 ± 0.07 $^a$ |
| Glucose mmol.l$^{-1}$ | 2.07 ± 0.49 $^a$ | 1.45 ± 0.12 $^a$ | 1.80 ± 0.24 $^a$ |
| Sodium mmol.l$^{-1}$ | 320 ± 2.96 $^a$ | 327 ± 1.28 $^a$ | 320 ± 3.17 $^a$ |
| Potassium mmol.l$^{-1}$ | 7.55 ± 0.38 $^a$ | 7.25 ± 0.41 $^a$ | 6.68 ± 0.26 $^a$ |
| Chloride mmol.l$^{-1}$ | 313 ± 1.03 $^a$ | 321 ± 1.63 $^a$ | 315 ± 3.84 $^a$ |
| *Muscle* | | | |
| IMP Load | 0.25 ± 0.07 $^a$ | 0.16 ± 0.03 $^a$ | 0.25 ± 0.12 $^a$ |
| AEC | 0.87 ± 0.02 $^a$ | 0.91 ± 0.01 $^a$ | 0.88 ± 0.01 $^a$ |
| Glucose mmol.kg-1 | 17.2 ± 4.72 $^a$ | 42.5 ± 2.07 $^b$ | 46.2 ± 8.00 $^b$ |
| Lactate mmol.kg-1 | 4.71 ± 4.68 $^a$ | 1.78 ± 1.20 $^a$ | 4.37 ± 3.31 $^a$ |

Base-line (n= 4); 15 minute trawl (n= 11); 45 minute trawl (n=10).

Table XIV. Physiological stress parameters measured in blood of Port Jackson sharks immediately after 30 minute trawls in October 2003, as compared to the Base-line group measured in July 2005. Mean ± SE, treatments sharing the same superscript are not significantly different.

| Parameter | Base-line | 30 min. trawl |
|---|---|---|
| Lactate mmol.l$^{-1}$ | 1.33 ± 0.27 [a] | 1.44 ± 0.11 [a] |
| pH | 7.01 ± 0.18 [a] | 7.67 ± 0.02 [b] |
| Glucose mmol.l$^{-1}$ | 2.07 ± 0.49 [a] | 2.42 ± 0.18 [a] |
| Sodium mmol.l$^{-1}$ | 320 ± 2.96 [a] | 344 ± 1.86 [b] |
| Potassium mmol.l$^{-1}$ | 7.55 ± 0.38 [a] | 7.02 ± 0.47 [b] |
| Chloride mmol.l$^{-1}$ | 313 ± 1.03 [a] | 341 ± 1.95 [b] |

Base-line (n=4). 30 min. trawl lactate, glucose, potassium and chloride
(n=31) and pH, sodium (n= 30).

*Port Jackson shark- Experiment 2*

Measures of physiological stress at 0, 2 and 4 hours after capture from 15 and 45 minute trawls, April 2004.

Blood parameters

Plasma potassium levels for all groups were significantly lower at four hours post-capture than those measured at 0 hours; however there was no significant difference between levels measured at 0 and two hours, or two and four hours, or between trawl groups and Base-line group (Table XV). There were no other significant differences in any other parameter measured, either between trawl groups and Base-line group, or over the four hour sampling period.

Table XV. Physiological stress parameters measured in blood of Port Jackson sharks at 0, 2 and 4 hours after capture from line caught fish (Base-line group) and 15 and 45 minute trawls (mean ± SE). Significant differences are not indicated.

| Parameter | Base-line | | | 15 minute trawl | | | 45 minute trawl | | |
|---|---|---|---|---|---|---|---|---|---|
| | *0 hrs* | *2 hrs* | *4 hrs* | *0 hrs* | *2 hrs* | *4 hrs* | *0 hrs* | *2 hrs* | *4 hrs* |
| Lactate mmol.l$^{-1}$ | 2.35 ± 0.73 | 2.93 ± 0.90 | 2.75 ± 0.82 | 2.34 ± 0.33 | 2.75 ± 0.81 | 2.88 ± 0.71 | 4.76 ± 0.91 | 6.81 ± 1.07 | 5.18 ± 1.08 |
| Glucose mmol.l$^{-1}$ | 2.03 ± 0.21 | 2.26 ± 0.18 | 2.35 ± 0.17 | 2.05 ± 0.25 | 1.93 ± 0.11 | 2.18 ± 0.20 | 2.17 ± 0.18 | 2.34 ± 0.26 | 2.58 ± 0.28 |
| Sodium mmol.l$^{-1}$ | 335.7 ± 8.3 | 331.0 ± 1.5 | 322.7 ± 4.2 | 326.5 ± 3.1 | 329.3 ± 9.9 | 327.8 ± 1.7 | 330.0 ± 3.8 | 323.7 ± 7.5 | 331.57 ± 3.6 |
| Potassium mmol.l$^{-1}$ | 6.63 ± 1.25 | 6.10 ± 0.55 | 6.40 ± 0.76 | 7.50 ± 0.33 | 5.25 ± 0.35 | 5.30 ± 0.52 | 7.51 ± 0.47 | 6.64 ± 0.78 | 5.24 ± 0.30 |
| Chloride mmol.l$^{-1}$ | 325.3 ± 4.2 | 326.7 ± 1.9 | 313.7 ± 6.1 | 322.8 ± 1.8 | 323.0 ± 0.9 | 322.5 ± 2.1 | 330.0 ± 4.1 | 320.1 ± 5.8 | 328.4 ± 2.9 |

Base-line (n= 3); 15 minute trawl (n=4); and 45 minute trawl (n= 7).

*Degens leatherjacket- Trawl stress*

15 and 45 minute trawls, April 2004

Blood parameters

Plasma lactate values for the 15 and 45 minute trawl groups (4.4 ± 0.5 and 5.5 ± 0.4 mmol.l$^{-1}$, respectively ) were significantly higher than the Base-line group (1.3 ± 0.1 mmol.l$^{-1}$), (Figure 10). Similarly, plasma glucose levels for the 15 and 45 minute trawl groups (3.1 ± 0.4 and 3.5 ± 0.3 mmol.l$^{-1}$, respectively) were significantly higher than Base-line group (1.1 ± 0.1 mmol.l$^{-1}$), as were plasma pH values . Plasma pH values for the 15 and 45 minute trawl groups were 7.73 ± 0.06 and 7.71 ± 0.04, respectively, as compared to the Base-line group level of 7.43 ± 0.03 (Figure 11). However, there were no significant changes in any of these parameters between the 15 and 45 minute trawl groups.

Muscle parameters

Muscle lactate level for the 45 minute trawl group was significantly higher than the Base-line group level (Figure 12), while there was no significant difference between the trawl groups or between the 15 minute trawl group and the Base-line group. Muscle glucose levels for the 15 and 45 minute trawl groups, 13.9 ± 0.9, 13.1 ± 1.0 mmol.kg$^{-1}$, respectively, (Mean ± SE) were both significantly lower than the Base-line group at 31.8 ± 1.9 mmol.kg$^{-1}$, (Mean ± SE). Similarly, IMP load for the 15 and 45 minute trawl groups, 1.77 ± 0.20 and 2.11 ± 0.14, respectively, (Mean ± SE) were significantly higher than the Base-line group level (0.37 ± 0.07; Mean ± SE, Figure 13), while AEC values for the 15 and 45 minute trawl groups, at 0.82 ± 0.02, 0.80 ± 0.01, respectively (Mean ± SE), were significantly lower than the Base-line group level (0.93 ± 0.00 Mean ± SE).

Figure 10. Blood lactate and glucose measured in Degens leatherjacket immediately after 15 and 45 minute trawls, April 2004, as compared to base-lines, April 2005, mean + SE. Treatments sharing the same superscript are not significantly different.



Figure 11. Blood pH measured in Degens leatherjacket immediately after 15 and 45 minute trawls, April 2004, as compared to base-line, April 2005, mean + SE. Treatments sharing the same superscript are not significantly different.

Figure 12. Muscle lactate and glucose measured in Degens leatherjacket immediately after 15 and 45 minute trawls, April 2004, as compared to base-lines, April 2005, mean + SE. Treatments sharing the same superscript are not significantly different



Figure 13. Adenylate energy charge (AEC) and the IMP load measured in Degens leatherjacket immediately after 15 and 45 minute trawls, April 2004, as compared to base-lines, April 2005, mean + SE. Treatments sharing the same superscript are not significantly different.

## Discussion

Investigation of the stress response in an animal implies that the normal resting levels of the parameters to be measured are known. If they are not, as noted by Campbell *et al.* (1993), they must be obtained within the investigation. The difficulty of obtaining resting levels of metabolites in fish is well covered by Love (1970) and although this text is now dated, the scenario described there is still relevant today. To the best of the author's knowledge there are no recorded normal or resting levels for the parameters used to measure stress in the species investigated in this study. Thus in order to obtain them, each species was sampled following as little capture disturbance as possible and from an environment where the animals would be expected to be in their normal active state. In addition, as with all the species examined in this study, during the trawling experiments, there is no way of ascertaining when an individual animal was captured in the trawl net. For this reason the results can only be indicative of the effect of the maximum trawl duration.

### Trawl stress and post-discard survival

*Blue swimmer crab- Base-line*

The blue swimmer crab (*Portunus pelagicus*) is a large (maximum carapace 210 mm) crab that inhabits sheltered sand and seagrass beds. This crab species is caught by recreational and commercial fishers throughout a distribution, which includes tropical waters and the South Australian gulfs (Edgar, 1997). This crab is a highly mobile aggressive animal that grows rapidly and reaches maturity in one year. Although this crab is a good swimmer, it is unlikely that it would be able to escape the trawl net and on capture it is probably swept to the cod end of the net or it may become entangled in the net on contact.

The collection of samples from resting active crabs was more difficult to achieve than anticipated. In October 2004 the crabs that were caught in traps at Wallaroo were necessarily handled during sampling more than what was considered desirable. For this reason, these crabs were not considered good candidates for the establishment of base-line parameters. In addition the hemolymph lactate level of this group and that caught in January 2005 (RV *Ngerin*), were higher than the LMSC group and the April

2005 group. The April 2005 group was chosen to best represent the Base-line for this species because they were quickly captured individually in drop nets and sampled immediately. This sampling regime is reflected in the parameters measured as the hemolymph lactate is lower than those crabs caught off the *Ngerin*; however unexpectedly the hemolymph pH is also lower than for any other group. This result is surprising as a low lactate level would indicate basal anaerobic metabolism and metabolic acid production. Bergmann et al. (2001) noted that the hemolymph pH and lactate level of creel – caught swimmer crabs, *Liocarcinus depurator* was also lower than trawl-caught animals. Bergmann et al. (2001) went to the trouble of running their experiment again but came up with a similar result. This result confirmation suggests that perhaps there is a mechanism for the regulation of hydrogen ion in exercised or stressed crustaceans (perhaps species specific) that is not understood. In the current study, the hemolymph and muscle parameters of the LMSC group and the April 2005 group of crabs are very similar. This result is surprising as the LMSC group were noted as being lethargic (i.e. moribund) when sampled. The LMSC crabs had a much higher muscle glucose level than the other groups and this might be a result of the low activity level of these crabs. Nevertheless, other than the higher muscle glucose and the noted lethargy of the surviving crabs, there were no obvious sign that these crabs were not in good health. Despite the low hemolymph pH of the April 2005 crabs, this group was identified as best representing active resting levels of the parameters measured for this species in the current study. This decision was based mainly on the appropriateness of the capture and sampling regime, rather than on any between-group comparisons of physiological parameters measured.

*Blue swimmer crab- Experiment 1*
Physiological stress effects of 15 and 45 minute trawls, April 2004, and 30 minute trawls, October 2003

15 and 45 minute trawls
Blood parameters
The Base-line group level of the hemolymph lactate is higher than that reported by Bergmann *et al.*, (2001) in trap caught swimming crabs (*L. depurator*) and squat lobsters (*Munida rugosa*) but similar to tank held western rock lobsters (*Panulirus cygnus*). In the current study, based on the sampling of crabs caught using different

methods, the lactate level of the Base-line group was considered be a good representation of the level of normal active blue swimmer crabs (*P. pelagicus*). The increase in hemolymph lactate level in crabs subject to trawl capture is indicative of animals subject to anoxia resulting in an increase in anaerobic metabolism. This switch to increased anaerobiosis is normally also associated with an increase in hemolymph glucose (Bergmann *et al.*, 2001, Paterson and Spanoghe, 1997, Vermeer, 1987) but this was not observed in the current study. The hemolymph sodium and chloride levels of the Base-line group crabs was within the range recorded for western rock lobster (*Panulirus cygnus*) by Paterson *et al.*, (2005) however the potassium level in the Base-line group crabs was slightly higher than the that recorded by Paterson *et al.* (2005) and Roberts (2001), for the unstressed southern rock lobster (*Jasus edwardsii*). It was also noted that hemolymph pH was within the range recorded for crustaceans (Bergmann *et al.*, 2001, Roberts, 2001). It is not possible to compare the levels of parameters measured in the current study to those of other studies on blue swimmer crabs as to the best of the authors knowledge there is no published data. In the current study the change in hemolymph pH in response to trawl stress was surprising as it was higher than the Base-line group level despite the higher level of hemolymph lactate. This occurrence may be accounted for by noting that the Base-line group pH may well have been inexplicably lower than expected for unstressed crabs. There was some indication that potassium level dropped from the Base-line group level in trawl-caught crabs, particularly the 45 minute trawled group. As potassium is generally thought to be intracellular, the mechanism by which it would drop in response to trawl capture is not known. Wheatly and Henry, (1992) point out the there may be extracellular compartments within the carapace of crustaceans that contain intracellular fluid. If this is the case it may well be that ionic regulation in crustaceans may not be conventional or conform to that which is understood for vertebrates. Aside from this there is evidence that the trawl capture of crabs in the current study does cause changes in some of the parameters measured, indicating an increase in anaerobic metabolism and altered ionic regulation.

Muscle parameters

The Base-line group AEC of the muscle of the crabs in the current study was similar to that recorded for unstressed prawns *Penaeus monodon* and *P. japonicus* (Paterson, 1993) and rock lobsters *J. edwardsii* (Morris and Oliver, 1999, Speed *et al.*, 2001).

The extrapolated IMP Load of the unstressed crustaceans investigated within these studies was also similar to that reported for the Base-line group crabs within the current research. Interestingly in the current study the IMP Load and AEC of the crabs subjected to trawl capture were very different to that recorded for the Base-line group indicating a shift in the energy level of the muscle cells of the trawled crabs. The IMP Load increase indicates that there was a utilisation of ATP that resulted in the enzymatic conversion of accumulating adenosine monophosphate to inosine monophosphate. The change in AEC for the trawled crab muscle is an indication that the homeostasis of the muscle cells was challenged, which resulted in an imbalance of the energy compounds within the cells that helps to maintain normal cell function. It is difficult to determine if this disruption to cellular energy balance was a result of anoxia or increased activity during the capture process but it is likely that it was a result of a combination of factors associated with the trawl capture. This explanation is supported by the increase in hemolymph lactate; however contrary to this there was no rise in muscle lactate and this would be expected in crabs that were operating more anaerobically than usual. Overall the results here indicate that trawl stress can cause changes in the muscle energy balance in blue swimmer crabs which may influence the ultimate survival of the fish post release.

Damage

There was a higher level of damage sustained by crabs that were trawled than that found in net caught animals sampled in order to establish base-line parameters. This result is not surprising as the appendages of the blue swimmer crab are easily lost; that is except for the swimmerets, which the crab must maintain if it is to remain mobile. Even though there was no significant difference between the total damage score of the 15 and 45 minute trawl groups it is interesting to note that number of crabs with 2 or more legs missing increases dramatically from the 15 to 45 minute trawl. This rise in limb loss suggests that trawl length will have an effect on leg loss of this crab and it is likely that this may also affect the ability of the crab to survive predation post release. However the current study did not examine post release predation of by-catch.

Blood parameters

When the hemolymph parameters of crabs captured during a 30 minute trawl was compared to Base-line group levels, there is, as with those captured during 15 and 45 minute trawls, an associated increase in plasma lactate level. Again there was not the expected drop in hemolymph pH that is associated with the acidosis of anaerobic metabolism. Also, hemolymph glucose level remained unchanged by trawl capture and this supports the idea that glucose may not be a particularly good indicator of stress in crustaceans (Paterson *et al.*, 2005) and that stressed crustaceans are not necessarily hyperglycaemic (Hall and van Ham, 1998). There was some evidence that the crabs caught within the 30 minute trawl had a disruption to osmoregulation. Sodium and chloride hemolymph levels were higher in trawled crabs than the net-caught Base-line group crabs and again hemolymph potassium level was higher in the net-caught crabs than the trawled. In combination these hemolymph ion concentrations show a shift from the Base-line group levels and may well indicate that trawled crabs suffer a disruption to ionic regulation following capture by trawling.

*Blue swimmer crab- Experiment 2*

Post-trawl physiological stress response and post-discard survival in the blue swimmer crab, *Portunus pelagicus*, October 2004.

Survival

That all the crabs subjected to the trawl survived indicates that this species is quite hardy. There is no better way to estimate the post-release survival of an animal following capture than to observe if it dies after it is released back into the environment it came from. However there is considerable debate about the length of time that the released fish need to be observed before they are considered "survivors" (Ryer, 2004) and there is also the problem of how you keep the released fish under observation. In the current study, we chose to contain the animals in cages; however this is never exactly the same as the conditions the fish would be exposed to if they were simply returned to the sea, from the deck of the fishing vessel, following the trawl. We also chose to keep the animals under observation for 3 days post release. It is not known if crabs would have eventually died if they were held for a longer observation period.  On the other hand, if there had been deaths, that occurred later

than 3 days post release, it would have been more difficult to attribute the deaths to the effects of the trawl.

Damage

That there was no difference in the damage scores of trawled crabs that were killed and sampled and those deployed in the sea-cages for 3 days provides a consistent start point for the observations made within this experiment. The slightly higher damage score of the crabs held for 3 days in cages indicates that there was some limb loss associated with the experimental recovery procedure that was followed. However the increase in damage was small and apparently had no impact on the survival of the crabs, over the recovery period of 3 days. This result is not surprising, as it was observed that these crabs readily lose their limbs, which is probably a survival mechanism associated with allowing escape from predators. It is most likely that limb loss would only be a significant problem when the limb loss included swimmerets (loss of mobility) or claws (loss of defence mechanism). A hole in the carapace may also be a problem in that the loss of hemolymph might be more likely and the damage may provide a portal of entry into the crab's body cavity. In the current study there was some claw loss and swimmeret loss was not categorised; however there was no recorded carapace damage.

Blood parameters

From the results of the hemolymph parameters, the crabs that were placed into the sea-cages for 3 days appeared to recover. In these crabs, the hemolymph lactate level fell and the pH rose and this suggest a reduction in anaerobic metabolism and removal of metabolic acid from the hemolymph. It was also noted that following 3 days in sea-cages the hemolymph lactate level was similar to the Base-line group level established for this species; however the pH of the hemolymph was still well above the Base-line group level. There was little change to hemolymph ion concentrations from the Base-line group levels with the exception of potassium, which fell in crabs held in the sea-cages. Because this meant that the potassium level of the sea-cage crabs was lower than that recorded for Base-line group, it is difficult to know if this indicated a recovery response for this species.

Muscle parameters

The muscle parameters changed little in the sea-cage crabs compared to those recorded in crabs following the trawl; with the exception of muscle lactate which fell in sea-caged crabs to a level much lower than that previously measured in any crabs (including the Base-line group). The reason for this dramatic change in lactate may have been that the crabs were housed in such a manner that they were almost completely immobile. As this is a highly mobile species this lack of activity may have resulted in a shift in the partitioning of aerobic and anaerobic metabolism and the subsequent lactate production was reduced to a very low level.

*Blue swimmer crab- Experiment 3*

The effect of physical interaction at capture on the post-discard survival of the blue swimmer crab, *Portunus pelagicus*, July 2005

Survival

This experiment investigated the effect of the physical interaction of crabs that may occur during capture and sorting. We obtained all the crabs used in this experiment from 45 minute trawls but screened the crabs so as to remove those that may have been damaged during the trawl. This protocol allowed us to look specifically at the effect of the on-deck interaction on crab damage and survival. That there were more deaths in the groups of crabs that were allowed to interact than those that were kept apart from one another tells us that the interaction of crabs during capture and sorting contributes to the death rate. Further to that the longer the crabs were allowed to interact the more likely they were to suffer a wound that would result in death.

Damage

The actual survival rate of the crabs tells us little about the reason for their death other than it was directly connected to the interaction time. The damage rating gives us more information about the effect of the interaction time on the crabs. The total damage score was higher than the control than for all interaction times, but there was no difference in damage score for any length of interaction. However if we look at the actual damage categories and note the observation that the crabs that died were clearly eaten out by sea lice, it can be seen that the 'hole in the carapace' category accounted for the deaths in the crabs. This relationship exists because a hole in the carapace

provides a portal of entry for the sea lice to attack the crab. It should be noted that although the crabs with a hole in the carapace were those that died and were attacked by sea lice, we have no way of knowing if the crabs were dead prior to the sea lice attack.

<u>Blood and muscle parameters</u>

That there was no difference in the hemolymph, other than the pH, and muscle parameters of the crabs subjected to the interaction treatments indicates that the treatment had little effect on the parameters measured, or that the 3 days spent in sea-cages provided adequate time for crabs to recover from the interaction treatment. There is an indication that hemolymph pH may be lower in crabs that were subject to the interaction treatment; that is, in the recovered crabs, the hemolymph pH appeared to be lower in those that were subject to the extended interaction treatments.  As there was no evidence of increased lactate in the crabs with the lowered pH the result is unlikely to be due to metabolic acidosis associated with anaerobiosis.  Although the lower hemolymph pH of the crabs appears to be associated with the interaction treatment the reason for this effect is not clear.  It was noted that the blood and muscle parameters of the crabs recovered from the sea-cages in this experiment indicated that these crabs could be considered more "rested" than the crabs sampled for base-line comparisons in the previous experiments.  This scenario would seem to indicate that the compartments used to house the crabs in the sea-cages were appropriate in terms of providing a suitable holding environment, which also restricted crab movement. As previously noted, the results here indicate that interaction between crabs that results in a hole in the body carapace or retained limbs is likely to result in crab death through sea lice attack.

*Sparsely spotted stingaree- Experiment 1*
<u>15 and 45 minute trawls</u>
This ray can be described as a small, i.e. a maximum length 38cm, (Edgar, 1997) shy benthic fish species that spends most of its time partially buried in the sand or foraging for food. The ability of this fish to avoid the trawl net is probably very low as would its ability to swim within the confines of a moving trawl net. It is most likely that, on capture, this fish is immediately swept to the cod end of the net and remains there until catch is sorted on deck. The Base-line group levels recorded for this

species were one of the most difficult to obtain of those studied. This difficulty was experienced because these fish could not be caught on handlines and were not readily found or easily captured, other than during the trawls. Therefore to establish the base-line parameters, we caught animals during trawls and allowed them to recover in captivity, for 4 weeks, prior to sampling. As the survival of the fish post-trawl and in the deck tanks was low we also had difficulty obtaining an adequate number of fish that we were confident had recovered from the trawl capture method. The base-line parameters for this fish should be viewed with this information considered.

Blood parameters

The increase in plasma lactate and glucose over the Base-line group level indicates that there was a mobilisation of energy and an increase in anaerobic metabolism in fish that were subject to trawling. Although the level of lactate following the trawl was low compared to the levels recorded for other elasmobranches (Jenkins *et al.*, 2004), the level in trawled rays of the current study is twice and three times as high, for the 15 and 45 minute trawled fish respectively, as the Base-line group. This increase in carbohydrate metabolites may have been due to sustained swimming during the trawling process but is more likely due to a hypoxia induced by crushing in the cod end of the trawl net. The drop in plasma pH for fish that were trawled is consistent with the increase in plasma lactate and the accumulation of hydrogen ions in the plasma, associated with the production of lactate in the muscles. In a fish that is noted as having limited ability to metabolise or excrete lactate (Murdaugh and Robin, 1967), the rise in plasma lactate concentration and indicative drop in plasma pH is likely to represent a significant stress response.

The lack of change in the plasma electrolytes in fish subject to the trawl was a little surprising as this species is relatively soft bodied and subject to physical damage (see *Damage* section) during the trawl and this may result in potassium leakage into the extracellular fluid. In addition Eddy, (1981) notes that capture stress can have a profound effect on the salt and water balance of sea-water fish that results in elevated sodium and chloride levels. Piiper and Schumann, (1967) also note that in elasmobranches water shifts out of the vascular compartment in response to raised intracellular lactate or increased sodium in the blood.

<u>Muscle parameters</u>

Although the rise in muscle lactate concentration in trawled rays was not significant compared to the Base-line group, the levels in the trawled fish did tend to be higher and more variable than the base fish level. This variability is probably due to the fish being caught at different times during the trawl and may be due to their position within the mass of trapped matter that ends up in the cod end of the net. Interestingly, if the muscle glucose and lactate concentrations are looked at together, within the groups of fish, it can be seen that in the Base-line group fish glucose is higher than lactate. The 15 minute trawl group have similar levels to each other and lactate concentration is higher than glucose following 45 minute trawl. This indicates a utilization of the available glucose resulting in an anaerobic accumulation of muscle lactate that increases as a result of trawl duration.

The level of inosine monophosphate in comparison to the adenylate total pool (i.e.IMP Load) remains much the same for the trawls and in comparison to the Base-line group. This is similar to the AEC of the muscle of the groups as although the 15 and 45 minute trawl group have a significantly higher AEC than the Base-line group, the difference is small and may not be biologically significant in this situation. Utilisation of ATP in fish induced to exercise may show a disrupted adenylate pool ratio, which indicates a disruption of homeostasis. As IMP is formed through the deamination of adenosine monophosphate by AMP deaminase, the maintained IMP Load of trawled stingaree may reflect the enzymatic capability of this animal to convert AMP to IMP. This lack of enzymatic capability has been noted in other species that have behaviour described as sedentary or low activity (Paterson, 1993).

<u>Damage</u>

That the damage sustained during the trawl did not increase with trawl length was not surprising. These fish end up in the cod end of the net and once there are immobilised by the weight of other trapped animals and organic material (personal observations). When this happens they have likely sustained all the physical damage they are likely to; therefore longer trawl time will not change that. Nevertheless, a ~40% damage score represents a considerable amount of trauma and is likely to contribute to stress responses and survival of the fish (Wassenberg and Hill, 1989). However it was also surprising that the damage sustained here by the trawled fish did not result in muscle

damage and a subsequent increase in the plasma potassium concentration as a result of muscle cell disruption and damage.

*Sparsely spotted stingaree - Experiment 2*

<u>Survival</u>

That only one stingaree, left for three days in the sea-cage, survived was interesting but an inconclusive result in determining the survival of released fish post-trawl. Because it was not possible (due to rough weather) to make diver observations of the moored sea-cages containing the stingarees, it was also not possible for us to know when the fish were eaten and if they were dead prior to being eaten. We can be reasonably certain that the stingarees were consumed by sea lice, as previous observations by Svane (pers com) indicate that sea lice are an active consumer of carrion in this area. However, it is not known if sea lice will consume live fish. One of the shortcomings of our survival test methods is that although the fish are placed back in their natural environment they are caged. This cage environment excludes large predators that may prey on fish, which may be moribund or wounded from the trawl, but allows no escape for the fish from predators that can enter the cages, such as sea lice. There has been a great deal of attention focused on estimating the survival of fish released by recreational anglers (Mcleay *et al.*, 2002). Nevertheless, the unavoidable bias associated with the methods necessary to house fish for a reasonable post release observation period makes it difficult to relate observed survival directly and exclusively to the method of capture and or post-capture treatment. In the current study we are able to combine the survival results of this experiment with our observation of the hardiness of this species. We noted that the stingarees that were caught by trawling were very difficult to keep alive in deck tanks on the *Ngerin*. On one occasion that we were successful in maintaining this species in tanks, those that we were able to keep alive were transported back to the laboratory and maintained for 4 weeks prior to killing and sampling in order to provide base-line levels of our measured parameters. As previously noted, obtaining base-line levels for any species examined for their response to stressors is imperative. If more conclusive information were to be obtained on the post-trawl survival of the sparsely spotted stingaree it would be necessary to direct more effort into obtaining base-line information and the appropriate design of open sea-cages that address the problem of providing a suitable habitat for the released post-trawl animals to be observed and assessed.

<u>Blood parameters</u>

There was approximately an 8-fold increase in the plasma lactate level in the stingarees that were subjected to the 30 minute trawls. The level of plasma lactate increase is similar to the 10-fold increase reported in exercised dogfish by Zammit and Newsholme, (1979) and the Base-line group level is very close to that recorded for the shovelnose ray *Rhynobatos typhus* by Lowe *et al.*, (1995). Plasma glucose level also rose and plasma pH was lower than the Base-line group, and these combined with the increased plasma lactate indicate that the 30 minute trawls were a significant stressor for this species. That there was no change in plasma sodium or chloride as a result of the 30 minute trawl probably indicates that the branchial mechanics of the gill retained regulatory functions in this situation, or it is possible that there was insufficient time for the effect of the trawl to be seen before the fish were sampled. Eddy, (1981) notes that in most marine teleosts, even a moderate stress may disrupt ionic regulation, however earlier work by Beggs *et al.*, (1980) and Oikari and Rankin, (1985) shows that plasma sodium and chloride remains fairly constant in teleosts during stress. However the response of stingaree is likely to be different to teleosts as in the current study, despite obvious metabolic responses to the trawl, plasma sodium and chloride in 30 minute trawled fish changed very little and was not interpreted as being disrupted. Generally a proper level of potassium is essential for normal cell function and as potassium is a major intracellular cation, fluctuations in plasma potassium level may indicate muscle cell damage or leakage from muscle cells that may have been a result of intracellular acidosis or changes to carbohydrate metabolite level (Cliff and Thurman, 1984, Houston *et al.*, 1971). In the current study the level for the Base-line group ($\sim$7.9 mmol.l$^{-1}$) was higher than that recorded for resting level in the shovelnose ray *R. typus* (Lowe *et al.*, 1995) and the bonehead, blacktip and bull shark levels noted in Manire and Hueter, (2001). Cliff and Thurman, (1984) note that in terrestrial animals a plasma potassium level approaching 7 mmol.l$^{-1}$ (considered hyperkalemia) can result in myocardial dysfunction. Later, Lowe *et al.*, (1995) suggest that this plasma potassium level may also have implications for cardiac function in elasmobranches. In the current study we observed an increase in plasma potassium level that appears to mirror the increases in plasma carbohydrate metabolites that resulted from trawl capture. In addition, following the 30 minute trawl the plasma potassium level ($\sim$9.3 mmol.l$^{-1}$) is well above the level

expected to affect myocardial / muscle function. It is not known for sure if plasma potassium level increases of the magnitude seen in the stingarees following the trawls have the capacity to cause mortality, however considering the implications of increased extracellular potassium for terrestrial animals, it is possible that the response to trawl capture, recorded in the current study, is indicative of a response to a stressor that has the capacity to result in fish death.

Muscle parameters

In contrast to the sparsely spotted stingaree- Experiment 1 results, there was an increase in IMP Load and a decrease in AEC ratio in the muscle of fish subject to 30 minute trawls compared to the Base-line group. As in Experiment 1, the change in AEC ratio was small but in combination with the relatively large in IMP Load indicates that there was a shift in the adenylate levels in the muscle in response to the trawl capture. The increase in IMP Load indicates a shift in the total adenylate pool to inosone monophosphate (IMP) through the action of AMP deaminase. The muscle carbohydrate metabolite levels also change in response to the 30 minute trawl, with glucose declining and a concurrent accumulation in muscle lactate. This scenario is consistent with the utilisation of muscle energy through anaerobic metabolism and the accumulation of the anaerobic metabolite, lactate that occurs in teleost and elasmobranch fish (Milligan and McDonald, 1988). The relative level of lactate and glucose in the muscle and plasma of the stingarees, and the changes that can be attributed to the trawl capture in Experiments 1 and 2 indicate that this species is likely to deal with increased muscle lactate via in situ glycogenesis within the muscle. This metabolic mechanism has also been noted as being important in sedentary teleosts, particularly when compared to active species (Barnett and Pankhurst, 1998) and is associated with minimising the loss of energy from the muscle to other tissues or to the environment. In the current study the apparent build up of muscle metabolites in stingarees, similar to other sedentary elasmobranches that have limited capacity to deal with stress related metabolite accumulation (Murdaugh and Robin, 1967), may be a critical factor in the post-trawl recovery and ultimate survival of this species.

*Port Jackson shark- Base-line*

This small shark is most common around rocky areas but is also found over sand and mud and amongst seagrass. As with the sparsely spotted stingaree, the ability of this fish to avoid the trawl net is probably low, as would its ability to swim within the confines of a moving trawl net. However based on its' physical attributes and swimming ability, it was considered that this species would be able to cope with the physicality of the trawl capture operation better than that of the stingaree. Unlike the stingaree this fish has a robust exterior in that it has a thick leathery skin over a muscular elongate body. Nevertheless in common with the stingaree, it is most likely that on capture, this fish is almost immediately swept to the cod end of the net and remains there until the catch is sorted on deck.

The base-line levels recorded for this species were obtained from sharks caught opportunistically on handlines between trawls in July 2005 although, as reported, other methods of capture were also used. There were some differences in the level of plasma parameters measured at each sample collection however muscle samples were only taken from sharks caught in July 2005. For this reason the results of the blood and tissue samples collected form the sharks caught in July 2005 were used to represent the base-line levels for this species.

In contrast to the stingarees the survival of the shark post-trawl and in the deck tanks was high (no deaths reported). In addition, as this shark was the subject of intensive study that involved ample opportunity to observe post release survival of trawled individuals, a specific survival experiment was not carried out on this species.

*Port Jackson shark- Experiment 1*
15 and 45 minute trawls July 2005
Blood parameters
The base-line group level of plasma lactate for the Port Jackson sharks (~1.3 mmol.l$^{-1}$) was higher than for the stingarees measured within this study, but similar to the levels in line caught dogfish (Murdaugh and Robin, 1967) and considerably lower than the shark species investigated by Manire (2001). These differences in Base-line group plasma lactate levels are probably due to interspecific variation rather than sampling artefacts and the levels recorded for the Port Jackson sharks in the current study were

considered low and a good representation of resting levels for this species. In the current study the only parameter indicating that the sharks responded to being captured by trawls was the $\sim$ 60% increase in the plasma lactate over the Base-line group level, of those subject to the 45 minute trawl duration. This situation, coupled with a trend of declining plasma pH with increasing trawl length, indicates that there was some movement of hydrogen ions to the plasma, which was probably as a result of lactic acid formed through anaerobic metabolism and initiated in response to the trawl capture. In a trawl capture situation it would be expected that there would be a mobilisation of stored energy (glycogenolysis) through the action of catecholamines and corticosteroids (Janssens and Waterman, 1988), which would be seen as an increase in plasma glucose. In the current study there was no evidence of an increase in the glucose concentration in response to trawl capture. This apparent lack of response may indicate that the sharks did not have an endocrine response to the trawl capture, or that the mechanism of that response is different to the model that is understood for teleosts. The plasma sodium and chloride levels of elasmobranches appear to vary with species. That is, the resting plasma sodium level of the sharks investigated by Cooper and Morris (1998) are similar to that recorded for the Port Jackson sharks in the current study and those recorded for the tropical sharks investigated by Manire and Hueter (2001). However the plasma the chloride levels are considerably higher in the Port Jackson sharks, reported currently and by Cooper and Morris (1998), than those reported by Manire and Hueter (2001). The significance of the differences between species is unknown, but the levels recorded for Port Jackson sharks in the current study appear to represent a base-line this species. In the current study, that there was no significant change to the plasma sodium and chloride levels of sharks subject to trawl capture indicated that there was no disruption to regulation of these ions associated with capture. This maintenance of ionic balance is consistent with the response of the stingarees and indicates that these species may not be susceptible to disruption of osmoregulatory function that is obvious immediately following capture stress. The Base-line group Port Jackson shark plasma potassium level recorded in the current study ($\sim$ 7.6 mmol.l$^{-1}$) is slightly higher than the resting level ($\sim$ 4.5 mmol.l$^{-1}$) recorded for the same species by Manire and Hueter (2001). In the current study there is a trend that indicates that plasma potassium may be falling with increased trawl time, however the changes are not significant and the mechanisms that would lead to a fall in plasma potassium following trawl capture are

not apparent. Overall, other than the slight increase in plasma lactate level of sharks captured following 45 minute trawls, the results obtained give little indication of a stress response in Port Jackson sharks that can be attributed to trawl capture or the duration of the trawl.

Muscle parameters

There was no evidence to suggest that trawls of 15 or 45 minute duration significantly changed the energy status of the muscle of captured Port Jackson sharks. This lack of response is in contrast to the stingarees captured during the 30 minute trawls, where there was evidence of a build up of inosine monophosphate (i.e. higher IMP Load) and a shift in the adenylate ratios that resulted in a lower AEC. However it should be noted that the stingarees captured in the 45 minute trawls of experiment 1 did not show any change in IMP Load or AEC either. The increase in muscle glucose following trawl capture is difficult to explain and was not associated with a change in muscle lactate, attributable to trawl capture. Nevertheless based on the results here it appears that overall the Port Jackson sharks are less affected than the stingarees by trawl capture.

30 minute trawls, October 2003

Blood parameters

There was no evidence to suggest that the sharks subject to the 30 minute trawl increased their level of anaerobic metabolism or had an endocrine response that resulted in an increase in plasma glucose. That is, the aerobic metabolic capacity did not appear to be exceeded and the need for an increase in the amount of immediately available energy did not appear to change from that of the Base-line group. The elevated plasma sodium and chloride level in the sharks subject to trawl capture may have been result of ion regulation changes that could possibly have been caused by the capture process. The lower plasma potassium concentration of the trawl-caught sharks is difficult to explain as cellular disruption and muscle cell damage is generally expected to result in the movement of potassium from the cells in to the extracellular fluid. That said the level of plasma potassium tended to fall from the Base-line group level in sharks caught in 15 and 45 minute trawls as well. This drop in potassium level is in contrast to the trawled stingarees where plasma potassium was noted as elevated in trawl-caught animals. It is possible that there is some mechanism of ion regulation

disruption in the Port Jackson sharks that causes a fall in plasma potassium in sharks subject to trawl capture, however at this time that mechanism is not understood.

*Port Jackson shark- Experiment 2*

Measures of physiological stress at 0, 2 and 4 hours after capture from 15 and 45 minute trawls, April 2004.

Blood parameters

The lack of change in the blood parameters of sharks sampled following hook capture (Base-line group) and those subject to trawls of 15 or 45 minutes gives an indication of the hardiness of this species. There was some indication that the plasma lactate was higher in sharks sampled following 45 minute trawls compared to the others but the differences were small and not statistically significant. It was noted that the plasma lactate levels of the line caught sharks sampled immediately following capture was generally higher than that measured in the previous experiments. However these lactate levels were still within the range reported for other elasmobranches (Murdaugh and Robin, 1967, Manire and Hueter, 2001). Plasma potassium levels of line caught sharks were slightly lower or similar, in this experiment, to sharks sampled in experiment 1, but still higher than that reported in Manire and Hueter (2001), for resting sharks. In the current experiment, there was no evidence to suggest a disruption to potassium regulation that could be attributed to the trawl duration; however there was a drop in the plasma potassium during the 4 h of recovery for every group. This fall in potassium level may indicate that there was a disruption to potassium regulation associated with capture which was compensated for during the 4 h the sharks spent in the on-deck tanks.

Overall there were only small changes to plasma and muscle parameters that could be attributed to trawl captures stress in the experiments carried out in this study. In addition the blood parameters measured in sharks, allowed to recover for 4 h in on-deck tanks, shifted very little from those measured immediately following capture. It appears that this shark is particularly robust and well able to cope with the physicality of trawl capture.

*Degens leatherjacket- Trawl stress*

15 and 45 minute trawls, April 2004

The Degens leatherjacket (*Thamnoconus degeni*) is reported to reach a maximum length of 29 cm (Edgar, 1997) but the fish caught during this study were all smaller than this. They are a schooling species and represent a significant component of the Spencer Gulf prawn fishery by-catch. The species is not considered to have an ability to sustain a swimming effort that would allow it to escape the trawl net and it is likely that it would be swept to the cod end of the net following a short period of swimming.

In the current study, the Degens leatherjackets that were sampled from the trawl were those that were deemed to be alive following the trawl. We previously established that approximately 46% of the leatherjackets subject to 15 minute trawls and 78% subject to 45 minute trawls did not survive. That established, the results that follow are relevant only to the 54% and 22% of the live leatherjackets from the 15 and 45 minute trawls respectively.

Blood parameters

The level of plasma lactate and glucose in the Base-line group fish was similar to that recorded in other resting/active finfish (Milligan and Girard, 1993, Lowe *et al.*, 1993, Barnett and Pankhurst, 1998) and was considered a reasonable indication of resting levels in this species. The response of leatherjackets subjected to trawl capture is consistent with that reported for fish subjected to capture, confinement and associated increased activity (Wood and Perry, 1985). Plasma lactate is an indication of anaerobic activity in fish and increased plasma glucose has been reported in fish subjected to stress (Robertson *et al.*, 1987) and is the end result of the endocrine initiated glycogenolysis that mobilises energy reserves within the fish. It would be expected that plasma pH would fall as the lactate increases in response to increased anaerobiosis subsequent to metabolic acidosis. That pH was higher in those fish that were subject to the trawl capture is unexpected but may have been due to the fish removing hydrogen ions across the gills to the surrounding water. That there was no difference between the plasma metabolites measured in fish sampled from the 15 and 45 minute may well be an artefact of the sampling regime. That is because we only sampled "survivors" and as there is no way of estimating the time of capture of individual fish, it is likely that the "survivor" from both the trawl times had been subject to similar capture times within the net. This situation would mean that their stress responses would also be similar.

Muscle parameters

The level of lactate in the muscle of the leatherjackets that were hook-caught was high but in the range of other finfish species. And although this range of lactate was used as an estimation of active resting leatherjackets, it is possible that these fish were exercised prior to capture. The glucose level is more difficult to compare to the reported literature but is within the range of that for glucose-based molecules found in the muscle of other fish species sampled following minimal capture disturbance, however the response of the muscle of the fish subject to trawl capture, was consistent with an increase in activity and the utilisation of glucose-based energy reserves.

Similar to the blood parameters, all of the muscle parameters measured in the 15 and 45 minute trawl groups were significantly different to the Base-line group; however the trawl groups were not significantly different to each other. Following trawl capture the available muscle energy in the muscle of the leatherjackets was depleted to less than half that of resting fish muscle. In addition, anaerobic metabolism increased and there was a subsequent build up of lactate in the muscle resulting in levels that were higher than that of the Base-line group. The IMP Load increased by 4 to 5 times that of the Base-line group fish muscle and this indicates that there was considerable muscular activity in these fish during capture, which resulted in a loss of ATP and subsequent build up of the metabolite of this reaction, inosine monophosphate (IMP). The conversion of ATP to IMP in fish muscle functions to maintain the ratio of the adenylate pool and a shift in this ratio indicated that the depletion of ATP exceeds the capacity of the muscle to maintain homeostasis. The change in homeostasis is indicated by a lower AEC. In the current study there was a small but biologically significant drop in the AEC of the trawl captured leatherjackets compared to the line-caught (Base-line) group. As with the changes to blood metabolites the similar level of the metabolites in the muscle of the leatherjackets subject to 15 and 45 min trawls is likely a result of the fish that were actually selected for sampling ('survivors') being subject to a similar level of time within the net and therefore capture stress. As a result of our sampling method, for this species of leatherjacket we have an estimate of survival that eliminates obviously compromised fish, which are likely to die when returned to the sea, from our tissue sampling regime.

# Conclusion

This research investigated the stress response of the blue swimmer crab, sparsely spotted stingaree, Port Jackson shark, and the Degens leatherjacket. We examined the relationship between trawl time and fish stress and where possible, estimating survival of fish collected by trawling in Spencer Gulf of South Australia.

Overall, irrespective of time, trawl capture resulted in a response that is consistent with that recognised in animals subject to stress. More specifically, for the blue swimmer crab, trawl capture generally resulted in higher hemolymph lactate and lower pH than the Base-line level. Muscle energy balance was generally disrupted in trawl-caught crabs and post-capture damage and crab death increased as post-capture time increased. Post-capture crab death was due almost totally due to holes in the carapace or retained limbs.

The sparsely spotted stingaree generally showed a clear stress response to trawl capture, with higher plasma glucose and lactate and lower pH than the Base-line group. In addition energy ratios changed, with AEC being lower and IMP Load higher in 30 minute trawled fish. Post-trawl survival of this species was estimated as being low.

The Port Jackson shark showed little physiological response to trawl capture. However muscle glucose level was elevated in sharks subject to 15 and 45 minute trawls compared to the Base-line group. In addition this species showed slightly elevated plasma lactate in response to trawl duration and small shifts in plasma ions. Generally, this species appeared unaffected by trawl capture and no shark death occurred during trawls or in tanks during post-capture observations and experiments. For trawl captured Degens leatherjacket approximately 50-75% did not survive. Of the surviving fish, subject to trawl times of 15 and 45 minutes, plasma land muscle parameter changes indicated a considerable stress response, with clear increases with trawl time, and this response was likely to contribute to the fish death.

This study shows that the stress response to trawling is species specific and it follows that the consequences of stress induced by trawling will also be interspecific. In this study, with the exception of the Port Jackson shark, the species investigated showed a considerable stress response and evidence of physical damage following trawling. The stress response was typified by a mobilisation of energy reserves and an increase in anaerobic metabolism. In addition, following trawling muscle adenylate ratios altered and there was a disruption to ionic regulation in some species. It is reasonable to expect that the level of physical damage sustained and magnitude of the stress response of any particular species will effect post trawl survival of released animals. However, although it is likely that may bycatch species will show signs of trawl induced stress similar to those demonstrated here, it is recommended that species identified as " vulnerable" or that may be of particular interest, should be examined individually.

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## Appendix I. Trawl stress in other species

Physiological stress indicators were measured in other, opportunistically sampled species, but it was not possible to collect base-lines for these species, apart from the southern fiddler ray. However, only one southern fiddler ray was collected from the trawl, which did not allow for statistical comparison. Data from those species collected from both 15 and 45 minute trawls- rough leatherjacket and sand trevally, were statistically compared using one way ANOVA SigmaStat, version 3.1. For species collected from one trawl duration, where statistical analysis was not undertaken, empirical comparisons only are discussed.

**Sampling**

*Rough leatherjacket and sand trevally*

Experimental sampling

Rough leatherjacket and sand trevally were collected from 15 and 45 minute trawls conducted in October 2003. Rough leatherjacket were caught at Site 4 (Wallaroo), and sand trevally were caught at Site 4 (Wallaroo) and Site 5 (The Gutter). Blood and muscle were sampled as per methods described for Degens leatherjacket. Due to the small size of the fish ($102.79 \pm 1.41$ and $109.94 \pm 1.68$ mm for rough leatherjacket and sand trevally respectively), low volumes of blood were collected. Subsequently, blood samples from fish collected in the same trawl were pooled in groups of three to provide sufficient sample volumes for laboratory analysis.

*Snapper*

Experimental sampling

Snapper were collected from 30 minute trawls conducted in January 2005 at Site 2 (Plank Point). Blood and muscle were sampled as per methods described for Degens leatherjacket.

*Smooth stingray and eagle ray*

Experimental sampling

Smooth stingrays and eagle rays were collected from 30 minute trawls conducted in January 2005. Smooth stingrays were caught at Sites 1 (Western Shoal), 3 (Middle Bank) and 4 (Wallaroo), and eagle rays were caught at Site 2 (Plank Point).

Immediately after removing each animal from the sorting table, blood was collected from the caudal artery in a 3ml syringe fitted with a 22 gauge needle, which were both heparinised. The needle was inserted into the ventral surface of the tail, approximately 5-10cm from the wings/body, at an angle of 60º towards the head. Muscle samples were not collected.

*Southern fiddler ray*

<u>Base-lines</u>

Base-lines of physiological stress parameters for southern fiddler rays were determined from fish that were line caught from the RV *Ngerin* in July 2005 at Site 3 (Middle Bank). Immediately after landing each animal, blood was collected from the caudal artery in a 3ml heparinised syringe fitted with a 22 gauge needle. The needle was inserted into the ventral surface of the tail, approximately 5-10cm from the wings/body, at an angle of 60º towards the head. Muscle samples were collected from the ventral surface of the upper portion of the tail using a 5 mm Ø stainless steel coring tool. Blood and muscle samples were prepared as described in the methods section.

<u>Experimental sampling</u>

One southern fiddler ray was collected from a 30 minute trawl conducted in July 2005, at Site 3 (Middle Bank). Immediately after removing the animal from the sorting table, blood and muscle samples were collected, prepared and analysed as described for base-line determination, above.

**Blood and muscle parameters**

*Rough leatherjacket and sand trevally*

Due to the small amount of sample collected for rough leatherjacket and sand trevally, samples for these species were pooled prior to analysis. There were no significant differences in any of the parameters measured between the two trawl groups, for eitherspecies (Table XVI). There were insufficient amounts of muscle to determine muscle glucose and lactate.

*Snapper*

Blood and muscle parameters measured in snapper after 30 minute trawls are presented in Table XVI.

*Smooth stingray, eagle ray and southern fiddler ray*

Physiological stress indicators for smooth stingrays, eagle rays and southern fiddler rays are presented in Table XVII.

Table XVI. Physiological stress parameters measured in blood and muscle of rough leatherjacket, sand trevally and snapper after 15, 45 or 30 minute trawls. Rough leatherjacket (n= 12) and sand trevally (n= 24) collected in October 2003, snapper (n= 19) collected in January 2005. Rough leatherjacket and sand trevally samples were pooled in groups of three fish prior to analysis. Mean ± SE.

| Parameter | Rough leatherjacket | | Sand trevally | | Snapper |
|---|---|---|---|---|---|
| | *15 min.* | *45 min.* | *15 min.* | *45 min.* | *30 min.* |
| *Blood* | | | | | |
| Lactate mmol.l$^{-1}$ | 3.68 ± 1.51 | 2.23 ± 0.29 | 6.65 ± 0.73 | 6.83 ± 0.80 | 8.83 ± 0.76 |
| pH | 7.58 ± 0.06 | 7.60 ± 0.04 | 7.11 ± 0.10 | 7.33 ± 0.07 | 7.46 ± 0.06 |
| Glucose mmol.l$^{-1}$ | 0.80 ± 0.36 | 1.91 ± 0.61 | 4.11 ± 0.31 | 4.32 ± 0.58 | 6.80 ± 0.46 |
| *Muscle* | | | | | |
| IMP Load | 1.38 ± 0.22 | 1.56 ± 0.15 | 3.94 ± 0.55 | 5.01 ± 0.54 | 0.95 ± 0.16 |
| AEC | 0.85 ± 0.02 | 0.80 ± 0.02 | 0.70 ± 0.03 | 0.65 ± 0.02 | 0.91 ± 0.009 |
| Glucose mmol.kg$^{-1}$ | na | na | na | na | 15.06 ± 1.19 |
| Lactate mmol.kg$^{-1}$ | na | na | na | na | 66.74 ± 3.85 |

Table XVII. Physiological stress parameters measured in blood and muscle of smooth stingrays, eagle rays and southern fiddler rays after 30 minute trawls, including base-lines for southern fiddler rays. Smooth stingrays and eagle rays collected in January 2005 (n= 9), southern fiddler rays (n= 1 from a 30 minute trawl, n= 4 for base-lines) collected in July 2005. Mean ± SE.

| Parameter | Smooth stingray | Eagle ray | Southern fiddler ray 30 min. | Base-line |
|---|---|---|---|---|
| *Blood* | | | | |
| Lactate mmol.l$^{-1}$ | 5.02 ± 2.17 | 3.49 ± 0.90 | 2.09 | 1.26 ± 0.36 |
| pH | 7.39 ± 0.12 | 7.19 ± 0.09 | 6.92 | 6.88 ± 0.22 |
| Glucose mmol.l$^{-1}$ | 1.15 ± 0.44 | 1.45 ± 0.18 | 2.37 | 1.98 ± 0.65 |
| Sodium mmol.l$^{-1}$ | 342.3 ± 15.64 | 336.3 ± 4.56 | 287 | 287.0 ± 8.44 |
| Potassium mmol.l$^{-1}$ | 5.43 ± 0.38 | 6.37 ± 0.26 | 7.7 | 6.25 ± 1.28 |
| Chloride mmol.l$^{-1}$ | 347.8 ± 22.46 | 329.3 ± 7.19 | 289 | 282.8 ± 14.25 |
| *Muscle* | | | | |
| IMP Load | na | na | 1.05 | 0.41 ± 0.25 |
| AEC | na | na | 0.82 | 0.67 ± 0.13 |
| Glucose mmol.kg$^{-1}$ | na | na | 21.24 | 20.82 ± 2.20 |
| Lactate mmol.kg$^{-1}$ | na | na | 3.12 | 7.57 ± 3.17 |

# CHAPTER 6: THE EFFECT OF PRAWN TRAWLING AND DISCARDED BY-CATCH ON THE BEHAVIOUR OF PORT JACKSON SHARKS (*Heterodontus portusjacksoni*) IN SPENCER GULF, SOUTH AUSTRALIA

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# Abstract

Smaller sharks, skates and rays are common in the by-catch of Spencer Gulf prawn fishery. Population size estimates of sharks and rays from trawling are potentially biased because trawling operations may influence behaviour, resulting in inaccurate approximations of catchability, survival and other population characteristics. In this study we used Radio Acoustic Position Telemetry (RAPT) to determine whether the behaviour of the Port Jackson shark (*Heterodontus portusjacksoni*) was influenced by discarded by-catch and prawn trawl operations, two factors that may affect behaviour. Movements of tagged Port Jackson sharks were recorded after exposure to by-catch cues and prawn trawler operations. In both experiments, the results showed there was no effect of by-catch or prawn trawl operations on the movements of Port Jackson sharks. However, laboratory experiments showed that sharks were attracted to by-catch, but that by-catch composition had little influence on attraction. In addition, by-catch volume did not have an effect on attraction, although the feeding biology of Port Jackson sharks, coupled with the constraints of using large animals in laboratory food preference experiments, were believed to confound this particular result. This study also demonstrated that RAPT data are amenable to statistical analyses rather than descriptive analyses alone.

## Introduction

Commercial fishing for prawns, *Penaeus (Melicertus) latisulcatus* (see Poore, 2004) in Spencer Gulf is undertaken using a double demersal otter trawl, each with a headline length of 14.63 m and a minimum mesh size of 4.5 cm. The trawl consists of two cone shaped nets closed at one end by a codend, and lateral wings extending forward from the opening that are held open horizontally by two otter boards. Although trawling of this nature is one of the most widely used methods of prawn fishing, its environmental effects and sustainability are increasingly being questioned (Andrew and Pepperell, 1992). The environmental effects can be divided into three main groups: 1) the effects of by-catch on trophic linkages and food web dynamics; 2) the effect of trawling on resuspension and nutrient regeneration, and 3) the direct effects of trawling on the benthic and pelagic assemblages (Svane, 2003). An awareness of these ecological impacts has alerted most fisheries to the importance of ecosystem-based management and the concept of ecologically sustainable development. Understanding of the ecosystem effects of fishing is essential to ensure prudent management, and hence the sustainability of the exploited resource.

Population size estimates of sharks and rays made from trawl surveys are potentially biased because trawling operations may influence the behaviour of the target species. For example, it is common for trawlers to pass over areas that have previously been trawled, and it is therefore possible that they are trawling sharks and rays in numbers greater than those representative of the area because the sharks and rays are aggregating around discarded by-catch. Sharks and rays may also be attracted to the noise of prawn trawl operations (engine/propeller noise, wire hydraulic noise etc.) because they have developed a learned response to discarded by-catch associated with the noise. Clearly, both of these factors may affect catchability, survival, and other population characteristics of sharks and rays that potentially bias population estimates.

This study aimed to determine whether the behaviour of a major by-catch consumer, the Port Jackson shark (*Heterodontus portusjacksoni*), is influenced by prawn trawl operations. If so, this could affect their survival and have major implications for population estimates obtained in the current FRDC funded prawn by-catch project. Radio Acoustic Position Telemetry (RAPT) was used to track Port Jackson sharks

during exposure to discarded by-catch and the noise of prawn trawlers. Telemetry tracking has been successfully used on various marine animals in descriptive studies (e.g. Mather and O'Dor, 1991; Wartzok *et al.* 1992; Lagardère *et al.* 1995; Sauer *et al.* 1997; Klimley *et al*, 2001; O'Dor *et al.* 2001; Aitken *et al.* 2002; O'Dor, 2002; O'Dor *et al.* 2002; Parsons *et al.*2003) but the methods have not been used experimentally. The extent to which Port Jackson sharks are attracted to by-catch is likely to be dependent on various factors, especially by-catch volume and composition. Both of these variables differ between seasons and sites. Therefore, the influence of by-catch volume and composition on the behaviour of Port Jackson sharks was examined in a series of laboratory preference trials.

## Materials and methods

### Sampling of experimental animals

Port Jackson sharks and by-catch for both field and laboratory experiments were caught using a standard otter trawl. The by-catch for RAPT and laboratory experiments was immediately frozen on board the vessel. The sharks were kept alive on board until required for the RAPT trials or transported to the Lincoln Marine Science Centre for use in laboratory experiments. Each shark was individually coded using livestock roto tags™ (Dalton Pty. Ltd.), which were attached to the first dorsal fin. All fieldwork was completed aboard the research vessel RV *Ngerin* and all laboratory studies were carried out at the Lincoln Marine Science Centre, Port Lincoln, South Australia.

Tracking experiments were completed during a series of four research cruises (April 2004, October 2004, January 2005 and April 2005) over a period of 12 months. During this period, two experiments were conducted using six Port Jackson sharks for each experimental trial and a further six sharks for a set of control trials (a total of 18 Port Jackson sharks). All trials were conducted during daylight hours.

### Field experiments

*RAPT study site*

The radio acoustic position telemetry (RAPT) study site was situated in Spencer Gulf, South Australia, approximately 5.2 kilometres north of Pt. Riley (Figure 1). This site

was chosen because the sea-floor is relatively featureless and the depth was almost constant over the entire site, averaging approximately 22 metres. These are important considerations because variations in both bottom features and depth can interfere with the RAPT system (Aitken *et al.*, 2005). Experiments were undertaken at neap tide so there was minimal tidal movement; however, no measurements of tidal movement over the study period were taken. Tidal direction was determined by visual observation.



Figure 1: Location of the study site in Spencer Gulf, South Australia.

*Tracking*

A VEMCO® radio acoustic positioning telemetry system (RAPT) was used to monitor the movement of sharks during field experiments. Three RAPT buoys were deployed 500 m apart in an equilateral triangle. The layout of this system was similar to that described in Parsons *et al.* (2003) and only a brief description will be given here. A hydrophone was attached to each buoy at a depth of 0.5 m and received acoustic transmitter signals from the tag every 20 s. Signals were transmitted from the buoys to a base station that calculated the position of the tag by triangulation using the arrival times of the acoustic pulses to each of the three buoys. This information was displayed and saved onto a laptop computer. This system is capable of determining position with an accuracy of ± 1 to 2 m (O'Dor *et al.*, 1998). A control tag was placed in the centre of the RAPT triangle and used as a reference against which the system was tested and the movements of sharks were monitored.

*Tag attachment*

VEMCO V16 acoustic transmitters were glued to livestock roto tags™ (Dalton Pty. Ltd.) using epoxy glue. Transmitters were programmed to emit a signal every 2 seconds at unique frequencies (51 - 84 kHz). Tags were then attached to the first dorsal fin of Port Jackson sharks (Figure 2). Roto tags were selected because they can be attached to the first dorsal fin without causing noticeable trauma to the shark (Heupel et al., 1998). Port Jackson sharks were left to recover in continuous flow, 300 litre fibreglass holding tanks aboard the research vessel for a period of at least 24 h before release at the study site.



Figure 2: A VEMCO V16 acoustic transmitter attached to the first dorsal fin of a Port Jackson shark via a livestock roto tag™ (Dalton Pty. Ltd.).

*Control sharks*

The movement of control sharks was assessed during April 2004. After the recovery period, a shark was randomly selected from the holding tank and transported in a 60 litre plastic tub filled with seawater to the centre of the RAPT triangle. Once at the desired position, the shark was placed inside a release trap and lowered to the sea-floor where it was allowed to recover from handling stress *in situ* for a period of at least 30 minutes. The release trap was then triggered and the shark was set free. The movement of the shark was recorded using the RAPT tracking equipment. The trial was terminated once the shark had left the study triangle. This method was repeated for all control sharks. Net tidal movements were recorded for each trial, and successful attempts to retrieve the sharks and tags were made by SCUBA divers using an underwater tracking device (VEMCO® VUR 96) that pinpointed the direction of the individual frequency signal.

*Collection of by-catch*

By-catch was collected during prawn trawling carried out near the study site using the same fishing gear used to catch the sharks. The by-catch was mixed demersal and pelagic fish and blue crabs, and separated into lots of 22 kg (± 1.5 kg) that were then frozen until required for experiments. Percent composition (by weight) of by-catch used during this experiment can be seen in Figure 3. Prawns were released, as they are not typically discarded as by-catch during commercial prawn trawl operations. Elasmobranchs were also released because they were being tagged in a concurrent research project and because they did not constitute a part of the diet of Port Jackson sharks.

*Effect of discarded by-catch on movements of Port Jackson sharks*

This experiment was carried out during October 2004 and January 2005. Sharks in this experiment were treated the same way as control sharks. However, for at least 20 minutes prior to release, a 22 kg (± 1.5 kg) bag of thawed by-catch was placed 65 m up-current from the release site. The shark was then released and positional data was recorded using RAPT tracking equipment. The hypothesis tested was that the behaviour of Port Jackson sharks, as defined by movement, was not affected by the

presence of discarded by-catch. This experiment was repeated using six different Port Jackson sharks.



Figure 3: By-catch composition of bait used in RAPT by-catch experiment during October 2004 and January 2005. Error bars are 95% CI.

*Effect of prawn trawler activity on movements of Port Jackson sharks*

Sharks in this experiment were treated the same way as control sharks; however, prior to, and after the release of the shark, a prawn trawler was operating approximately 250 m away from the release location. These trials were conducted in April 2005. The trawler was operating as it would during a typical fishing night, although only one net was being used and the cod end was left open to prevent unnecessary capture of animals. Once the shark was released, positional data were recorded using RAPT tracking equipment. This experiment was repeated using six different Port Jackson sharks, and the trawler operated back and forth over the same transect for all six trials. The hypothesis tested was that the behaviour of Port Jackson sharks, as defined by movement, was not affected by prawn trawler activity.

**Laboratory experiments**

*Laboratory experimental set-up*

An experimental tank (dimensions: $5 \times 1.6 \times 0.6$ metres, volume = 4800 L) with a central holding section (603 L) created by 2 removable mesh panels was utilised for

all laboratory experiments (Figure 4). A white line painted on the tank bottom marked the central position of the tank. To allow time for sharks to recover from handling before experiments, one randomly selected shark was placed in the holding section of the experimental tank for 24 hr with no food prior to testing. Water-flow and aeration were continuous throughout the recovery period. Only one shark was tested at a time because the use of two or more individuals in a feeding trial may cause interference between individuals (Peterson and Renaud, 1989). Three experiments were carried out testing: 1) attraction to by-catch, 2) by-catch preference, and 3) preference of by-catch volume. After the recovery period, water flow and aeration were stopped and a PVC tube was placed at either end of the tank. Different arrangements of by-catch were placed in the tubes depending of the factor being tested. The end of the tank at which the by-catch was placed was randomised for each trial. Both tubes had numerous holes that allowed the scent of the by-catch to be released into the tank, thus ensuring that any response by the shark was the result of olfactory cues (chemotactic response) rather than a tactile response. The holding panels were released 30 seconds after deploying the tubes and the behaviour of the shark was recorded on a remote video camera to prevent any observer effect on their behaviour. At the termination of each trial, the shark was removed and placed into an aquarium and fed. By-catch tubes were also removed and water from the experimental tank was drained and replaced to eliminate any left-over scent that may affect the next trial.



Figure 4: Design of the experimental tank.

*Hypothesis 1: Test of attraction to by-catch*

To test whether sharks are attracted to by-catch, 500g squid (*Sepioteuthis australis*) + 500g sand trevally (*Pseudocaranx wrighti*) + 500g blue crab (*Portunus pelagicus*) were placed in one tube while the other tube was empty. The trials were terminated after 30 seconds because preliminary observations of shark behaviour have shown that this amount of time was adequate for sharks to display a preference. Each shark was tested twice. A total of 14 trials were carried out using seven sharks.

*Hypothesis 2: Test of species preference*

Different species of by-catch were placed at either end of the tank (there was no empty tube in these trials) to determine if Port Jackson sharks display a preference for particular by-catch species. These trials were run for a period of 15 minutes allowing the shark sufficient time to display a preference. The by-catch species tested were squid (*Sepioteuthis australis*), blue crabs (*Portunus pelagicus*) and Degens leatherjackets (*Thamnaconus degeni*). Each by-catch species was tested against each other (*i.e.* squid vs blue crab, squid vs leatherjacket and blue crab vs leatherjacket) and each shark was tested with each combination of by-catch. A total of 45 trials were carried out using 15 different Port Jackson sharks.

*Hypothesis 3: Test of by-catch volume*

To determine whether Port Jackson sharks are attracted to larger by-catch volumes, 1000g of sand trevally was placed at 1 end of the tank and 100g of sand trevally was placed at the other end. The movement of sharks was remotely videotaped over 15 minutes using the methods outlined previously. A total of 10 trials were carried out using 10 different Port Jackson sharks.

**Analysis**

*Field experiments*

Erroneous positions were given by the RAPT system at some stage of all trials. This is common for this system and occurs when features such as rocky projections and waves cause the transmitter pulses to 'scatter'. The erroneous data points were identified as those that showed the position to be more than 300 m away from the previous positions, after a time frame of only 20 seconds. Subsequent positions were observed to resume along the identified track. These data were filtered from the raw

RAPT files prior analyses. The edited data were analysed at 5, 10, 15, 20 and 25 mins ($\pm$ 1 min) using a non-parametric Watson's $U^2$ test (see circular statistics – Zar, 1999) by comparing treatment data to control data. Where a position was not available for a particular time interval, a position was interpolated using the sharks swimming speed and the closest positions recorded before and after this time interval. A Chi-squared goodness of fit test for circular data (Zar, 1999) was carried out on control data to ensure movements during these trials were not different from random.

The distances travelled by sharks per unit of time in the RAPT experiments were analysed to determine the effect of each treatment on shark swimming speed. A one-way ANOVA was carried out on the distance travelled (in 5 minute blocks) by each shark with treatment as the main factor. When data failed to meet Levene's test of homogeneity, a square root transformation was carried out to obtain homogeneity of variances.

*Laboratory experiments*

In each of the laboratory experiments, video footage was analysed for the time that the shark spent in each half of the tank after the trial period. A one-tailed non-parametric binomial test (Zar, 1999) was carried out to test each null hypothesis:

1) Port Jackson sharks will not spend more than half the total trial time (15 secs) at the by-catch end of the tank.
2) Port Jackson sharks will not spend more than half the total trial time at one particular end of the tank during each of the preference experiments.
3) Port Jackson sharks will not spend more than half the total trial time at the end of the tank that has the 1 kg tube of sand trevally.

The length of the by-catch preference and volume experiments was 15 mins, which may have resulted in the water becoming saturated with by-catch scent, thereby masking potential preferences. Therefore, the analyses of these trials were carried out at 3-minute intervals to determine whether sharks display any preferences prior to the possible saturation of the tank with scent.

Due to the repeated use of sharks in the first laboratory experiment (attraction to by-catch), Spearman's rank order correlation was carried out to investigate whether there was any correlation between time at by-catch during the first and second set of trials.

## Results

### Field Experiments

*Control sharks*

Chi-squared analysis showed that the movement patterns of six Port Jackson sharks during control trials were random in relation to their release position at 5, 10, 15, 20 and 25 minutes (Figure 5; Table I).

*Effect of discarded by-catch on movements of Port Jackson sharks*

Two sharks initially moved towards the by-catch before heading off in another direction (Figure 6). The movement pattern of the other four sharks showed no directional movement patterns toward the by-catch (Figure 6). These observation were confirmed by Watson's $U^2$ test which showed that the distribution of Port Jackson sharks during by-catch trials was not significantly different from that of sharks during the control trials at 5, 10, 15, 20 and 25 minutes (Table II).

*Effect of prawn trawler activity on movements of Port Jackson sharks*

Four of six sharks appeared to move away from the trawler, while the movement patterns of the other two tracks appeared to be random (Figure 7). Watson's $U^2$ test revealed that the distribution of Port Jackson sharks during the trawl passing trials was not significantly different from the distribution of sharks during the control trials at 5, 10, 15, 20 and 25 minutes (Table III). Thus, their movement patterns were unrelated to trawler activity.

Figure 5: Tracks of six Port Jackson sharks recorded during control trials. The coordinates (0,0) represent the location where the sharks were released. Numbers on the vertical and horizontal axes represent distance (m).

Table I: Results of goodness of fit tests for circular data obtained during RAPT control trials. $\chi^2_{0.05\,(5)} = 11.07$.

| Time | df | $\chi^2$ | P |
|---|---|---|---|
| 5 | 5 | 4.0 | >0.05 |
| 10 | 5 | 4.0 | >0.05 |
| 15 | 5 | 4.0 | >0.05 |
| 20 | 5 | 4.0 | >0.05 |
| 25 | 5 | 4.0 | >0.05 |

Figure 6: Tracks of six Port Jackson sharks recorded during by-catch experiment. The coordinates (0,0) represent the location where the sharks were released. Numbers on the vertical and horizontal axes represent distance (m).

Table II: Results of the Watson's $U^2$ test for circular data obtained during RAPT by-catch trials. $U^2_{0.05\ (6,6)} = 0.206$.

| Time | df | $U^2$ | P |
|------|-----|-------|------|
| 5 | 6 | 0.120 | >0.05 |
| 10 | 6 | 0.099 | >0.05 |
| 15 | 6 | 0.081 | >0.05 |
| 20 | 6 | 0.081 | >0.05 |
| 25 | 6 | 0.067 | >0.05 |

Figure 7: Tracks of six Port Jackson sharks recorded during prawn trawler experiments. The coordinates (0,0) represent the location where the sharks were released. Numbers on the vertical and horizontal axes represent distance (m).

Table III: Results of the Watson's $U^2$ test for circular data obtained during RAPT trawler trials. $U^2_{0.05\,(6,6)} = 0.206$.

| Time | df | $U^2$ | P |
|------|----|-------|-----|
| 5 | 6 | 0.048 | >0.05 |
| 10 | 6 | 0.056 | >0.05 |
| 15 | 6 | 0.090 | >0.05 |
| 20 | 6 | 0.093 | >0.05 |
| 25 | 6 | 0.067 | >0.05 |

*Distance travelled*

The distance travelled by sharks in 5-minute blocks was variable (Figure 8). The mean distance travelled by sharks during the control and by-catch experiment increases over time; however, during the trawl experiment, the distance travelled by sharks started high and then decreased. A one-way ANOVA showed no significant effect of treatment on distance travelled for the various time intervals (Table IV). Partial eta squared values were between 0.108 and 0.207 showing that only between 10-20% of the variance in the dependent variable (distance travelled) can be explained by treatment (Table IV).



Figure 8: Distance travelled by sharks during each treatment (error bars are ± 95% CI).

Table IV: One-way ANOVA (carried out per 5 minute block) on the distance travelled by Port Jackson sharks with treatment as the main effect. Data are square root transformed.

| Source | df | F | P | Partial eta squared | Observed Power |
|--------|----|----|----|----|----|
| 1st 5 minutes | 18 | 0.908 | 0.425 | 0.108 | 0.177 |
| 2nd 5 minutes | 18 | 0.903 | 0.426 | 0.108 | 0.177 |
| 3rd 5 minutes | 18 | 0.109 | 0.898 | 0.014 | 0.064 |
| 4th 5 minutes | 18 | 0.840 | 0.451 | 0.101 | 0.167 |
| 5th 5 minutes | 18 | 1.961 | 0.175 | 0.207 | 0.342 |

**Laboratory experiments**

*Test of attraction to by-catch*

Binomial analysis showed that the median time spent by sharks at the end of the tank containing by-catch was significantly higher (P = 0.013) than at the control end (Figure 9). Spearman's rank order correlation showed that the time spent at by-catch during the first set of trials was not associated with time spent at by-catch during the second set of trials (r = - 0.249, n = 7, P>0.05). Therefore, repeated use of each shark in trials 1 and 2 was not likely to confound the result.



Figure 9: Time spent in each sector of the tank during 30-second by-catch trials (N = 14). Error bars are ± 95% CI.

*Test of species preference*

While recognising that performing multiple statistical tests increases the probability of a type II error, the results for experiments on preference for by-catch species and preference for by-catch volume are unambiguous. Binomial analysis for by-catch preference revealed no significant preference between squid and leatherjackets at 3 mins (P = 1.0), 6 mins (P = 1.0), 9 mins (P = 1.0), 12 mins (P = 0.607) and 15 mins (P = 0.607) (Figure 10). Similarly, sharks displayed no preference for blue crab or squid at 3 mins (P = 0.607), 6 mins (P = 0.607), 9 mins (P = 0.607) or 12 mins (P = 0.302) (Figure 11). Only at the 15-minute time interval were sharks found to spend significantly more time at the squid end than the blue crab end (P = 0.035) (Figure 11). The binomial analysis of preference between leatherjackets and blue crabs revealed that there was no significant attraction for either species at 3 mins (P =

0.607), 6 mins (P = 0.302), 9 mins (P =607), 12 mins (P = 1.0) or 15 mins (P = 0.607) (Figure 12).



Figure 10: Time spent in each sector of the tank during 15-minute preference trials of squid and leatherjackets (N = 15) including results of the binomial test. Error bars are ± 95% CI.



Figure 11: Time spent in each sector of the tank during 15-minute preference trials of squid and blue crabs (N = 15) including results of the binomial test. (P value in bold was significant). Error bars are ± 95% CI.

*Test of by-catch volume*

Sharks spent similar amounts of time at each end of the experimental tank irrespective of the volume of by-catch present (Figure 13). This observation was supported by the binomial test, which showed that by-catch volume did not significantly affect time spent at either high or low by-catch volume at 3 mins (P = 0.109), 6 mins (P = 0.344), 9 mins (P = 0.754), 12 mins (P = 0.109) and 15 mins (P = 0.344).



Figure 12: Time spent in each sector of the tank during 15-minute preference trials of leatherjackets and blue crabs (N = 15) including results of the binomial test. Error bars are ± 95% CI.



Figure 13: Time spent in each sector of the tank during 15-minute preference trials of by-catch volume (N = 10) including results of the binomial test. Error bars are ± 95%

## Discussion

### Field experiments

*Effect of discarded by-catch on movements of Port Jackson sharks*

RAPT trials suggest that Port Jackson sharks are not attracted to discarded by-catch. Statistical analyses showed that there was no directional swimming to by-catch displayed by Port Jackson sharks. Although two of the six sharks in this experiment appeared to display directional swimming toward the by-catch after release, they continued swimming past it on both occasions. Kleerekoper and Gruber (1975) and Carrier *et al.* (2004) have demonstrated that to locate an olfactory source, sharks commonly use a chemical gradient and exhibit positive rheotaxis (*i.e.* face into the water current). Directional swimming is facilitated by the ability of the shark to detect small differences in the concentration of odorous materials in each nostril and by their ability to head upstream in the direction of equal olfactory stimulation (Bleckmann and Hofmann, 1999). The experimental approach (*i.e.* placing 22 kg of by-catch up-current from the sharks) in this study should have facilitated the detection and location of the by-catch by the sharks. Therefore, it is surprising that the sharks did not respond. If the by-catch could be detected, it is likely the sharks would display an attraction towards it because stomach content analyses of Port Jackson sharks caught when the prawn fleet is operating have shown that blue crabs, fish, and cephalopods are commonly consumed by the sharks (Chapter 7, Table V). These species made up a large percentage of the by-catch composition used in experiments here (Figure 3). Therefore, it is likely that the sharks did not detect the by-catch in these experiments. The failure of sharks to detect by-catch may be due to a number of factors: 1) there may not have been enough by-catch used; 2) the distance the by-catch was placed in relation to the sharks (65 metres) was too great for them to detect it or; 3) the tidal conditions were unfavourable for the detection of by-catch. Because the method used in this study is representative of commercial prawn fishing conditions, it can be assumed that when discarded by-catch is situated 65 metres or more up-current from Port Jackson sharks, it is unlikely that they will be able to detect it. Under these conditions, it is likely that Port Jackson sharks will not aggregate around prawn trawl by-catch, and therefore not get caught in numbers greater than those estimated using prawn trawl data. Due to the cost of tracking studies, I was unable to determine how

Port Jackson sharks may respond when by-catch is discarded at distances of less than 65 metres and under different tidal regimes.

*Effect of prawn trawler activity on movements of Port Jackson sharks*

Shark swimming patterns were not affected by the noise produced by a prawn trawler during RAPT trials carried out in this study. Only one of the six sharks appeared to show directional swimming towards the area in which the trawler was operating. It is likely that the sharks were able to hear the trawler at the distance it was operating (250 m) because Myrberg *et al.* (1972) demonstrated that artificial sounds could be localized by silky sharks (*Carcharahinus falciformis*) at distances of over 400 m. Although these are two different species of shark, their sensory biology is likely to be similar. Evidence from the trials suggests that the sharks may be deterred by the noise of the trawler because three of the six tracks showed definite movement away from the area the trawler was operating in, with another shark initially moving away from the trawler and then remaining stationery. While not statistically significant, there was also a trend for a greater distance travelled after the first five minutes in those trials where the trawler was active compared to the by-catch experiment and control trials. This may indicate that the sharks swim away from the trawler until they reach a distance at which they feel comfortable. Therefore, there is a possibility that instead of Port Jackson sharks making the association that trawl noise = free food, they make the association of trawl noise = stress and trauma of capture. However, it should be stressed that despite an apparent negative response to trawler noise, movement of sharks away from trawlers was not statistically significant and the distances they travelled were highly variable; results should therefore be interpreted cautiously. Like many large-scale field experiments, this experiment suffered from a lack of sufficient replication due to limited resources and logistical constraints. Greater replication may therefore yield different results.

*Application of RAPT system*

The RAPT system was developed to allow tracking and monitoring acoustically tagged aquatic animals. To date, no experimental studies have been undertaken using the RAPT system to test hypotheses. Instead, investigators have used the data for descriptive purposes only. This study demonstrates that experimentally designed statistical analyses can be applied to movement data of acoustically tagged animals.

The maximum spatial scale of the RAPT triangle is limited (~ 500m between buoys) and largely determined by conditions under which the system is being used; therefore, the range of studies it can be used for may be limited by the behaviour of the study animal and prevailing sea conditions. Despite these limitations, the RAPT system does provide a useful tool for studying animals in environments where direct observations are not possible. The successful application of this system is largely dependent on understanding these limitations.

**Laboratory experiments**

*Test of attraction to by-catch*

In contrast to the field experiments, the first set of laboratory trials revealed that sharks were attracted to by-catch. The different scales of both experiments may be responsible for these differing results; therefore, the potential for comparisons between the experiments may be limited. However, the results of the laboratory trials do support the suggestion that sharks were unable to detect the by-catch during the RAPT trials because they were clearly attracted to by-catch in the laboratory trials. While only three species (blue crab, squid and sand trevally) were used to test the responses of Port Jackson sharks, they provided a good representation of by-catch from prawn trawlers because they are the most commonly caught species, often making up the largest percentage of overall composition, and were found to be a significant component of stomach contents in over 25 Port Jackson sharks examined (Chapter 7, Table V).

The repeated use of each shark in the first laboratory experiment (attraction to by-catch) violates the assumption of independent samples, but was necessary to obtain an adequate sample size given the limited availability of sharks and the limited holding capacity at the Lincoln Marine Science Centre. However, the use of the PVC tubes ensured the sharks did not develop a learned response that PVC tube = food because the tubes did not allow the sharks to access the food, and the empty tube did not contain any food. We believe that this eliminated potential biases created from the repeated use of sharks in this experiment because Spearman's rank order correlation showed that there was no association between time spent at by-catch during the first and second trials for each shark. The repeated use of sharks in the second experiment

was not considered a serious violation of the assumption of independent samples because sharks in this experiment were never exposed to the same by-catch arrangement; therefore, there was no opportunity for learning.

*Test of species preference*

When squid and leatherjackets were presented to Port Jackson sharks, the laboratory experiment on species preference showed that there was no preference for either species after 3, 6, 9, 12 or 15 mins (Figure 10). When sharks were presented with squid and blue crabs, there appeared to be a preference for squid (Figure 11); however, this was only statistically significant after 15 mins. This is likely to indicate that saturation of the water with scent of by-catch did not confound the laboratory trials because if saturation were occurring it would be expected that the significant difference would be found early in the trials, and then once saturation had occurred, there would be no significant difference. In contrast, trials where the sharks were presented leatherjackets and blue crabs, there was no preference for species after 3, 6, 9, 12 or 15 mins (Figure 12).

Ideally, all by-catch species should be presented at the same time in preference experiments (Peterson and Renaud, 1989). The design of the experimental tank precluded this possibility. However, results of stomach content analyses indicated that this did not matter because a total of 12 different by-catch species were found in the guts of Port Jackson sharks (Chapter 7, Table V), supporting the conclusion that they do not show preferences for any particular by-catch species. Given that sharks displayed a preference for squid over blue crabs at 15 mins, there is a possibility that by-catch composition plays a minor role in the way sharks respond to discards. It is acknowledged that the by-catch species used in this experiment only represent a small number of species caught as prawn trawl by-catch, and that by-catch is always discarded as a mixture of species. It is therefore difficult to comment on how the sharks would respond when there is a mixture of odorous material. To do this would prove to be a complex task and was not the objective of these trials. Instead, these findings provide an indication of whether the strength of attraction of Port Jackson sharks to by-catch is likely to be influenced by variability in by-catch composition between seasons and locations.

*Test of by-catch volume*

The results from the experiment examining whether Port Jackson sharks responded to differences in by-catch volume, revealed that sharks were not significantly attracted to the end that contained more sand trevally after 3, 6, 9, 12 or 15 mins (Figure 13). This result was unexpected because it is widely documented that the concentration of a chemical cue can have profound effects on the ability of marine organisms to use chemoreception (*e.g.* Moore *et al.* 1991; Moore & Grills, 1999; Sherman and Moore, 2001; Weissburg *et al.* 2002). It is likely that this result was due to one of the many constraints of carrying out laboratory experiments on large animals. It is possible that sharks were detecting a chemical gradient running from either end of the tank, and were confused as to which end to swim. The result of this is likely to be continual searching up and down the tank, and hence the failure of the sharks to show a preference for the larger volume of by-catch. It is possible that this problem was exacerbated by the large volumes selected because of the highly sensitive nature of the olfactory system of Port Jackson sharks. This was not considered a problem in the by-catch preference experiment because the qualities of the gradients were different for different food choices; therefore, a preference for by-catch species should still be displayed.

*Challenges associated with the design and analysis of preference experiments*

The problems associated with carrying out food preference experiments have been widely documented (*e.g.* Peterson and Renaud, 1989; Roa, 1992; Manly, 1993). However, these problems are mainly associated with studies in which food preference is determined from the amount of food consumed, and were therefore not an issue in this study. However, the way in which by-catch scent may disperse within the experimental tank presented a significant design and analytical challenge. To eliminate the problem of inconsistent flow conditions, water flow to the tank was turned off during all trials. A pilot study indicated that, during 15-minute trials, oxygen levels would not reach critical levels described by Rodda (2000), hence respiratory distress from a cessation of water flow was not a problem. Saturation of the tank with by-catch scent presented the biggest experimental obstacle. If saturation were to occur, there would be no concentration gradient to be utilised by the sharks for directional orientation to the by-catch. Short trials of 30 seconds duration in the first experiment avoided the saturation problem, however, in the second and third

experiments, trials had to go for longer times to allow sharks sufficient opportunity to display a preference.

While the use of PVC tubes in the laboratory trials were crucial for ensuring that responses of sharks to by-catch was purely olfactory, they may have a confounding influence on the experiments. McLaughlin and O'Gower (1971) state that Port Jackson sharks require food to come in contact with the mouth region for final localisation. As sharks could not physically contact the by-catch in these experiments, they may have kept swimming in search of the food instead of stopping to feed as they would under natural conditions. This suggestion is supported by Carrier *et al.* (2004) who states that olfactory involvement in elasmobranch feeding can be roughly categorized as arousal, directed approach and attack, and if the bait is not located or lost, usually continued searching. If this were the case, it would mean that sharks were more likely to swim into the half of the tank that contained less preferable by-catch species/volume, thus making it more difficult to detect any by-catch/volume preference in the analysis of these trials. In addition, Kleerekoper and Gruber (1975) found that nurse sharks required flowing water to pinpoint the source of odorous material and when the flow was removed, only generalized location of the release site was possible. However, it was not possible to introduce flow to this experimental tank in a way that would not cause preference biases.

## Conclusion

This study showed that the use of by-catch data obtained during commercial prawn trawling in the Spencer Gulf is adequate for making population size estimates of Port Jackson sharks because they are not attracted to either discarded by-catch or the noise of prawn trawlers. However, there was evidence (although not statistically significant) that sharks may be deterred by prawn trawler noise. If this is the case, there is a possibility that population size estimates made from commercial trawling, may underestimate Port Jackson shark numbers. Greater replication is required for both of the field experiments to thoroughly investigate this possibility, and to determine how sharks behave when by-catch is situated at distances less than 65 m from the sharks. Laboratory trials indicated sharks were attracted to by-catch, and that attraction may occur at distances of less than 65 m. However, as this is such a short distance, any

overestimation of shark numbers caused by shark aggregations is likely to be minimal. Irrespective of the distances over which sharks are attracted to by-catch, by-catch composition is likely to have little influence on the strength of this attraction. Instead, it appears that Port Jackson sharks are non-selective scavengers when it comes to the consumption of discards. Similarly, laboratory trials indicate that the volume of by-catch has little influence on shark behaviour. However, the feeding behaviour of Port Jackson sharks coupled with artefacts of the laboratory experimental design, mean that results of these experiments should be interpreted cautiously.

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# CHAPTER 7: STABLE ISOTOPE AND DIET ANALYSIS OF DOMINATING BY-CATCH AND SCAVENGER SPECIES IN SPENCER GULF

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## Abstract

With the aim to testing whether the trophic position of major consumers of discarded by-catch in the Spencer Gulf prawn fishery varies among sites with a history of different trawl intensity (hours), the stable isotope composition, $^{13}$C and $^{15}$N, of seven quantitatively important consumers of by-catch (blue crab, *Portunus pelagicus*, bridled leatherjacket, *Acanthaluteres spilomelanurus*, rough leatherjacket, *Scobinichthys granulatus*, toothbrush leatherjacket, *Acanthaluteres vittiger*, sand trevally, *Pseudocaranax wrighti*, Degens leatherjacket, *Thamnoconus degeni*, Port Jackson shark, *Heterodontus portusjacksoni*), and the target species, western king prawn, *Melicertus latisulcatus*, were analysed at five sites exposed to different fishing intensity. In addition, gut contents were analysed for five of the species. For comparison, samples for stable isotopes of plankton, seagrass, and sealice were collected. The results showed a distinct trophic fractionation of both $\delta^{13}$C and $\delta^{15}$N separating the species into four distinctively different trophic groups. Seagrass $\delta^{13}$C values were much higher than plankton indicating that seagrass contribution to the food chain is through other pathways. The linkage between plankton and secondary production appears to be through the consumption of detritus by crustaceans. $\delta^{13}$C and $\delta^{15}$N values declined gradually from the northern site towards the southern site showing spatial variation in trophic position. Leatherjackets showed a gradual decline in $\delta^{15}$N-values towards Site 4 but then an increase towards Site 5 indicating a marked change in feeding pattern. Disregarding Port Jackson sharks at the highest trophic level, there was a general trend of negative correlation between $\delta^{13}$C and $\delta^{15}$N and historical trawl intensity, and a positive correlation between $\delta^{13}$C and $\delta^{15}$N and habitat complexity, implying that discarded by-catch and/or habitat complexity affects diet composition. The studied species can be characterised as omnivorous with more than 10 diet categories. Port Jackson sharks had a higher occurrence of blue crabs and calamary in their diet during periods of trawling, and prawns during periods of no trawling, demonstrating that discarded by-catch affects the diet. Other factors than the inter-correlated habitat complexity and trawl intensity, such as functional patterns of feeding and competition for space and food, may affect trophic position.

# Introduction

One environmental impact of prawn trawling is the provision of discards to the ecosystem, which may subsidise large scavenger populations, affecting trophic linkages and food web dynamics (Andrew & Pepperell 1992, Alverson et al. 1994, Hall 1999). It is therefore important to gain knowledge about how exploitation of both target species and discarded by-catch affects food web structure (Christensen 1998). There are few studies that report on trophodynamic consequences of commercial fishing because baseline data for comparison is usually not available. Badalamenti et al. (2002) were able to compare the trophic levels at two points in time, namely before and after a trawl ban was implemented, causing an increase in biomass of some target species. Badalamenti et al. (2002) found that after nine years of the trawling ban, the observed increase in numerical abundances were not associated with substantial changes in the trophodynamics of the three species studied.

A study of a marine food web usually involves gut content analysis of the dominant species in combination with direct observations of feeding, but this only provides a snapshot of the individuals immediate past consumption. The analysis of stable isotope ratios provides an alternative whereby an organism's trophic position (level) may be identified by a simple tissue sample (Peterson & Fry 1987, Michener & Schell 1994). In most cases, $\delta^{13}C$ values are used as an indicator of the primary carbon source, which increases early in a food chain at a level of 1‰ or less, while $\delta^{15}N$ values are used to determine the relative trophic position with an enrichment of 3-4‰ per trophic level (Peterson & Fry 1987). Knowing the base isotope ratios is required for comparisons between ecosystems (Post 2002).

The basis for using stable isotope ratios is founded on the observation of a pattern of isotopic enrichment with increasing trophic level. However, trophic levels may not be discrete and fixed but rather dynamic, particularly in ecosystems with high levels of omnivory (Jennings et al. 1997, Post 2002). Isotopic composition is also known to vary with latitude and along gradients in estuaries. The latitudinal shift in isotopic composition is primarily in $\delta^{13}C$ correlated with the characteristics of phytoplankton (Takai et al. 2000) while sources of variations along gradients of both $\delta^{13}C$ and $\delta^{15}N$

in estuaries is less clear (Riera 1998, Connolly et al. 2005a). Deegan & Garritt (1997) found that consumer $\delta^{13}C$ values for species common to all regions showed lower values in the oligohaline upper Plum Island estuary than the middle or lower estuary consistent with a change from oligohaline to marine phytoplankton, while $\delta^{15}N$ values were correlated with trophic level rather than region within the estuary. Riera (1998) found that $\delta^{15}N$ in particulate organic matter increased slightly from riverine to oceanic environments while in oysters the $\delta^{15}N$ values decreased due to a change in the source of particulate food. However, Connolly et al. (2005a) found no statistically significant relationship between $\delta^{13}C$ values and positions along transects (400m) and between transects situated along a north-south gradient in the upper Gulf St Vincent in South Australia contradicting the results of Guest et al. (2004) who showed variation within the scale of meters. Connolly et al. (2005a) concluded that other environmental variables (e.g. tidal currents) than openness to the sea (Odum et al. 1979) are important in determining the extent of carbon movements and subsequent assimilation of consumers.

Spencer Gulf is an oligotrophic inverse estuary with higher salinities in the upper gulf than further south (Bye 1981, Nunes Vaz et al. 1990, El-Sabh et al. 1997). The physical characteristics of the environment are also different in temperature and tidal currents. During the summer months the upper Spencer Gulf experiences higher temperatures than further south (Bye 1981, Smith and Veeh, 1989). There is little evidence of autotrophic sources of nutrition for fisheries in Spencer Gulf and terrestrial run-off is limited due to low rainfalls. Connolly et al. (2005a) studied the nutrition of the yellowfin whiting (*Sillago schomburgkii*) occurring in shallow waters in Spencer Gulf and Gulf St Vincent and found that seagrass and epiphytes were the most important contributors while mangrove, saltmarsh, and microalgae/microphytobenthos played a minor role. The energy and nutrient contribution of seagrass to the growth and reproduction of quantitatively and commercially important species occurring in deeper waters is not known. In the Spencer Gulf prawn fishery, which is a trawl fishery, variable volumes of by-catch are discarded throughout the gulf during a 60-day fishing season (November-December, March-June), depending on prawn population dynamics, effort (trawl hours) and subsequent depletion by fishing. Discarded by-catch is consumed by several

scavenger species, which themselves may be caught by the prawn fishing fleet and discarded as by-catch.

The aim of this study was to investigate the isotopic gradient in an inverse estuary and to test whether the isotopic composition and hence trophic position of major consumers of discarded by-catch varies among five sites along a North-South gradient with a history of different trawl intensity.

## Material and Methods

### Selection of Study Areas

Five sites were selected representing areas of different fishing intensity (trawl hours) along a North-South gradient in Spencer Gulf (Figure 1 and Table I in Chapter 1). Data were obtained using logbook recordings of trawling hours reported from administrative fishing blocks for the period 1999-2004. Blocks that include a site, plus data from the neighbouring blocks, were pooled and the 4-year annual mean with the 95% confidence interval was calculated for each site (Figure 2 in Chapter 2). The five selected sites also represent different habitats with different heterogeneity/complexity (see McCoy and Bell 1991) demonstrated by the catch rates of benthos (sessile organisms dominated by sponges) obtained from replicated trawl samples (Figure 6 in Chapter 2).

### Sampling

Sampling of dominating by-catch and scavenger species that occur at all five sites (Table I) was carried out at each site using one standard prawn trawl with a headline length of 14.63 m and a 4.5 cm diamond mesh cod end. Vessel speed was maintained at 3 knots. After each 30-minute trawl shot the catch was dropped on a sorting table and sorted into groups. For comparison, samples of seagrass, plankton, and sealice were obtained by diving, plankton net tows and baited traps, respectively.

The species and number of samples analysed for spatial patterns of stable isotopes are listed in Table I. Four samples from adults of each of the species analysed were collected. The size-range of blue crabs was 85-109 mm carapace width and for

prawns between 40-50 mm carapace length. Port Jackson sharks weight-range was 1.18-1.59 kg. All muscle tissue samples were collected from mature animals. In addition, gut content samples were analysed from blue crabs, sand trevally, rough leatherjacket, Degens leatherjacket and Port Jackson shark. After collection, the samples were dissected and the muscle tissue and gut content immediately frozen. As a standard tissue we chose muscle because metabolically active tissue, such as muscle, has a more rapid isotopic turnover rate than less metabolically active tissues (Estrada *et al.* 2005, Yokoyama *et al.* 2005). On return to the laboratory all samples were freeze-dried. All samples were ground and subsequently analysed for $^{13}C$ and $^{15}N$ in addition to total C and N at CSIRO (Department for Land and Water). Lipid extraction was not carried out. Although lipid extraction can cause a positive shift in the food web placement it does not change the overall interpretation of the trophic structure (Davenport & Bax 2002, Murry et al. 2006).

The results were calculated as delta values, which are expressed as the deviation from standard reference material (stable isotope ratio), where

$\delta X = [(R_{sample}/R_{std})-1] \times 1000;$

$X = {}^{13}C$ and $^{15}N;$

$R = {}^{13}C/{}^{12}C, {}^{15}N/{}^{14}N.$

The standards used were Pee Dee Belemnite for carbon and atmospheric $N_2$ for nitrogen.

$\delta^{13}C$ is not considered a useful indicator of trophic position beyond the first two trophic levels, however $\delta^{15}N$ is generally accepted as a much stronger signal (Hobson & Welch 1992). The following formula was used to estimate trophic level (TL) according to Grall et al. (2006):

$TL = [(\delta^{15}N_{consumer} - 6.17)/3.4] + 1,$

where 6.17 is the mean value for plankton in Spencer Gulf (N=10; SD=1.56) and 3.4 is the assumed $^{15}N$ trophic enrichment factor (Minawaga and Wada 1984). Plankton is designated as TL1. This approach provides only a rough estimate of trophic level considered sufficient to give information of the analysed species.

Table I. Species collected in Spencer Gulf for stable isotope analyses, their abbreviation, number of samples (N), and trophic level.

| Species | Species codes | N (tissue) | N (gut) | Trophic level |
|---|---|---|---|---|
| Blue crab (*Portunus pelagicus*) | BC | 50 | 15 | 1.50 |
| Prawn (*Melicertus latisulcatus*) | PRA | 30 | - | 1.60 |
| Sand trevally (*Pseudocaranax wrighti*) | SAT | 20 | 20 | 2.07 |
| Rough leatherjacket (*Scobinichthys granulatus*) | RLJ | 45 | 15 | 1.55 |
| Degens leatherjacket (*Thamnoconus degeni*) | DLJ | 35 | 4 | 2.09 |
| Bridled leatherjacket (*Acanthaluteres spilomelanurus*) | BLJ | 25 | - | 1.32 |
| Toothbrush leatherjacket (*Acanthaluteres vittiger*) | TBLJ | 25 | - | 1.48 |
| Port Jackson shark (*Heterodontus portusjacksoni*) | PJS | 15 | - | 2.35 |
| Sealice (Amphipoda) | AMP | 3 | - | 1.59 |
| Sealice (Isopoda) (*Natatolana sp.*) | ISO | 3 | - | 2.06 |
| Seagrass (*Posidonia australis*) | Seagrass | 3 | - | - |

**Gut samples**

Individuals for stomach and gut content analyses were sampled haphazardly at each station. Numbers of samples are given in the Table IV-VII. Prey items were identified to the lowest possible taxon. In addition to samples obtained from the trawl surveys, samples of the stomach contents of Port Jackson sharks from industry returned tagged individuals were included to allow for comparisons between periods of commercial trawling and no trawling. Samples of sand trevally were obtained from all five sites and blue crab from four sites. Samples of Degens leatherjacket and rough leatherjacket were only obtained from Sites 2 and 4. The stomachs/intestines were dissected and assessed visually for fullness. Stomach fullness was described on a scale of 0-3, where 0 = empty, 1 = some food items, 2 = half full, and 3 = completely full.

**Statistical Analysis**

Stable isotope concentrations from species sampled at all five localities were statistically analysed using two-way ANOVA's with site and species as fixed effects. The data were tested for normality and homogeneity of variances using Kolmogorov-Smirnov test and Levene's test, respectively, as provided in the statistical software package SPSS v. 14.0 (SPSS Inc., Chicago, Illinois, USA). Post-hoc tests were done using Tukey HSD test when variances were homogeneous and Dunnett C test when variances were not homogeneous. In SPSS, partial eta squared values are provided as a substitute for effect size (see Pallant 2005). Additional analyses using Spearman Rank correlations were performed. A hierarchical cluster analysis on the mean $\delta^{13}$C and $\delta^{15}$N values using Ward's minimum variance method was used to identify trophic groups (Davenport & Baxt 2002, Grall et al. 2006). Gut content was analysed using the PRIMER v. 6.1.6 package (PRIMER-E Ltd, Plymouth Marine Laboratory, 2006). A similarity matrix was constructed using the Bray-Curtis similarity coefficient and the matrix was ordinated using multidimensional scaling techniques. One and two-way analyses of similarities (ANOSIM) were employed to test whether dietary composition differed among species and sites. Stomach fullness comparisons were analysed using $\chi^2$ test for independence.

## Results

**Trophic groups**

The relationships between $\delta^{13}$C and $\delta^{15}$N concentrations for dominating by-catch and scavenger species that occur at all five sites are shown in Figure 1 and their trophic levels based on $\delta^{15}$N in Table 1. Assuming a 3.4‰ increase in $\delta^{15}$N per trophic level, the results showed a trophic fractionation separating the species into three different trophic subgroups with Port Jackson shark (PJS) at the highest level (TL=2.35) followed by Degens leatherjacket (DLJ), sand trevally (SAT) and the isopods *Natatolana woodjohnsii* and *N. viridis* (ISO) with TL = 2.09, 2.07 and 2.06, respectively. At the lowest level were prawns (PRA; TL=1.60), a group of unidentified amphipods (AMP; TL=1.59), rough leatherjackets (RLJ; TL=1.55), blue crabs (BC; TL=1.50), toothbrush leatherjackets (TBLJ; TL=1.48) and bridled leatherjackets (BLJ; TL=1.32). Plankton values of $\delta^{13}$C decreased with increasing

particle size while $\delta^{15}N$ values were relatively constant while seagrass values were considerable lower.

A cluster analysis of the mean $\delta^{13}C$ and $\delta^{15}N$ values of the eight species and two sealice groups is shown in Figure 2. The analyses separate four main groups comprised of 1: BC, AMP and PRA; 2: PJS and ISO; 3: RLJ, TBLJ and BLJ; 4: DLJ and SAT.



Figure 1. Stable isotope composition from plankton, seagrass and muscle tissue of quantitatively important by-catch species in the Spencer Gulf prawn fishery. Trophic groups are encircled. See Table I for species codes. Error bars are 95% CI.

Figure 2. Hierarchical clustering the mean $\delta^{13}C$ and $\delta^{15}N$ values of 8 species and 2 species groups from Spencer Gulf.

**Effect of site and species - carbon**

Figures 3, 4 and 5 show the distribution of $\delta^{13}C$ for eight species as a function of site and species. The data are presented in three graphs for clarity. To test the null-hypothesis of no difference in $\delta^{13}C$ between site and species, a two-way ANOVA on a normally distributed data set with homogeneous variances, with site and species as the main factors, and $\delta^{13}C$ as the dependent variable was carried out. The results showed a significant effect of site ($F_{[4, 254]} = 8.170$, P < 0.001, partial eta squared = 0.132) and species ($F_{[7, 254]} = 56.346$, P < 0.001, partial eta squared = 0.648) with no significant interactions ($F_{[28, 254]} = 0.819$, P = 0.728, partial eta squared = 0.097). The effect size for the factor site was moderate to large, and for the factor species large, with low probability for type I error. The effect of site for $\delta^{13}C$ was consistent among species. Accordingly, the null-hypothesis can be rejected. A Tukey HSD post hoc test separated Site 1 with the highest $\delta^{13}C$ value from Site 2, 3 and 4, which were significantly separated from Site 5. The results showed that, with the exception of PJS (Figure 5), $\delta^{13}C$ values declined gradually from the northern site (Site 1) towards the southern site (Site 5). TBLJ and BLJ showed large variations in $\delta^{13}C$ values between sites compared to the other species (Figure 4). A Tukey HSD post hoc test separated the eight species into three groups with respect to $\delta^{13}C$, 1: SAT, 2: RLJ, TBLJ, DLJ and BLJ, 3: PRA, PJS and BC (Figure 1).

Figure 3. Stable isotope composition ($\delta^{13}C$) of blue crab (*Portunus pelagicus*) and prawn (*Melicertus latisulcatus*) at five sites in Spencer Gulf. Error bars are 95% CI.



Figure 4. Stable isotope composition ($\delta^{13}C$) of bridled leatherjacket (*Acanthaluteres spilomelanurus*), rough leatherjacket (*Scobinichthys granulatus*), toothbrush leatherjacket (*Acanthaluteres vittiger*) and Degens leatherjacket (*Thamnoconus degeni*) at five sites in Spencer Gulf. Error bars are 95% CI.

Figure 5. Stable isotope composition ($\delta^{13}$C) of sand trevally (*Pseudocaranax wrighti*), and Port Jackson shark (*Heterodontus portusjacksoni*) at five sites in Spencer Gulf. Error bars are 95% CI.

**Effect of site and species – nitrogen**

Figures 6, 7 and 8 shows the distribution of $\delta^{15}$N for eight species as a function of site and species. The data are presented in three graphs for clarity. To test the null-hypothesis of no difference in $\delta^{15}$N between site and species a two-way ANOVA with site and species as the main factors and $\delta^{15}$N as the dependent variable was carried out. The data were normally distributed but the variances were not homogeneous. Transformation and removal of outliers could not solve this problem. However, ANOVA is usually robust against violation of this assumption so the analysis was carried out regardless. The results showed a significant effect of site  ($F_{[4, 254]}$ = 9.695, P < 0.001, partial eta squared = 0.153) and species  ($F_{[7, 254]}$ = 26.278, P < 0.001, partial eta squared = 0.462) with no significant interactions  ($F_{[28, 254]}$ = 1.270, P = 0.175, partial eta squared = 0.142). The effect size for the factor site and species were both large with low probability of type I error. Accordingly, the null-hypothesis was

rejected. A Dunnett C-test separated Site 1 from 4 and 5 and Site 2 from 4. The results showed that $\delta^{15}$N-values declined gradually from Site 1 (north) towards Site 5 (south). However, this pattern was less consistent for leatherjackets and SAT, which showed a gradual decline in $\delta^{15}$N-values towards Site 4 but then an increase at Site 5, although this increase was not evident for TLJ (Figure 7). However, PRA, BC and PJS showed a consistent pattern of decline towards south in $\delta^{15}$N enrichment. The results indicate that in addition to a gradual decline, the difference in environment influence $\delta^{15}$N enrichment, particularly between Site 4 and 5. A Dunnett-C test separated the eight species into two groups with respect to $\delta^{15}$N-values, 1: PJS, DLJ and SAT with the highest values, and 2: BC, PRA and the remaining leatherjackets, with the lowest values (Figure 1).



Figure 6. Stable isotope composition ($\delta^{15}$N) of blue crab (*Portunus pelagicus*) and prawn (*Melicertus latisulcatus*) at five sites in Spencer Gulf. Error bars are 95% CI.

Figure 7. Stable isotope composition ($\delta^{15}$N) of bridled leatherjacket (*Acanthaluteres spilomelanurus*), rough leatherjacket (*Scobinichthys granulatus*), toothbrush leatherjacket (*Acanthaluteres vittiger*) and degens leatherjacket (*Thamnoconus degeni*) at five sites in Spencer Gulf. Error bars are 95% CI.



Figure 8. Stable isotope composition ($\delta^{15}$N) of sand trevally (*Pseudocaranax wrighti*), and Port Jackson shark (*Heterodontus portusjacksoni*) at five sites in Spencer Gulf. Error bars are 95% CI.

374

**Stable isotope contents of gut samples**

The gut contents for SAT and BC were analysed for $\delta^{13}C$ and $\delta^{15}N$ and the results from samples collected at the five studied sites are shown in Table II. The results showed that the mean stable isotope enrichment of SAT was $\delta^{13}C_{tissue}$- $\delta^{13}C_{gut}$=1.30‰ and $\delta^{15}N_{tissue}$- $\delta^{15}N_{gut}$=3.02‰. However, the isotopic content of the BC gut samples was variable and most samples collected at Site 2 failed to produce values. Nevertheless, the results showed that the mean stable isotope enrichment of BC was $\delta^{13}C_{tissue}$- $\delta^{13}C_{gut}$=-4.13‰ and $\delta^{15}N_{tissue}$- $\delta^{15}N_{gut}$=2.03‰.

Table II. Stable isotope content of gut content of sand trevally (*Pseudocaranx wrighti*) and blue crab (*Portunus pelagicus*) from Spencer Gulf. * = one sample, NA= no samples. Errors are 95% CI.

| Species | Sand trevally | | Blue crab | |
|---|---|---|---|---|
| Site | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C$ | $\delta^{15}N$ |
| 1 | -15.95±1.65 | 6.85±0.56 | -13.26±1.12 | 6.03±1.25 |
| 2 | -21.70±1.47 | 7.25±0.51 | -12.35* | 6.74* |
| 3 | -22.25±1.38 | 7.75±0.67 | -6.86±0.53 | 5.39±0.65 |
| 4 | -23.63±0.25 | 5.58±2.02 | -16.28±1.17 | 5.90±0.65 |
| 5 | -12.80±4.75 | 6.53±0.71 | NA | NA |
| Mean | -19.27±4.80 | 6.79±0.53 | -12.10±2.19 | 5.84±0.49 |

**Trawl hours, habitat complexity and stable isotope relationships**

The results of Spearman Rank correlation analyses between habitat complexity (see McCoy & Bell 1991) defined as catch rates of benthos obtained by replicated trawl samples (Chapter 2, Figure 6), trawl hours and stable isotopes is shown in Table III. With the expectation of PJS at the highest trophic level, there was a general trend of negative correlation between $\delta^{13}C$ and $\delta^{15}N$ and trawl hours and a positive correlation between $\delta^{13}C$ and $\delta^{15}N$ and habitat complexity. The pattern for PJS is similar for habitat complexity and trawl hours (positive correlation for $\delta^{13}C$ and negative correlation for $\delta^{15}N$) but these correlations were small. DLJ showed a medium negative correlation between trawl hours and $\delta^{15}N$ but otherwise no correlation

pattern. The correlation can be explained by the $\delta^{15}N$ pattern of gradual reduction towards Site 4 and the step increase at Site 5 where trawl effort is relatively low.

SAT showed a large positive correlation between $\delta^{15}N$ and habitat complexity and medium to large negative correlation between both $\delta^{15}N$ and $\delta^{13}C$ and trawl hours. Because SAT is not a scavenger on discarded by-catch, trawl hours would not be expected to have any effect on trophic position.

Table III. Spearman rank correlations between stable isotope values and habitat complexity (benthos biomass) and mean trawl hours 1999-2004 in the Spencer Gulf prawn fishery. Rho-values indicate the strength of the correlations ($\pm0.10$-$0.29$ = small, $\pm0.30$-$0.49$ = medium, and $\pm0.50$-$1.0$ = large). Values are highlighted for medium and large strength.

| Species | Trophic group | N | Habitat Rho ($\delta^{13}C$) | Habitat Rho ($\delta^{15}N$) | Trawl hours Rho ($\delta^{13}C$) | Trawl hours Rho ($\delta^{15}N$) |
|---|---|---|---|---|---|---|
| Port Jackson shark | 1 | 15 | 0.222 P=0.427 | -0.223 P=0.423 | 0.241 P = 0.386 | -0.166 P = 0.555 |
| Degens leatherjacket | 2 | 37 | 0.088 P=0.604 | 0.157 P=0.355 | -0.076 P = 0.657 | **-0.424** P = 0.009 |
| Sand trevally | 3 | 20 | 0.188 P=0.427 | **0.580** P=0.007 | -0.267 P = 0.255 | **-0.618** P = 0.004 |
| Bridled leatherjacket | 4 | 25 | **0.477** P=0.016 | **-0.490** P=0.013 | -0.185 P = 0.375 | **-0.669** P = 0.000 |
| Rough leatherjacket | 4 | 47 | -0.084 P=0.573 | 0.003 P=0.983 | **-0.310** P = 0.034 | **-0.367** P = 0.011 |
| Toothbrush leatherjacket | 4 | 25 | -0.055 P=0.794 | 0.218 P=0.295 | **-0.369** P = 0.069 | -0.367 P = 0.071 |
| Blue crab | 5 | 52 | -0.087 P=0.538 | 0.062 P=0.660 | -0.289 P = 0.038 | -0.214 P = 0.127 |
| Prawn | 5 | 33 | **0.368** P=0.035 | 0.254 P=0.154 | **-0.387** P = 0.026 | **-0.348** P = 0.047 |

Habitat complexity and trawl hours: Spearman's rho = **-0.511**, P=0.000

The scavenger leatherjacket group BLJ, RLJ and TLJ showed consistent negative correlations to trawl hours for both $\delta^{13}$C and $\delta^{15}$N while the pattern for habitat was less consistent with the exception of BLJ. Both BLJ and TLJ are not as commonly occurring in prawn trawls as RLJ.

The final group constitutes blue crabs and prawns. For $\delta^{13}$C and $\delta^{15}$N both BC and PRA showed a negative correlation with trawl hours but only PRA showed a positive correlation to habitat complexity. The overall pattern of correlations indicates that factors other than the inter-correlated habitat complexity and trawl hours, such as competition for space and food, may contribute to determining trophic position and how the environment affects it.

**Gut content and stomach fullness**

The gut contents were analysed for sand trevally, Port Jackson shark, blue crab, and two species of leatherjackets, Degens and rough leatherjacket.

*Sand trevally*

Diet composition and stomach fullness of sand trevally (N=220) are shown in Table IV. The results showed that SAT had a varied diet of more than 12 categories of food items and that the diet composition varied significantly between sites. A statistical test of differences in diet composition among sites could not be carried out due to data loss. However, Sites 3 and 4 showed a different diet composition than Sites 1 and 2. A $\chi^2$ test for independence showed a significant difference in frequency of stomach fullness between the five sites ($\chi^2 = 42.561$, $\chi^2_{crit.} = 21.026$, df $= 12$, P<0.001). The results showed that stomach fullness was generally higher at Site 3 and 5 than elsewhere (Table IV). A further analysis of independence between day and night showed a significant difference in frequency of stomach fullness between day and night ($\chi^2 = 108.275$, $\chi^2_{crit.} = 7.815$, df $= 3$, P<0.001), indicating that SAT have significantly more content in the gut during the day than during the night.

Table IV. Diet composition (%) as a function of site and stomach fullness (%) as a function of site and time (day/night) of sand trevally (*Pseudocaranx wrighti*) at five sites in Spencer Gulf. Stomach fullness: 0=empty, 1=some content, 2=half full, 3=full. N=no of individuals analysed.

| Species\Site | 1 | 2 | 3 | 4 | 5 | Stomach fullness \Site | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Porifera | 5.8 | 0 | 12.7 | 0 | 1.4 | 0 | 13.8 | 48.9 | 11.5 | 23.1 | 6.0 |
| Foraminifera | 8.7 | 10.8 | 0 | 0 | 8.3 | 1 | 37.9 | 25.5 | 30.8 | 41.0 | 32.0 |
| Hydrozoa | 14.4 | 7.5 | 0 | 1.7 | 8.3 | 2 | 43.1 | 21.3 | 38.5 | 33.3 | 46.0 |
| Anthozoa | 2.9 | 3.2 | 0 | 1.7 | 8.3 | 3 | 5.2 | 4.3 | 19.2 | 2.6 | 16.0 |
| Bryozoa | 1.9 | 2.2 | 9.1 | 0 | 0 | N | 59 | 47 | 26 | 39 | 51 |
| Gastropoda | 9.6 | 10.8 | 0 | 13.6 | 6.9 | | | | | | |
| Bivalvia | 17.3 | 17.2 | 0 | 5.1 | 22.2 | | | | | | |
| Polychaeta | 7.7 | 7.5 | 0 | 3.4 | 4.2 | | Day | Night | | | |
| Crustacea | 17.3 | 21.5 | 36.4 | 32.2 | 27.8 | 0 | 0.0 | 38.7 | | | |
| Teleosts | 8.7 | 7.5 | 20.0 | 27.1 | 6.9 | 1 | 18.5 | 47.1 | | | |
| Plant matter | 4.8 | 11.8 | 20.0 | 15.3 | 5.6 | 2 | 67.0 | 10.9 | | | |
| Other | 1.0 | 0 | 1.8 | 0 | 0 | 3 | 14.6 | 3.4 | | | |
| N | 59 | 47 | 26 | 39 | 51 | N | 105 | 117 | | | |

*Port Jackson shark*

Diet composition and stomach fullness of 63 PJS sampled during periods with commercial trawling and periods with no trawling is shown in Table V. Following ordination of the dietary data (present/not present) the dietary samples of PJS formed two groups according to "trawling" and "no trawling" with overlap and outliers. However, "no trawling" was more dispersed than "trawling" (Figure 9A). ANOSIM demonstrated that the dietary compositions between "trawling" and "no trawling" were significantly different overall ($P = 0.018$). The result showed that blue crabs and Cephalopoda (primarily southern) were more abundant in the diet when trawling

occurred, while prawns were more abundant in the diet when no trawling occurred. No difference in stomach fullness between trawling and non-trawling periods was evident ($\chi^2 = 3.570$, $\chi^2_{crit.} = 7.815$, df = 3, P = 0.312).

Table V. Port Jackson shark (*Heterodontus portusjacksoni*). Diet composition and stomach fullness during commercial trawling and no trawling. Stomach fullness: 0=empty, 1=some content, 2=half full, 3=full. N=number of individuals analysed.

| Species | No trawling (%) | Trawling (%) | Stomach fullness | No trawling (%) (N) | Trawling (%) (N) |
|---|---|---|---|---|---|
| Vermes | 26.3 | 14.7 | 0 | 23.8 (5) | 14.6 (6) |
| Anthozoa | 0 | 2.9 | 1 | 38.1 (8) | 36.6 (15) |
| Polychaeta | 19.3 | 15.7 | 2 | 28.6 (6) | 19.5 (8) |
| Blue crab | 3.5 | 13.7 | 3 | 9.5 (2) | 29.3 (12) |
| Crustaceans | 5.3 | 5.9 | N | 21 | 41 |
| Prawn | 14.0 | 7.8 | | | |
| Cephalopoda | 1.8 | 17.7 | | | |
| Other Mollusca | 1.8 | 0 | | | |
| Echinodermata | 8.8 | 2.0 | | | |
| Teleost | 14.0 | 14.7 | | | |
| Other | 5.3 | 4.9 | | | |
| N | 21 | 41 | | | |

Figure 9. Non-metric MDS ordination of the dietary data (present/not present) of PJS sampled during periods of no trawling and trawling (A) and BC sampled at four different sites in Spencer Gulf (B).

Figure 10. Non-metric MDS ordination of the dietary data (present/not present) of Degens and rough leatherjacket (A) and both species at two sites (B). DLJ=Degens leatherjacket; RLJ=rough leatherjacket.

Table VI. Blue crab (*Portunus pelagicus*). Diet composition (%) as a function of site and stomach fullness (%) as a function of site and time (day/night) at five sites in Spencer Gulf. Stomach fullness: 0=empty, 1=some content, 2=half full, 3=full. N=number of individuals analysed.

| Species\Site | 1 | 2 | 3 | 4 | Stomach fullness\Site | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|
| Foraminifera | 28.6 | 31.9 | 29.1 | 31.3 | 0 | 23.3 (7) | 40.0 (14) | 13.3 (4) | 32.1 (9) |
| Porifera | 1.4 | 0 | 0 | 1.6 | 1 | 46.7 (14) | 28.6 (10) | 56.7 (17) | 46.4 (13) |
| Hydrozoa | 2.9 | 10.6 | 3.8 | 1.6 | 2 | 23.3 (7) | 2.9 (1) | 23.3 (7) | 10.7 (3) |
| Bryozoa | 1.4 | 0 | 2.5 | 7.8 | 3 | 6.7 (2) | 28.6 (10) | 6.7 (2) | 10.7 (3) |
| Polychaeta | 1.4 | 4.3 | 3.8 | 3.1 | N | 30 | 30 | 30 | 30 |
| Bivalvia | 21.4 | 23.4 | 8.9 | 12.5 | | Day | Night | | |
| Gastropoda | 20.0 | 14.9 | 15.2 | 12.5 | 0 | 35 | 22.4 | | |
| Crustacea | 15.7 | 14.9 | 7.6 | 17.2 | 1 | 53.3 | 43.1 | | |
| Echinodea | 5.7 | 0 | 27.9 | 9.4 | 2 | 6.7 | 22.4 | | |
| Other | 1.4 | 0 | 1.3 | 3.1 | 3 | 5.0 | 12.1 | | |
| N | 30 | 33 | 30 | 30 | N | 60 | 60 | | |

*Blue crab*

Diet composition and stomach fullness of 123 BC sampled at four sites is shown in Table VI. Following ordination of the dietary data (present/not present) the dietary samples of BC formed a pattern where Site 4 was more aggregated and consequently less similarity with Site 1, 2 and 3 (Figure 9B). ANOSIM demonstrated that the dietary compositions among sites were significantly different overall (P=0.002). However, pair wise tests showed that only Site 4 was significantly different from the other sites indicated by a more evenly distribution of the presence of food items among dietary categories (Table VI). The results showed that the diet of BC was diverse, with more than 10 food categories, and varied between sites. Meiofauna and small fragments of larger animals dominated the food items. Small bivalves dominated the diet at Sites 1 and 2, while sea urchin fragments dominated at Site 3.

Foraminifera were a major component in all samples. Stomach fullness varied between sites. A $\chi^2$ test for independence of site showed a significant difference in frequency of stomach fullness between sites ($\chi^2 = 21.111$, $\chi^2_{crit.} = 16.919$, df = 9, P = 0.012). This pattern is primarily affected by the samples at Site 2, where BC guts were either empty (0-1) or full (3). An analysis of independence of stomach fullness between day and night showed a significant difference ($\chi^2 = 9.075$, $\chi^2_{crit.} = 7.815$, df = 3, P = 0.028). The results showed that more BC had a full stomach at night than during the day.

Table VII. Degens leatherjacket (*Thamnoconus degeni*) (DLJ) and rough leatherjacket (*Scobinichthys granulatus*) (RLJ). Diet composition (%) and stomach fullness (%) (N) at two sites in Spencer Gulf. Stomach fullness: 0=empty, 1=some content, 2=half full, 3=full. N=number of individuals analysed.

| Species\Site | DL 2 | DL 4 | RL 2 | RL 4 | Stomach fullness\Site | DL 2 | DL 4 | RL 2 | RL 4 |
|---|---|---|---|---|---|---|---|---|---|
| Foraminifera | 10.5 | 2.4 | 19.1 | 13.6 | 0 | 33.3 (4) | 10.0 (1) | 0 (0) | 0 (0) |
| Algae | 0 | 11.9 | 0 | 16.7 | 1 | 50.0 (6) | 30.0 (3) | 16.7 (1) | 6.7 (1) |
| Cnidaria | 0 | 0 | 9.5 | 0 | 2 | 16.7 (2) | 20.0 (2) | 66.7 (4) | 40.0 (5) |
| Bryozoa | 5.3 | 7.1 | 0 | 7.6 | 3 | 0 (0) | 40.0 (4) | 16.7 (1) | 53.3 (8) |
| Polychaeta | 0 | 0 | 0 | 6.1 | N | 12 | 10 | 6 | 14 |
| Bivalvia | 15.8 | 11.9 | 23.8 | 13.6 | | | | | |
| Gastropoda | 15.8 | 4.8 | 4.8 | 1.5 | | | | | |
| Cephalopoda | 0 | 2.4 | 0 | 0 | | | | | |
| Crustacea | 7.9 | 4.8 | 0 | 4.6 | | | | | |
| Echinoderms | 2.6 | 11.9 | 9.5 | 4.6 | | | | | |
| Teleost | 2.6 | 11.9 | 0 | 6.1 | | | | | |
| Shell sediment | 5.3 | 9.5 | 23.8 | 13.6 | | | | | |
| Plant matter | 21.1 | 19.1 | 4.8 | 6.1 | | | | | |
| N | 12 | 10 | 6 | 14 | | | | | |

*Leatherjackets*

Diet composition and stomach fullness of 22 DLJ and 21 RLJ sampled at two sites is shown in Table VII. Following ordination of the dietary data (present/not present) the dietary samples of the two leatherjackets formed a pattern of larger overlap between the Site 2 and 4 but less overlap between the two species (Figure 10A, B). ANOSIM demonstrated that the dietary compositions between sites were not significantly different overall (P=0.078) but significantly different between the two species (P=0.023). The diet of both leatherjackets was diverse, with more than 13 food categories. The major differences in diet between the two species of leatherjackets were for the groups Foraminifera, Bivalvia, Cephalopoda, Teleost and shell sediment. Both species of leatherjacket were found to be benthic omnivores, with a varied diet, most likely reflecting differences in the benthic assemblages between sites. However, squid and fish dominated the stomach contents of a few individuals indicating feeding as scavengers, which affected the results of the statistical analyses. No statistical difference in stomach fullness between sites for either species was found (DLJ: $\chi^2 =$ 6.673, $\chi^2_{crit.} = 7.815$, df = 3, P = 0.083; RLJ: $\chi^2 = 1.091$, $\chi^2_{crit.} = 7.815$, df = 3, P = 0.779).

## Discussion

The introduction of discards from the Spencer Gulf prawn fishery is likely to affect the ecosystem by subsidising scavenger populations (Ramsay et al. 1997). The total volume of discards available to scavengers is unknown but likely to be in the range of 50-300 kg/h during 20,000 hours trawling without considering local depletion effects and discard survival. The Spencer Gulf ecosystem appears to have a high degree of interconnectivity among a large number of generalist species (omnivory) with a broad diet covering more than one trophic level. Baited video experiments have shown that on Spencer Gulf prawn trawling grounds the most numerically common scavengers on discarded by-catch were leatherjackets (notably Degens leatherjacket), blue crabs and sealice (notably *N. woodjonesi*). Of larger species, Port Jackson sharks and stingrays play an important role.

**Trophic groups**

Stable isotopes can provide useful information about trophic linkages and feeding patterns (Peterson & Fry 1987, Cabana & Rasmussen 1996, Hobson & Wassenaar 1999). However, variability in isotopic composition both between and within ecosystems makes stable isotope analyses complementary to other methods for determining trophic relationships (Davenport & Bax 2002, Connolly et al. 2005b). When using $\delta^{15}N$ alone, the species studied here separated into three trophic groups (subgroups) with PJS at the highest level followed by DLJ, SAT and ISO. At the lowest level were PRA and AMP, BC, and a group of leatherjackets (RLJ, TBLJ, BLJ). However, a hierarchical cluster analysis using the mean $\delta^{13}C$ and $\delta^{15}N$ values separated the species into four main groups (Figure 1 and 2). With the exception of SAT and PRA, all the other species have been observed to feed on discarded by-catch presented as bait during video experiments.

The five species where gut content analyses were performed (SAT, PJS, BC, DLJ, RLJ) can be characterised as omnivorous with more than 10 diet categories (Tables IV-VII) (Williams 1982, Last 1983, Edgar 1990, Officer & Parry 2000). The gut content of SAT was dominated by small benthic organisms particularly crustaceans. However, a large component in the stomach content was skeletal parts of bony fishes indicating that SAT is also a scavenger, probably during the day. The largest fraction of bony fish elements was observed at Site 3 and 4 with 20.0 and 27.1%, respectively (Table IV). These two sites are exposed to the highest trawl effort. In addition to a large variety of the remains of smaller benthic organisms, the stomach content of DLJ contained parts of bony fishes and squids demonstrating that DLJ is scavenger on discarded by-catch (Table IV). The stomach content of SAT and DLJ thus explain their position at a high trophic level (Figure 1). Of the group of leatherjackets at a lower trophic level only the stomach content of RLJ was analysed. The stomach content of this species also included parts of bony fishes but not of squids, and the content of shell fragments and Foraminifera was higher than DLJ. This shows a different feeding pattern from DLJ as indicated by the trophic position of the leatherjacket group.

The diet of PJS includes a variety of benthic animals justifying its trophic position (Table V). However, the stomach content of PJS caught during commercial trawling

included a significant larger fraction of blue crabs and squids (southern calamary). It is unlikely that PJS (and leatherjackets) should be able to catch live squids and the origin of both squids and the larger component of BC are most likely from discarded by-catch. It is noteworthy that the fraction of prawns found in the stomachs of PJS was larger during the period of no commercial trawling.

Sealice constitutes a group of marine scavengers composed of several isopod and amphipod carnivorous species. They are active swimmers and voracious scavengers, which burrow in the sediment during day and are active at night where they aggregate at carrion in swarms. In Spencer Gulf, the dominating species are isopods of the genus Natatolona (Cirolanidae) (ISO) and a group of unidentified amphipods (AMP). The hierarchical cluster analysis group ISO with PJS at a high trophic position, and for $\delta^{15}$N alone at the same level at SAT and DLJ indicating these species feeding preference for carrion. However, AMP was found to cluster with BC and PRA (Figure 1).

Seagrass and plankton are the likely primary sources of carbon in Spencer Gulf because terrestrial and intertidal contributions from mangroves and saltmarsh are low in the arid environment (Connolly et al. 2005a). The linkage between plankton and secondary consumers appears to be through the consumption of detritus by the crustaceans BC, PRA and AMP. Considering that BC have been identified as consumers of discarded by-catch (Wassenberg & Hill 1987), discards appear to contribute little if anything to this species trophic level, which is consistent with the results of the gut analyses (Table VI). BC showed a significant higher degree of stomach fullness at night than during the day. This is not likely to indicate that BC feeds at night because when the foregut is full it takes a crab about six hours before feeding resumes (Wassenberg & Hill 1987).

The stable isotope values of prawns and blue crabs are similar to what have been reported for the Portunid swimmer crab *Ovalipes molleri* and Carid shrimps by Davenport & Bax (2002), but comparisons between ecosystems are difficult because the source of $\delta^{13}$C and $\delta^{15}$N can vary significantly between systems (Post 2002). This study has shown that benthic crustaceans (PRA, BC and AMP) in Spencer Gulf broadly share the same food source, and have a stable isotopic composition within the

range found for tropical prawns by Loneragan et al. (1997). Loneragan et al. (1997) found for the offshore prawns *Penaeus merguiensis*, *P. esculentus*, and *P. semisulcatus* from the Gulf of Carpentaria the primary source of carbon being benthic microalgae, which are thought to be an important source for marine food webs (Newell et al. 1995), and concluded that mangrove/terrestrial carbon makes a minor contribution to the food web supporting prawns in coastal waters. The relatively high content of $\delta^{13}$C in both ISO and AMP can be explained by detritus feeding when they bury in the sediment during day.

In general, the trophic fractionation for $\delta^{15}$N is 3-4‰ and for $\delta^{13}$C 1‰ relative to the diet irrespective of habitat (see Michener & Schell 1994). In this study, trophic enrichment relative to gut content for SAT was 3.02‰ for nitrogen and 1.30‰ for carbon in accordance with values reported in the literature (reviewed by Post 2002). However, the isotopic content of the BC gut samples was variable with a negative enrichment (-4.13‰) for $\delta^{13}$C and a positive enrichment of 2.03‰ for $\delta^{15}$N indicating a broad spectrum in diet composition. The stable isotope analysis of the gut contents of the other studied species failed to produce any results.

Davenport & Bax (2002), who studied the shelf ecosystem off south-eastern Australia, reported stable isotope values for silver trevally (*Pseudocaranx dentex:* –17.1‰ $\delta^{13}$C and 12.3‰ $\delta^{15}$N), which is closely taxonomically related to the sand trevally studied here (*Pseudocaranx wrighti*), and for Port Jackson shark (–15.2‰ $\delta^{13}$C and 12.0‰ $\delta^{15}$N). The values from this study are significantly lower for both $\delta^{15}$N and $\delta^{13}$C indicating feeding at lower trophic levels in Spencer Gulf compared to the south-eastern shelf. Davenport & Bax (2002) reported stable isotope values for POM to be -21.5 for $\delta^{13}$C and 6.1 for $\delta^{15}$N for south-eastern Australian waters. In this study we found POM (plankton) values to be higher for $\delta^{13}$C decreasing with particle size ($\delta^{13}$C -15.5 to 18.2).

**Spatial variability**

Large-scale geographical variation in $\delta^{13}$C but not $\delta^{15}$N values has been related to latitudinal characteristics of phytoplankton with lighter values at higher latitudes (Takai et al. 2000). In estuaries, sources of variation along gradients of $\delta^{13}$C with a

shift towards lighter values in oligohaline habitats have been shown to be consistent with a change from marine to oligohaline phytoplankton (Deegan and Garritt 1997). However, Connolly et al. (2005a) found no significant differences in $\delta^{13}C$ values between transects situated along a north-south gradient in the upper Gulf St Vincent in South Australia. Variation in $\delta^{15}N$ appears to be correlated with trophic level rather than region within the estuary (Deegan and Garritt 1997, Riera 1998, Connolly et al. 2005a).

In this study the stable isotope composition for both $\delta^{13}C$ and $\delta^{15}N$ generally showed a significant decline from Site 1 to 5. The decline in stable isotope concentration indicates an overall feeding at successively lower trophic levels from north to south in Spencer Gulf. For PRA and BC the decline was about 1‰ for both $\delta^{15}N$ and $\delta^{13}C$, while for leatherjackets the decline was about 2-4‰ but with large variations between sites. However, for leatherjackets, with the exception of TBLJ, $\delta^{15}N$ increased dramatically from Site 4 to 5 (Figure 7) indicating a shift to higher trophic levels further south. A consistent decline in SAT $\delta^{13}C$ values was evident but not for PJS. Both of these species showed little change in $\delta^{15}N$ values as a function of site. The overall pattern of change in trophic position from north to south indicates that for a given species, trophic position can vary.

The decline in $\delta^{13}C$ enrichment is in contrast to what has been reported for estuaries elsewhere (Deegan and Garritt 1997), and is likely to be a consequence of Spencer Gulf being an inverse estuary with minimal freshwater input and higher salinity in the upper gulf than further south (El-Sabh et al. 1997). Water with the highest salinity have experienced the most extended isolation from mixing with shelf waters, which may last up to 270 days (Smith and Veeh 1989, Nunes Vaz et al. 1990). The primary production in Spencer Gulf is largely believed to be sustained by internal recycling of essential plant nutrients, but the mixing process with the up welling system off southern Australia is not understood (Smith and Veeh 1989, Corlis et al. 2003, Ward et al. 2006). In intertidal habitats, the source of carbon appears to be local, originating from seagrass and seagrass epiphytes, while mangrove/saltmarsh and macroalgae/microphytobenthos only play a minor role (Connelly et al. 2005a, b).

The variation in $\delta^{15}N$ enrichment from north to south can generally be explained by the diversity of food sources (Jennings et al. 1997, Riera 1998). However, a consistent decline was pronounced in PRA, BC and PJS, which may be explained by successive food deprivation (Michener & Schell 1994).

The stable isotope composition of the gut content of BC and SAT showed large variations between site, as would be expected because these values represent the diet of the immediate past while the stable isotope composition of muscle tissue accumulates through an individuals lifetime (Peterson & Fry 1987, Estrada et al. 2005, Yokoyama et al. 2005). The difference in mean stable isotope content for SAT gut content and muscle tissue was one trophic level (1.29‰ $\delta^{13}C$ and 3.02‰ $\delta^{15}N$) but slightly less for BC (4.18‰ $\delta^{13}C$ and 2.04‰ $\delta^{15}N$) (Table 2). However, the variations between sites were large, reflecting a broad diet of these species (Tables IV and VI).

Data on gut content between sites are available for SAT, BC, RLJ and DLJ. Significant differences between sites were evident for all species except RLJ. However, a statistical comparison between the two species of leatherjackets for each site failed to separate the diet composition. Stomach fullness was significantly different between sites for SAT and BC but not for leatherjackets. These results do not appear to reflect a consistent preference for a specific food items and volume, but rather reflect a difference in food availability between sites, because large variations between individuals were evident.

**Effects of habitat and trawling**

The correlation analysis between trawling hours and habitat complexity, measured as the abundance of benthos (primarily sponges) (see McCoy & Bell 1991), showed a general trend of negative correlation between $\delta^{13}C$ and $\delta^{15}N$ and trawl hours and a positive correlation between $\delta^{13}C$ and $\delta^{15}N$ and habitat complexity, disregarding the largest species, PJS, on the top of the food chain. These correlations are not conclusive but tentative and require further studies to be substantiated. Spearman Rank correlations can be used to give an overall view of correlation patterns because there is no assumption of linearity and it is insensitive to proportional and additive differences between samples (Krebs 1999). The Spearman rho values should be interpreted as a measure of the strength and direction of the relationship, as indicated

in Table III, and the significance levels should be treated cautiously due to the strong influence of sample size (see Cohen 1988, Pallant 2005). The negative relationship between habitat complexity and trawl hours cannot easily be verified because no data before fishing commenced are available. It is likely, however, that trawling has a negative effect on habitat complexity (Watling & Norse 1998). The results nevertheless showed a negative correlation between $\delta^{13}C$ and $\delta^{15}N$ and trawl hours indicating a general reduction in trophic levels as a function of trawling, implying that discarded by-catch and/or habitat complexity affects diet composition. SAT, which is not a scavenger on discarded by-catch, also showed a large positive correlation between $\delta^{15}N$ and habitat complexity and a medium to large negative correlation between both $\delta^{15}N$ and $\delta^{13}C$ and trawling hours, pointing towards habitat complexity as a possible driving force rather than discarded by-catch. A comparison of the diet of PJS between trawling and no trawling periods showed a significant effect of trawling, caused by a higher occurrence of blue crabs and calamary in the diet during periods of trawling, and prawns during periods of no trawling, demonstrating that discarded by-catch affects the diet (Table IV). However, no effect of stomach fullness between periods of trawling and no trawling was found. The overall pattern of correlations indicates that other factors than the inter-correlated habitat complexity and trawl hours, such as functional patterns of feeding and competition for space and food, may determine trophic position and how the environment affects the trophic position.

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# Chapter 8: Conclusions

## Benefits and adoption

This project provides essential information for the development of a spatial by-catch risk assessment for the Spencer Gulf prawn fishery, thereby benefiting both industry and regulators. The project furthermore provides important information on by-catch mortality and recapture, allowing for the incorporation of trawl duration and by-catch handling as an operational management tool. The results of this project provide an assessment of the ecological links between quantitatively important by-catch species and their populations allowing a broader understanding of the Spencer Gulf environment where trawl fishing takes place. The data collected will allow for the development of a basic Ecopath (mass-balance) model with the prospect of further development of an Ecosim model. The prawn fishing industry has benefited from this through an enhanced understanding of the consequences of prawn trawling and the capture of by-catch. The flow of benefits was partitioned between the commercial and recreational sector in NSW (8+2%), QLD (8+2%), WA (3+1%) and SA (62+2%). The flow of benefits was furthermore partitioned to the Northern prawn fishery, the recreational and the traditional sector benefiting by 8+2+2%. The primary beneficiary in the commercial sector is the prawn fishing industry, with data and analyses provided to aid in the further development of ESD and EBM management strategies, and improving community perceptions of prawn fisheries. The results are also useful for other fisheries in South Australia; in particular the blue crab and marine scale fisheries as well as other Australian prawn fisheries more broadly.

## Further development

The concept of ESD and EBM provides a scientific challenge because it requires a shift from managing a single species as an isolated component to managing a complex multi-species system. It seems difficult to resource, and within a short time frame obtain knowledge of exploited ecosystems, to permit the same kind of mechanistic approach usually followed in single species stock assessment. It is therefore important to avoid setting unachievable goals in research and management, but allow parallel studies of ecosystem functioning and ecosystem effects of fishing with new research of a precautionary nature (e.g. ecological risk assessment) with the aim of developing

operational frameworks for industry and management. An important part of understanding ecosystem effects of fishing is to obtain a historical perspective because the base for management is a "fished ecosystem" with a largely unknown baseline. To avoid the so-called "Shifting Baseline Syndrome" brought about by management systems without clear or drifting performance indicators, a research focus on causes of change and variability both temporally and spatially is essential. Accordingly, two parallel lines of study are required to develop a basic understanding of the ecosystem impact of prawn trawling. 1) Establishment of a simple risk assessment framework underpinned by a highly replicated data set from before and after trawling periods. This is essential in order to measure depletion effects and test these effects against temporal effects allowing the use of rigours statistical methods. A risk assessment will not provide an understanding of broader ecosystem effects because sampling will be restricted to areas where fishing occurs. Therefore a modelling approach is required. 2) Development of a framework for mass-balance model allowing ecosystem consequence analyses and predictions of changes in biomass. A supplementary tool available and widely used is the Ecopath/Ecosim model but further development is required to incorporate the results from this study. For a mass-balance model to be realistic continuous data input at the scale of the fishery is required, which reflects seasonality and fishing strategies. A mass-balance model will allowing ecosystem consequences analyses and prediction of changes in biomass as a response in a change in fishing methods or patterns. Furthermore, consideration has also to be given to marine reserves as a tool for EBM with the need to integrate knowledge on ecosystem functioning at a much broader scale to understand the real dynamics of exploited ecosystems. To achieve this a multidisciplinary approach is required.

**Planned outcomes**

The project's outputs have contributed to the following planned outcomes:
Improved Ecosystems Based Management through the provision of:

1. **An ecological assessment of the impact of prawn trawling on quantitatively important by-catch species and scavengers feeding on discarded by-catch.**

The project described the spatial and temporal distribution and abundance of quantitatively important by-catch species and correlated these variables to sites of different historical fishing effort. Aspects of the individual species biology such as age and growth parameters were determined in addition to their trophic position as a function of site. The analysis thus provides background data for an environmental risk assessment, and with an option of the development of a basic Ecopath model. This will provide the tools for industry and managers for EBM with respect to by-catch in the Spencer Gulf prawn fishery.

2. **An assessment of the relationship between trawl duration and handling on the survival of larger by-catch species (sharks and rays).**

The project provided detailed analysis of capture stress and survivorship of important by-catch species. This information is necessary to determine the impacts of trawling on by-catch species populations after being discarded. It will furthermore allow for the assessment of operational handling practices within prawn fisheries and thereby contribute to reductions in by-catch mortality.

3. **A methodology for the ecological application of AEC-ratios in fishery research, particularly in the assessment of sub-lethal effects on by-catch.**

The project provided a rigorous methodology to assess sub lethal effects including physiological "stress" and mortality of dominating by-catch species, with the capacity to quantify and understand the causes of fishing mortality.

4. **An improved capacity to assess bias in catchability and fishing mortality of larger by-catch species (sharks and rays) by testing movements and feeding behaviour during trawl operations.**

The project provided an assessment of catchability of smaller elasmobranchs through an extensive tagging program and subsequent recapture by the prawn fishing industry and others. The tendency for Port Jackson sharks to be affected by trawl activities and discarded by-catch was assessed in laboratory experiments and field experiments using acoustic tracking techniques. The information obtained is important to determine unbiased catch rates of discarded by-catch, particular smaller elasmobranchs.

5. **Development and promotion of environmentally responsible work practices that will follow from an empirical examination of ecosystem level effects of prawn trawling.**

The output provided by the project for this item is the final report and an acceptance of the results. Management action with implementation of the by-catch segment of the EBM risk assessment incorporating risk of trawl duration will be a desired outcome.

6. **Improved public acceptance of the prawn fishing industry through the provision of information to improve awareness and knowledge of the environmental effects of prawn fishing.**

After provision of the final report, the project steering committee will decide on a media strategy and releases, as well as publication of information material for the public. Scientific papers will be submitted for publication reflecting the individual chapters of the report and thus provide credibility to the information by international peer review.

## Conclusions

A summary of the project's key findings according to the objectives in the original application is detailed below.

**To obtain measurements of the trawling catchability and population parameters of important by-catch and scavenger species, particularly including smaller sharks, skates and rays.**

The research reported here provides an ecological assessment of quantitatively dominating by-catch species using a consistent rigorous statistical approach applied to data sampled at five sites on the Spencer Gulf prawn fishing grounds with a history of different trawling intensities. The dominating by-catch categories are benthos (sponges etc.), sand trevally, leatherjackets, blue crabs, sharks and rays. The mean catch rate of by-catch (total catch minus prawns) was 117-187 kg/h with the exception of one site (Middle Bank Channel) with about 30 kg/h for one standard prawn net with a 4.5 cm diamond mesh in the cod end. The wet weight of by-catch was significantly different between sites, with no statistical difference between surveys and day/night trawling. Twenty-two species of elasmobranchs were caught,

comprising 10 shark, 8 ray, 3 skate and 1 chimera species. Port Jackson sharks were the most common species (61.6%) followed by the sparsely spotted stingaree (17.6%). Other species of importance were cobbler wobbegong, elephant shark, fiddler ray and the large stingrays. The number of species per site ranged from 8 to 15. Diversity, species richness and distribution were strongly related to habitat heterogeneity/complexity measured as the abundance of benthos biomass. Abundance of elasmobranchs was lowest at the most northerly site and highest at the most southerly site. Seasonal variations in abundance were evident for most species.

Tagging of 1522 individual sharks, skates and rays, comprising nine species, was conducted in seven areas. Port Jackson sharks made up the majority of sharks tagged, and were the only species (with the exception of one stingaree) that were recaptured. A total of 79 Port Jackson sharks (6.3%) were recaptured after a period from 1 to 865 days. Dispersal of sharks was low, with 55% of individuals recaptured within 10 km of the original tag site, suggesting strong site fidelity. The majority of elasmobranch species in Spencer Gulf reached sexual maturity at a smaller size than reported elsewhere. Mature Port Jackson sharks were found to predominate in the most northerly sites and were significantly less abundant in the south, likely as a consequence of habitat heterogeneity. Ageing studies of Port Jackson sharks indicated longevity to be 40.7 years and age at maturity for males was 6-8 years and 8-11 years for females.

The quantitative important by-catch species sand trevally, bridled leatherjacket, rough leatherjacket, toothbrush leatherjacket, Degens leatherjacket, pygmy leatherjacket and blue crab showed a significant variation between sites in size, weight, biomass abundance. For the 2004/05 surveys, the correlation analyses showed that biomass of rough leatherjackets and Degens leatherjackets were positive correlated to trawl hours, with up to 31.5% of the variance explained by trawl hours, and negatively correlated with benthos, with up to 9% of the variance explained by this variable. Excluding the Oct 2003 survey, sand trevally showed a negative correlation with trawl hours, with 13 to 31.5% of the variance explained by trawl hours, and no correlation with habitat heterogeneity, which can be explained by a pelagic existence. The correlations for blue crabs with trawl hours and benthos were mixed. The observed patterns of abundance correlated to trawl hours and habitat

heterogeneity/complexity (benthos biomass), suggests that habitat affects abundance but the important correlation is with trawl hours. Trawl hours were negatively correlated to habitat heterogeneity, which explains 23.3% of the variance for the Oct 2004 survey and 28.6% for the Jan 2005 survey.

**To determine survival rates of key by-catch species using measures of physiological stress and mortality associated with capture and handling.**

A detailed assessment of physiological stress and mortality of quantitatively important by-catch species (blue crab, sparsely spotted stingaree, Port Jackson shark, and Degens leatherjacket) has been carried out in order to establish the relationship between trawl time and fish stress and where possible, survival. Additional species were opportunistically sampled and assessed as well. Animals subjected to trawl times of 15, 45 and 30 minutes were compared to a base-line group that had been line or net captured specifically in order to minimise capture stress prior to sampling. Blood samples were analysed for lactate and glucose and the ions sodium, chloride and potassium. Muscle samples were analysed for adenylate, glucose and lactate. The results show that, irrespective of time, trawl capture results in a response that is consistent with that recognised in animals subject to stress. More specifically, in the blue crab, trawl captures generally resulted in higher plasma lactate and lower hemolymph pH than the base-line but glucose varied little. Hemolymph potassium level varied in response to trawl but not consistently, and sodium and chloride sometimes increased in response to trawl capture. Muscle energy balance was disrupted in trawl caught crabs as IMP load was higher, and energy charge expressed as the ratio AEC was lower. Post-capture damage increased and survival in crabs declined, as post-capture interaction time increased. Post-capture crab death was due almost totally to the damage that caused holes in the carapace rather than the commonly occurring limb loss. In this study all crabs that died following post capture release were consumed by sealice. Sparsely spotted stingarees showed a clear stress response to trawl capture with higher plasma glucose and lactate and lower pH than the base-line. The plasma ions did not change in response to trawl capture except for potassium, which increased following the 30-minute trawls. Following 30-min trawls, muscle lactate was higher and muscle glucose lower than the base-line. In addition, energy ratios changed; with AEC being lower and IMP load higher in 30-minute

trawled fish. Post trawl survival of this species is probably low, as following trawl capture all but one stingaree died and/or were eaten by sea lice during the 3-day sea-cage survival test. Port Jackson sharks showed little response to trawl capture. This species showed elevated plasma lactate in response to 45-min trawls, and plasma sodium and chloride was lower in sharks following 30-min trawls than base-line levels. Muscle lactate was elevated in sharks subject to 15 and 45 min trawl durations, and this was consistent with an increase in anaerobic metabolism. Plasma and muscle parameters of sharks captured by line and 15 and 45 min trawls were similar following recovery in deck tanks for 2 and 4 hours. Generally, this species appears unaffected by trawl capture, and no shark death occurred during trawls or in tanks during post-capture observations and experiments. Of trawl captured Degens leatherjacket *Thamnaconus degeni,* we estimate that approximately 46% subject to 15-min trawls and 78% subject to 45-min trawls do not survive. Of the surviving fish subject to trawl times of 15 and 45-min, plasma lactate and glucose increased and inexplicably pH also increased. Muscle glucose decreased and muscle lactate concentrations increased following trawling. In addition, AEC values decreased and IMP load increased. These results indicate that discarded Degens leatherjackets are subjected to considerable stress during trawling, which clearly increases with trawl time, and is likely to contribute to the incidence of death in discarded fish. The results of this study show that the trawled species examined suffer stress following trawl capture, but that the severity of the response is species specific. With the exception of Port Jackson sharks, the investigated species suffered considerable stress during trawling, typified by a mobilisation of energy that was likely a result of an endocrine stress response, and followed by an increase in anaerobic metabolism. In addition, adenylate ratios in the muscle changed in some species, and sometimes there was a disruption to ionic regulation. This study has established that trawl capture contributes to post-capture mortality in released blue swimmer crabs, the sparsely spotted stingaree, and the Degens leatherjacket. There was little evidence to suggest that the Port Jackson shark was adversely affected by trawl capture.

**To determine whether trawling actually attracts or substantially affects the movement of smaller sharks, skates and rays to scavenge on discarded by-catch.** Field and laboratory experimental study of movement and feeding behaviour of Port Jackson sharks during trawl operations using Radio Acoustic Position Telemetry

(RAPT) showed there was no effect of either by-catch or prawn trawl operations on the movements of Port Jackson sharks. Laboratory experiments showed that sharks were attracted to by-catch, but that by-catch composition and volume had little influence on attraction.

**To incorporate the results into a marine tropho-dynamic model for sustainable resource utilization in the Spencer Gulf (EBM).**

The results obtained from FRDC 98/225 and the project reported here provide a data set that may contribute to the development of a simplified mass-balance (ECOPATH) model with the prospect of further development through ECOSIM and ECOSPACE by making certain assumptions and providing additional ecological data as well as fishery data. However, the sampling program undertaken in FRDC 98/225 and in this project is designed for hypothesis testing using fixed sites with known environmental and fishery characteristics. For a mass-balance model, a fully replicated stratified or randomised sampling program is required, covering a considerable area of Spencer Gulf, in order to obtain realistic data on population abundances. ECOPATH with ECOSIM is intended to lead to policy exploration for ecosystem-based fisheries management. With these types of biomass modelling it is possible to simulate and predict possible outcomes of management scenarios such as implementation of by-catch reducing devices and spatial restrictions. In the project agreement the development of a mass-balance model was not an output or an outcome and therefore awaits further development provided the fisheries managers and the fishing industries wish to pursue this path. However, the ecological assessment provided here and by FRDC 98/225 will allow the development of a formal risk assessment in line with the national ESD reporting framework using ecological consequence tables by interpolation the results from the five sites, which represents habitat types, fishing grounds and 73% of all effort in Spencer Gulf.

Stable isotopes ($^{13}$C and $^{15}$N) and stomach contents of quantitatively important by-catch species showed a distinct trophic fractionation, separating the species into five different trophic groups with crustaceans at the lowest level and Port Jackson sharks at the highest. The major source of food for crustaceans was found to be detritus. Stable isotope values for most species declined from the northern site towards the southern site, demonstrating that trophic position varied with site, violating one

assumption of the ECOPATH model, which needs to be considered. Disregarding Port Jackson sharks at the highest trophic level, there was a general trend of negative correlation between stable isotope concentrations and trawl hours and a positive correlation with habitat heterogeneity implying that discarded by-catch and/or habitat heterogeneity affects diet composition. The studied species are omnivorous with more than 10 diet categories. Port Jackson sharks had a higher occurrence of blue crabs and calamary in their diet during periods of trawling, and prawns during periods of no trawling, demonstrating that discarded by-catch affects the diet.

**Appendix 1.  Intellectual property**

This report will be available for public examination through FRDC and SARDI.
Components of this report were the subject of two Honours theses both achieving
First Class at the Flinders University of South Australia:

Barnett, J. 2005. The effect of prawn trawling and discarded by-catch on the
behaviour of Port Jackson sharks (*Heterodontus portusjacksoni*).

Izzo, C. 2005. Aging Port Jackson sharks, *Heterodontus portusjacksoni* (Meyers
1793) using calcified structures.


**Appendix 2  Project staff**

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|---|---|
| SARDI (Aquatic Sciences): | Dr Ib Svane (PI) |
| | Dr Kate Rodda (CI) |
| | Mr Jeremy Barnett (Technical Officer/Honours student) |
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