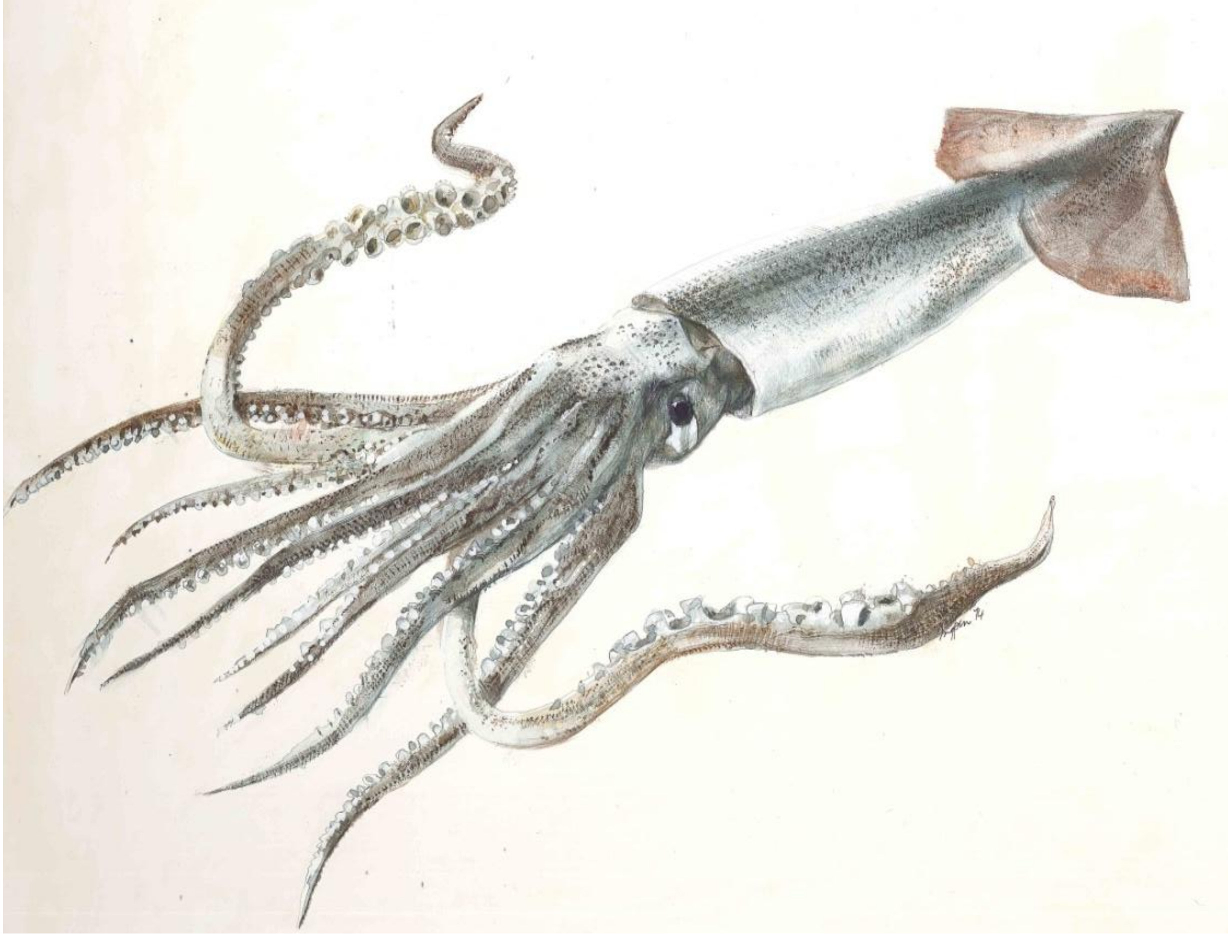
ARROW SQUID: STOCK VARIABILITY, FISHING TECHNIQUES, TROPHIC LINKAGES

FACING THE CHALLENGES



Patti Virtue, Corey Green, Heidi Pethybridge, Natalie Moltschaniwskyj,
Simon Wotherspoon and George Jackson

Final Report FRDC 2006/012

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

NON TECHNICAL SUMMARY

2006/012 Arrow squid: stock variability, fishing techniques, trophic linkages - facing the challenges.

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Objectives

1. To investigate the influence of environmental and oceanographic variables on arrow squid growth and recruitment.
2. To investigate the ecology, population dynamics of squid in the newly proposed mid-water trawl Great Australian Bight fishery.
3. To compare the biological composition of jig and trawl-caught squid.
4. To describe the key components, spatial and temporal variability and environmental influences on the diet of arrow squid.
5. To investigate the use of statolith shape for stock discrimination.
6. To develop an ecosystem model based on squid predator-prey relationships for squid stock assessment

OUTCOMES ACHIEVED

This work focused on the key fishing region off Portland as well as the Great Australian Bight where a pelagic trawl fishery for arrow squid is proposed. Results generated from modelling using both biological and oceanographic/environmental parameters provided a detailed understanding of the relationship between arrow squid populations and environmental conditions. The influence of oceanographic processes, quantified through satellite image derived data (e.g. sea surface temperature), revealed how arrow squid respond to environmental conditions in terms of recruitment, growth and reproduction. Growth and recruitment were determined through ageing of squid samples using statoliths and back-calculation of hatch dates. This information will contribute to monitoring, management and sustainable use of this resource. It was not only of interest to describe how squid respond to oceanographic changes through growth, reproduction and recruitment, but how these changes influence the availability of prey. Squid dietary analyses were also conducted in association

with a biochemical analysis of squid tissue to determine squid condition. Results here highlighted the need to investigate trophic interactions when considering a fishery resource such as squid. The generation of data on trophic interactions helped facilitate the development of a robust ecosystem model. This ecosystem model was developed to facilitate understanding of stock dynamics, and to determine the influences that both natural and fishery induced changes have on stock size. Results from this research will help fishery management facilitate the optimal use of the arrow squid resource through the development of an ecologically sustainable fishery. Project results were communicated through a number of national and international conferences, and are being published in the scientific literature.

The goal of this research was to conduct a stock assessment of Arrow Squid (*Nototodarus gouldi*) from southern Australia. Specifically we provide information on catch composition, growth, reproduction, recruitment, migration and diet of *N. gouldi* caught from the Great Australian Bight (GAB) and Victoria trawl and jig fisheries between 2007–2009. Results from this research will contribute to the information required to manage this fishery.

Squid caught inshore by the jig fishery were larger, consisted of a greater Male: Female ratio and had a greater percentage of mature females than those caught in the offshore trawl fishery. Juvenile squid less than the 220 mm dorsal mantle length were not represented in the catch.

Seasonal patterns in growth rates were similar for male and female squid caught in both locations. Squid grew faster and to a larger size in cooler waters off Victoria and matured later compared to those from the warmer waters of the GAB. Catch composition consisted of males which were predominantly mature. The degree of maturity for female squid however varied with season. Male and female squid were in better reproductive condition during summer than winter in Victoria. Reproductive condition did not vary with season for squid caught in the GAB. Female reproductive condition was better in the GAB during winter 2008; however, better in Victoria during summer 2008/09. This variability is a reflection of environmental and oceanographic conditions experienced during early life rather than those experienced during adult stages. Squid hatchlings that experienced warmer sea surface temperature grew faster and larger.

Distribution of hatch dates determined from back calculated statolith age estimates suggest that squid collected in Victoria and the Great Australian Bight (GAB) during 2007 – 2009, hatch year round. Aseasonal hatching events suggests that all ontogenetic stages are represented in the population at any one point of time. At least five cohorts were identified from Victoria and the GAB from mid 2007 to late 2008.

Fourier shape analysis of statoliths was used to determine whether discrete phenotypic stocks exist; whereas statolith element concentrations were used to determine where individuals from both

locations are likely to have hatched. Comparing statolith shape from squid collected in Victoria to squid collected in the Great Australian Bight suggested significant phenotypic heterogeneity in stocks; whereas elemental composition analysis suggests that squid hatched throughout their distribution. Although squid caught in the Great Australia Bight and Victoria appear as separate stocks, squid from both regions are important for recruitment.

Combined stomach content and fatty acid signature analyses provided clear evidence of seasonal dietary shifts in prey composition of squid. Teleost fish remains (mainly otoliths) were found in 67% of stomachs with the two mesopelagic planktivorous lightfish, *Lampanyctodes hectoris* and *Maurolicus mulleri* dominating. Cephalopods and crustaceans were supplementary dietary components, which increased over winter. Seasonal differences in total lipid content were related to summer upwelling events and local changes in productivity, while differences in lipid class and fatty acid composition were related to seasonal differences of prey consumption. Fatty acid analyses demonstrated dietary differences associated with gender, size and female maturation. Such relationships demonstrate that the diet of *N. gouldi* is closely linked to prey size, abundance and availability.

In this study we utilised ecosystem-based models to investigate the ecological impact of increased fishing pressure on arrow squid in the Great Australian Bight and in south east Tasmania. Results show that arrow squid populations were robust to high levels of fishing pressure and that the current fishing effort would need to increase substantially (i.e. by a factor of at least 500 times in the GAB) before noticeable population changes would occur. The abundance and feeding behaviour of arrow squid influences the population size, and distribution of its predators and prey. Hence increased fishing levels on squid would cause positive effects on principal prey groups (mainly sardines and myctophids) and negative effects on predators (New Zealand fur seals and predatory fish).

Key Words *Nototodarus gouldi*, ecosystem-based model, statolith, lipid class, fatty acid composition, stock assessment, fishing techniques

ACKNOWLEDGMENTS

This study was funded by the Fisheries Research and Development Corporation. The majority of this research (Appendices I, II, III, and V) was conducted by Corey Green, University of Tasmania PhD candidate, funded both through a University of Tasmania Postgraduate Award, and a FRDC top up scholarship from this grant. Funding for statolith microchemistry analysis was provided by the Holsworth Wildlife Research Endowment, granted to Corey Green. Aspects of this work were presented at both national and international conferences which were financially supported by the Graduate Research Candidate Conference Fund Scheme, University of Tasmania, Australian Marine Sciences Association and FRDC. Laboratory space and utilities were provided by the Victorian Marine Science Consortium, the Department of Primary Industries Victoria Fisheries, CSIRO Marine and Atmospheric Research and University of Tasmania.

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Dr Peter Nichols is gratefully acknowledged for his enduring support in terms of strategic advice and wisdom throughout the life of this research. Dr Ruth Casper helped manage this grant; she also played a major role both in organising squid collections and laboratory analysis. Thanks also to Dr Eric Grist for initial input to this research, and Naomi Flynn and Ty Hibberd for helping produce this report.

1. INTRODUCTION

1.1 Background

Squid fisheries have been rapidly expanding around the globe in recent years (Caddy & Rodhouse 1998). Whether this is in response to declining availability of traditional fish stocks or changing market preferences is debatable, however regardless of the cause, there is the need to monitor and manage these fisheries. Traditional stock assessment and management frameworks developed for finfish fisheries are not appropriate for squid stocks due to their unique biology and ecology, and present a challenge to managers. In contrast to fish, squid display a large degree of variability at an individual level through extreme plasticity in their biology and life history strategies, while at the population level this variability is amplified by a short life span (usually less than one year) and rapid population turnover. Such characteristics are reflected by the fluctuating commercial catches of Australia's largest squid fishery, the Commonwealth managed Southern Squid Jig Fishery (SSJF, Lynch 2005) that targets the arrow squid (*Nototodarus gouldi*).

In order to develop appropriate stock assessment techniques and management decisions for the SSJF, we need a thorough understanding of arrow squid biology and ecology, although some progress has been made on this (e.g., McGrath & Jackson 2002, Jackson *et al.* 2003, Jackson & McGrath-Steer 2004a, McGrath Steer & Jackson 2004, Triantafillos *et al.* 2004, Jackson *et al.* 2005). This research has provided information on the life span, broad scale spatial and temporal variation in growth rates, trends in reproduction and condition, mode of reproduction and genetic status of the population in Australia. In summary, we now know that this species lives for less than a year, is a multiple spawner, shows extreme variability in growth rates, size, age and condition, and appears to be genetically well mixed. Furthermore, preliminary research has found that sea surface colour (SSC, a measure of productivity) helped to explain differences in growth rates of female arrow squid. This was an exciting finding, although not unusual given the large numbers of studies from around the world that have shown a strong relationship between squid biology/ecology and environmental conditions (Waluda *et al.* 1999, Rodhouse 2001b). Sea surface temperature (SST) is likely to also be an important predictive parameter as squid growth is so closely linked with temperature. Because of their short life span, flexible growth and reproductive strategies, squid are able to respond rapidly to changing conditions (Jackson & Domeier 2003a). For many squid species around the world, the monitoring of environmental variables has proven to be a valuable predictive tool, critical to assessment and management (Agnew *et al.* 2000b, Anderson & Rodhouse 2001a, Agnew *et al.* 2002, Denis *et al.* 2002, Pierce & Boyle 2003). This is the next phase of research with a more detailed investigation of the influence of environmental variables on arrow squid populations and particularly growth, reproduction and recruitment.

The investigation of environmental control of arrow squid stocks is particularly relevant as the fisheries encompass regions of extreme oceanographic variability. The fishing grounds off Portland Victoria, at the eastern end of the Bonney Coast is subject to classic upwelling plumes between November and April each year (Butler *et al.* 2002b). Upwelling also occurs to a lesser degree in Eastern Victoria in another important SSJF region, off Lakes Entrance.

1.2 Need

Annual biomass estimations of Arrow squid cannot be determined due to lack of data, so setting annual total allowable catch rates is not possible. The arrow squid fishery is managed using total allowable effort restrictions determined annually. This research resulted from questions arising regarding differences in the biological characteristics of jig vs. trawled squid and the potential impact of a newly proposed pelagic squid fishery in the GAB. Likewise the inter-relationship between arrow squid and the environment in terms of their prey is also poorly understood. No ongoing annual stock assessment of the *N. gouldi* fishery is conducted hence there was a need for updated information to address these questions.

A special arrow squid ‘research needs’ meeting was held in Canberra 29 November 2005. This meeting was attended by a cross section of industry members and the need to understand many aspects of arrow squid biology was again reiterated. As a result of that meeting and by direct request of management and industry, this proposal was expanded to include ecosystem modelling to help provide predictive insights into stock fluctuations and fishing effort on stock size.

2. METHODS

2.1 Samples and data

Most of the analyses in this study (Appendices I - V) were conducted using arrow squid, *Nototodarus gouldi*, collected opportunistically from commercial fishers operating in the Great Australian Bite (GAB) (33°15S, 130°00E) from June 2008 – June 2009; and off the coast of Portland, Victoria, Australia (38°45S, 141°30E) from March 2007 – April 2009 (Figure 2-1). Animals were collected approximately bimonthly from the GAB; whereas samples from Victoria were collected approximately monthly (Table 2-1). Squid were frozen following capture and then transported to the University of Tasmania or the Marine and Freshwater Fisheries Research Institute in Victoria for dissection, sample preparation, and statolith analysis.

Data collected from each individual included total weight (g), dorsal mantle length (mm), mantle weight (g), ovary weight (g), testis weight (g), sex, maturity stage and age (d). Maturity stage was determined based on methods developed by Borges and Wallace (1993) with stages I – III classified as reproductively immature and stages IV and V classified as reproductively mature. Age was estimated using statolith pairs, which were removed from the squid's cranium, cleaned with distilled water, and stored dry at room temperature in cavity trays. Statoliths were ground and aged by counting incremental structure from the hatch mark to the edge of the dorsal dome.

From the total collection (GAB = 1142, Victoria = 2467 individuals), specimens were also randomly subsampled to assess the influence of fishing method (jig or trawl) on biological composition (Figure 2-2, Table 2-2; Appendix III), and to assess the population/stock structure of the two arrow squid fisheries using fourier shape analysis and elemental composition of statoliths (Table 2-3; Appendix V).

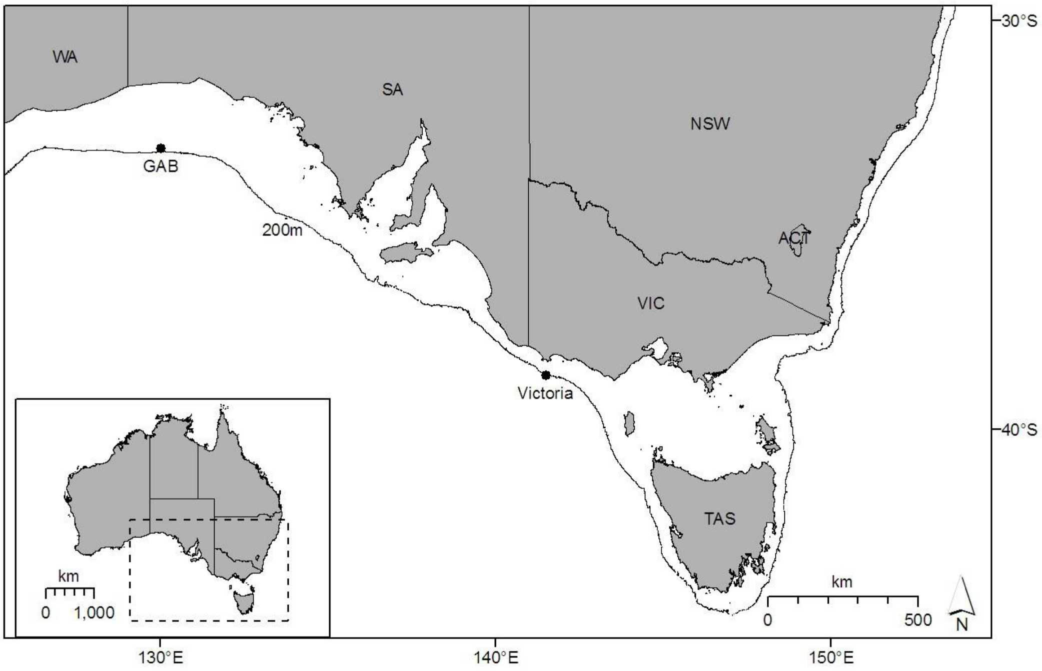

Figure 2-1 Map of southern Australia detailing the general location where *N. gouldi* were caught along the continental shelf from the Great Australian Bight (GAB) and Victoria.

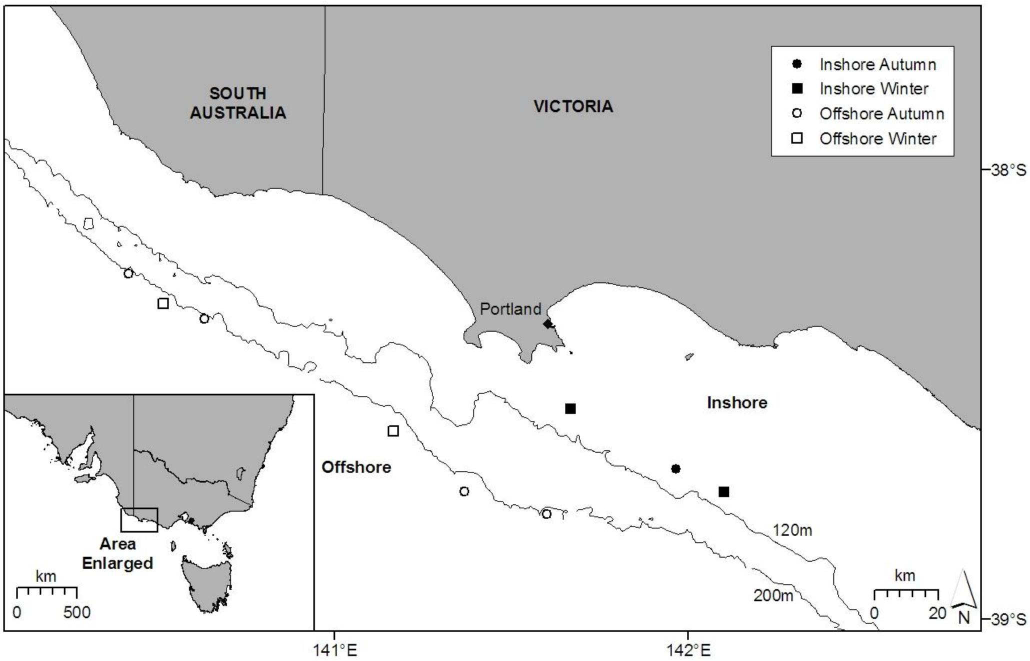

Figure 2-2 Map of south eastern Australia illustrating inshore (jig) and offshore (trawl) collection locations of *N. gouldi* during autumn and winter in 2007 and 2008.

Table 2-1 Details of *N. gouldi* collected from the trawl fisheries in the Great Australian Bight (GAB) and Victoria, Australia from March 2007 – June 2009.

COLLECTION DATE	GAB SAMPLES	VICTORIA SAMPLES	LATITUDE	LONGITUDE
01-Mar-2007		100	38.72	141.37
27-Mar-2007		100	38.77	141.60
07-Jun-2007		100	38.30	140.52
06-Aug-2007		101	38.58	141.17
19-Sep-2007		100	38.30	140.55
15-Oct-2007		100	37.93	139.98
2007 total		*601*		
19-Feb-2008		100	38.75	141.53
14-Mar-2008		101	38.93	142.18
14-May-2008		142	38.33	140.63
25-May-2008		100	38.23	140.42
22-Jun-2008		100	38.58	141.17
23-Jun-2008	112		33.25	130.03
25-Jul-2008		100	37.55	139.28
01-Aug-2008		100	38.62	139.27
15-Aug-2008	80		33.22	126.48
16-Aug-2008	90		33.28	128.58
17-Aug-2008	85		33.30	130.13
19-Aug-2008	99		33.28	130.13
28-Aug-2008		137	38.32	140.53
30-Sep-2008		121	38.68	141.27
06-Nov-2008	158		33.22	131.25
14-Nov-2008		140	38.62	141.20
11-Dec-2008		156	33.75	141.47
22-Dec-2008	83		33.35	132.10
2008 total	*707*	*1297*		
21-Jan-2009		130	38.70	141.30
12-Feb-2009	135		33.18	128.39
19-Feb-2009		175	38.68	141.32
25-Mar-2009		105	38.75	141.35
02-Apr-2009	96		33.16	128.30
30-Apr-2009		81	38.73	141.37
02-Jun-2009		78	38.77	141.57
10-Jun-2009	204		33.19	129.00
2009 total	*435*	*569*		
TOTAL COLLECTIONS	1142	2467		

Table 2-2 Details of when and where *N. gouldi* were randomly selected from jiggers operating inshore and trawlers offshore off the south east coast of Australia. Average dorsal mantle length (DML) and standard deviation (s.d.) for males and females sampled on each collection day is provided.

DATE OF CAPTURE	GEAR	LOCATION	YEAR	SEASON	NUMBER	DEPTH (M)	LATITUDE	LONGITUDE	MALE MEAN DML (±S.D.)	FEMALE MEAN DML (±S.D.)
14/03/2007	Jig	Inshore	2007	Autumn	95	75	38° 32`	141° 40`	280.9 (24.3)	316.6 (25.0)
17/06/2007	Jig	Inshore	2007	Winter	74	75	38° 32	141° 40`	301.8 (16.6)	338.9 (31.9)
14/05/2008	Jig	Inshore	2008	Autumn	100	80	38° 40`	141° 58`	285.0 (16.5)	325.5 (31.1)
2/06/2008	Jig	Inshore	2008	Winter	100	110	38° 43`	142° 06`	299.7 (19.5)	340.8 (25.0)
1/03/2007	Trawl	Offshore	2007	Autumn	100	384	38° 43	141° 22`	279.0 (16.6)	305.1 (24.7)
27/03/2007	Trawl	Offshore	2007	Autumn	100	250	38° 46	141° 36`	274.7 (17.3)	313.0 (26.9)
7/06/2007	Trawl	Offshore	2007	Winter	100	293	38° 18`	140° 31`	271.6 (24.7)	279.7 (37.2)
14/05/2008	Trawl	Offshore	2008	Autumn	142	290	38° 20`	140° 38`	266.9 (20.2)	291.7 (39.7)
25/05/2008	Trawl	Offshore	2008	Autumn	100	366	38° 14`	140° 25`	275.2 (23.1)	291.6 (36.7)
22/06/2008	Trawl	Offshore	2008	Winter	100	384	38° 35`	141° 10`	262.0 (32.2)	267.6 (42.8)

Table 2-3 Number of *N. gouldi* from which statoliths were used for shape analysis and/or statolith element analysis from each site (GAB and Victoria). Blank cells indicate no samples collected or used in that analysis.

			SHAPE ANALYSIS		ELEMENT ANALYSIS	
YEAR	MONTH	SEASON	*GAB*	*Victoria*	*GAB*	*Victoria*
2007	Mar	Autumn 07		149		
	Jun	Winter 07		91		
	Aug	Winter 07		88		
	Sep	Spring 07		82		
	Oct	Spring 07		86		
2007 Total				*496*		
2008	Feb	Summer 07/08		85		
	Mar	Autumn 08		84		
	May	Autumn 08		171		
	Jun	Winter 08	99	85	17	16
	Jul	Winter 08		88		
	Aug	Winter 08	248	195	17	16
	Sep	Spring 08		105		20
	Nov	Spring 08	102	108	17	
	Dec	Summer 08/09	64	110	17	15
2008 Total			*513*	*1031*	*68*	*67*
2009	Jan	Summer 08/09		89		
	Feb	Summer 08/09	126	121	18	18
	Mar	Autumn 09		86		18
	Apr	Autumn 09	76	66	14	
	Jun	Winter 09	114	63		
2009 Total			*316*	*425*	*32*	*36*
Total			**829**	**1952**	**100**	**103**

2.2 Growth and reproductive variability in relation to location and environment

Spatial and temporal comparisons of arrow squid growth rates from both fisheries were analysed using weight-at-age data (see Appendix I). To determine if growth rates of squid differed among fisheries and hatch season an analysis of covariance (ANCOVA) was used to assess the relationship between log mantle weight-at-age and log age of squid collected in the GAB and Victoria for males and females separately on a seasonal and yearly basis. Differences in the reproductive status (i.e. immature or mature) were also made by comparing the proportion of immature to mature squid as a function of season of capture for each sex and fishery using a Pearson's chi-square test, followed by 3-

way factorial analysis of variance (ANOVA) on significant results using location, sex and maturity (immature or mature) as factors.

Spatial and temporal differences in reproductive condition between the two fisheries for each season were analysed for each sex by calculating and comparing the standardized residuals from the regression between mantle weight and testis weight for mature males; and mantle weight and ovary weight for mature females a one-way ANOVA.

Growth data was compared to sea surface temperature (SST) to assess whether variability exists as a result of warmer or cooler conditions; sea surface colour (a measure of water productivity) was also assessed but was too difficult to interpret (see Appendix I). Generalized additive models (GAM) were used to determine the relationship between growth and SST. GAMs were fitted for each of the four combinations of location, SST and sex (i.e. SST (GAB) – Male) using log mantle weight-at-catch data and mean SST experienced by the individual in the first 30 days post-hatching. SST temperature data was acquired from the NOAA-CIRES Climate Diagnostic Centre (http://www.cdc.noaa.gov/).

2.3 Temporal and spatial recruitment variability

Analyses described in Appendix II aimed to identify periods of high recruitment and compare recruitment variability from the existing fishery in Victoria to the developing fishery in the GAB based on back calculated hatch dates of arrow squid. Hatch date for female and male squid at each location was determined by subtracting the estimated age from the date of capture (Appendix II).

First minimum age estimates were determined to indicate the age which arrow squid are susceptible to trawling gear (i.e. are most frequently caught). Mantle-length frequency distributions and age frequency distributions were then used to visually identify cohorts as well as to indicate if the size and age structure progressively gets larger and older over time. To further analyse the existence of cohorts within a collection, either a normal or log-normal mixture model was fitted to the distribution of hatch dates for the GAB and Victorian collections separately. To illustrate if hatching occurs throughout time, individual hatch date was plotted against capture date for each location of capture.

2.4 Catch composition and gear-selectivity

Catch composition of squid caught inshore from the jig fishery was compared to squid caught offshore from the trawl fishery using biological and statolith element analyses (see Appendix III).

2.4.1 *Biological comparisons*

Pearson's chi-square tests were used to determine whether size frequency distributions (i.e. dorsal mantle length), age frequency distributions, sex ratio (M:F) and the ratio of mature to immature arrow squid differed between inshore (jig) and offshore (trawl) locations for each combination of sex, year and season of collection (Appendix III). Growth rates and reproductive condition of squid captured by either method were analysed by comparing the mean standardized residuals calculated from the relationships between mantle weight and 1) testis weight for males, 2) ovary weight for females and 3) estimated age. The effects of year, season and location on these variables were also explored using ANOVAs. Trends in sex ratio between inshore and offshore locations for autumn and winter collections were also explored to determine whether females have greater choice in male selection in certain seasons.

2.4.2 *Statolith element composition between methods*

Statolith element/chemical composition was analysed to assess whether inshore and offshore animals have unique elemental signature in the statolith (see Appendix III). Different element concentrations among squid from inshore and offshore locations may infer that squid have been present at their location of capture for a period of time without migration, suggesting separate populations, provided that a unique elemental concentration exists at both locations.

A total of 40 statoliths (20 inshore, 20 offshore) from arrow squid caught during March 2007 were randomly selected and prepared to determine elemental concentrations at three statolith regions; 1) the 'core' representing the time when the squid hatched; 2) the 'mid-point' at approximately 120 days of age as determined along the transect; and 3) 'edge' representing the time period prior to death (Figure 2-3). Statolith element concentrations were determined for multiple elements like Magnesium (Mg), Manganese (Mn), Strontium (Sr) and Barium (Ba), using a mass spectrophotometer in conjunction with an ultraviolet laser microprobe; see Appendix III and Appendix V for analyses procedures.

To determine if elemental concentration present in the statolith edge differed between inshore and offshore collections, inferring separation, multivariate analysis of variance (MANOVA) was used. The average concentration of elements at the three different statolith regions was also analysed using MANOVA to determine whether ontogenetic migration (i.e. migration throughout development) occurs between inshore and offshore regions (and vice versa).

2.5 Feeding ecology

Stomach content and signature fatty acid analysis were conducted to evaluate the feeding ecology of arrow squid in sampled waters (Appendix IV). For stomach content analyses, prey groups and prey species were analysed using number of occurrences and percent frequency of occurrence. Statistical treatment of all data entailed the application of MANOVAs to assess the effects of season, sex, size and female maturity stage on diet composition, total lipid content, major lipid classes and fatty acid profiles.

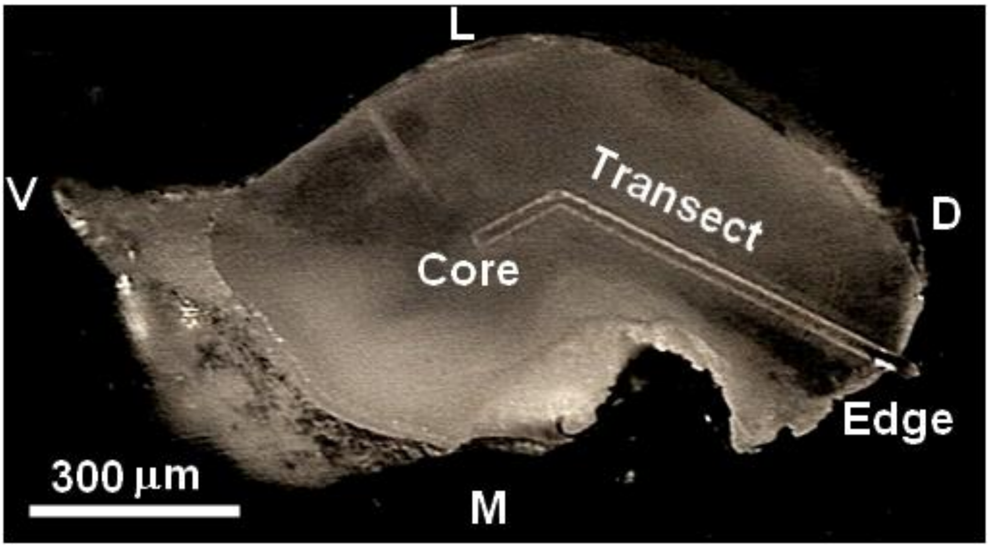

Figure 2-3 Posterior side of a ground *N. gouldi* statolith illustrating the location of the ablation transect from the core to the edge of the dorsal dome. D = Dorsal, V = Ventral, L = Ventral, M = Medial.

2.5.1 *Stomach content analyses*

Stomachs of dissected squid specimens were thawed, individually cut open and assigned a state of digestion and stomach fullness rating. Contents were sieved, and diagnostic prey items were removed and separated into teleosts, cephalopods or crustaceans. Teleost specimens were identified to genus or species where possible using otoliths, and otolith length was later used to estimate fish length (mm) using regression equations where possible.

2.5.2 *Lipid and fatty acid analyses*

Total lipid was extracted quantitatively by the modified Bligh and Dyer (1959) method. Total lipid content, lipid class composition and fatty acid profiles were determine using methods detailed in Appendix IV. Fatty acid profiles of squid digestive glands were then compared to published data on potential prey genera and species to determine diet composition.

2.6 Temporal and spatial population structure

Fourier shape analysis of statoliths was used for the first time to determine whether discrete phenotypic stocks exist; whereas statolith element concentrations were used to determine where individuals from both locations are likely to have hatched (see Appendix V). Ultimately these two analyses were combined to determine whether arrow squid caught in Victoria and the GAB are from a single population or are separate stocks.

2.6.1 *Fourier shape analysis*

Statoliths from more than 2500 individuals from Victoria and GAB were used in shape analysis prior to further preparation for aging and element composition analysis. Statoliths were first photographed using a compound microscope. Using image analysis software, the edges of the statolith were traced by assigning 128 x-y pixel coordinates (Figure 2-4). These x-y coordinates were then used to calculate relative statolith area and as a complex number input for a Fast Fourier Transform (FFT); resulting in an array of 128 Fourier descriptors which now represent the shape of the statolith. These descriptiors were then used in a number of formulae and multivariate analyses to calculate differences, or lack there of, in statolith shape between specified groups of squid and in relation to biological variables (i.e. mantle length) or sex.

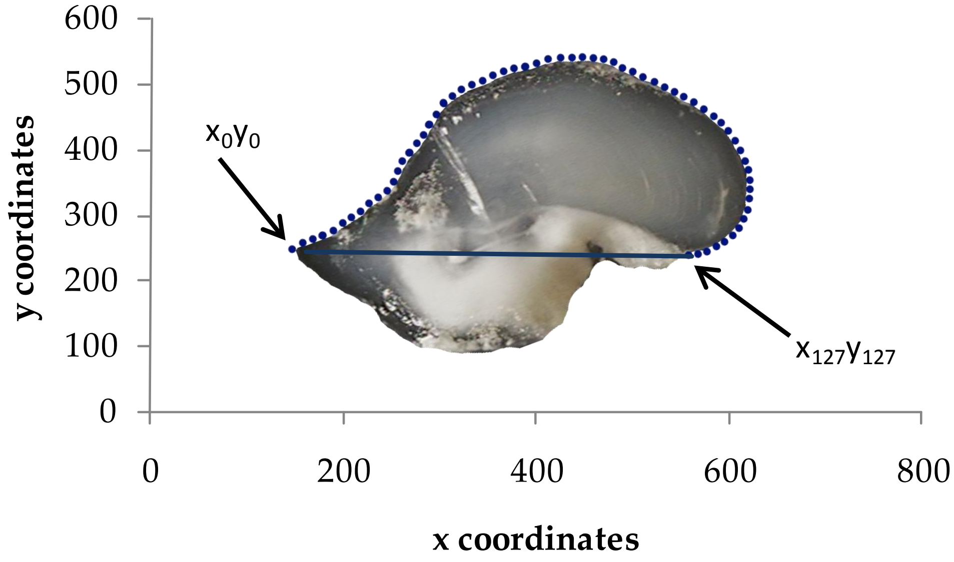

Figure 2-4 Posterior side of a *N. gouldi* statolith displaying the trace of the calcareous region from the rostrum tip (x_0y_0) to the margin between the dorsal dome and the statolith attachment point ($x_{127}y_{127}$).

2.6.2 *Statolith element composition between fisheries*

For comparison of the jig versus trawl caught squid, statolith elemental composition was used to determine the natal origin of individuals from specific times and locations to ascertain if there are significant spatial and temporal differences in the elemental composition within, and/or between, the Victorian and GAB fisheries. Squid collected from the GAB and Victoria in June 2008 – April 2009 were organised into approximately six bimonthly groups, and from each of these groups, up to twenty animals from each location were randomly selected for element composition analysis as per methods described in Appendix III and Appendix V (see Table 2-3). ANOVAs were used to illustrate differences between location and season of capture combinations.

2.7 Ecosystem modelling for GAB and south eastern Tasmania

Two ecosystem model frameworks were used to investigate the effects of increased fishing pressure on arrow squid, the role they have as both prey and predator, and to quantify the inter-reliance of squid stocks in different management areas (see Appendix VI). The Ecopath with Ecosim (EwE) model framework was used to examine squid-stocks in the GAB while the Atlantis framework was used to explore squid from waters off south east Tasmania (SETas).

2.7.1 *Ecopath with Ecosim model framework for GAB*

Ecopath with Ecosim (EwE) model framework was used to understand the ecological consequences (changes in trophic linkages and biomass flow) of increased fishing pressure on arrow squid stocks located in the GAB (Figure 2-5). Specifically, we aimed to estimate fishing mortality rates that would cause ecosystem instability and estimate maximum sustainable yields for arrow squid. In addition, we investigated the ability of arrow squid stocks to recover from high-fishing effort followed by a period of reduced fishing. In the process we aimed to determine the importance and function of arrow squid in the pelagic ecosystems in the GAB. A detailed description of the model dynamics is outlined in Appendix VI (Part I) which briefly describes the biophysical realm, trophic connections, time series and fisheries components enclosed in the model framework.

2.7.2 *Atlantis model framework for south eastern Tasmania*

The Atlantis ecosystem framework was employed to investigate the impact of different fishing scenarios and increased fishing pressure on stocks of arrow squid located off south eastern Tasmania (Figure 2-6), our goal being to determine the effects in terms of ecosystem impacts or changes in trophic structure. In addition we examined the effects of removing myctophid fish as common prey of squid. Parameters for initial abundance estimates of arrow squid were obtained from published literature.

The primary processes considered in Atlantis were consumption, production, migration, recruitment, waste production, habitat dependency, predation and (natural and fishing) mortality. Using these parameters, various fishing pressure scenarios including current fishing rates, high fishing rates and extreme fishing pressure, were modelled to explore the impacts on the surrounding ecosystem. Those parameters and scenarios examined are described in Appendix VI (Part II).

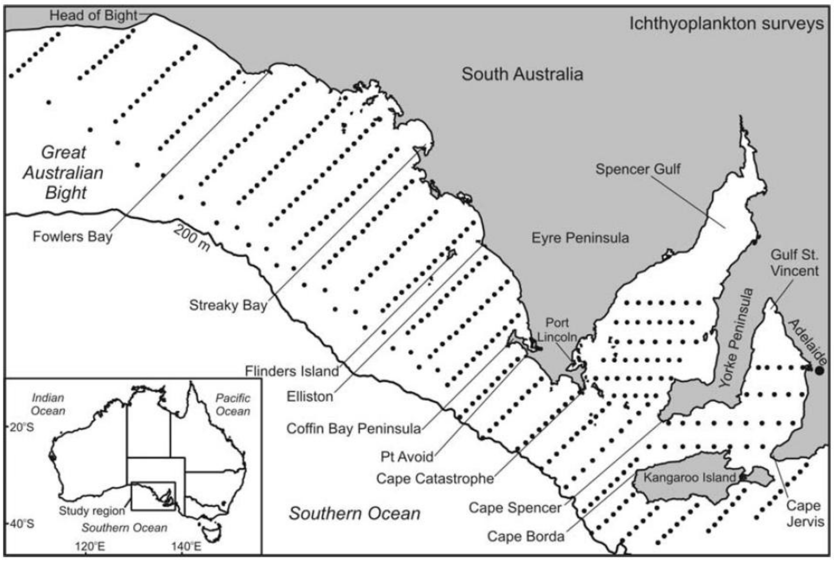

Figure 2-5 Map area of the model domain in the GAB covering 154,084km^2. Dots in horizontal lines refer to stations and transects during the SARDI Aquatic sciences ichthyoplankton survey conducted between 1998 and 2007.

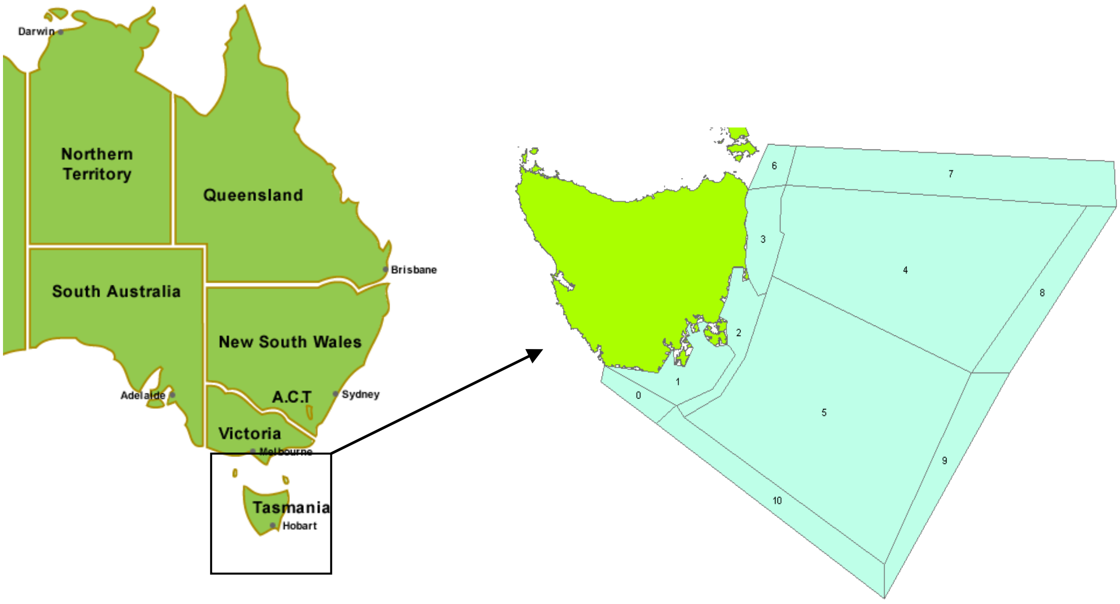

Figure 2-6 The spatial area of south eastern Tasmania that is covered by the model domain.

3. RESULTS

3.1 Growth and reproductive variability and the influence of season and sea surface temperature

Growth and reproductive data from arrow squid collected from Victoria and the GAB was compared with respect to oceanographic and environmental factors. Individuals collected from March 2007 – June 2009 in Victoria and the GAB had variable mantle weight frequency distributions over approximately monthly and bimonthly time scales (Figure 1-4; Appendix I). The size range of individuals collected was 25 – 525 g and 50 – 400 g in the GAB and Victoria respectively. Of the 1142 (GAB) and 2467 (Victoria) squid collected, 993 and 2177 age estimates were determined and used in calculating hatch date. The minimum age of individuals collected in the GAB was 140 d and 145 d for females and males respectively; whereas in Victoria, the minimum age was 145 d and 166 d for females and males respectively.

Seasonal patterns in growth rates were similar for male and female squid caught in both locations, although squid grew faster and larger in cooler waters off Victoria compared with individuals caught in the warmer waters of the GAB. Temporal variation in growth rates of squid from Victoria and the GAB was evident among hatch years and hatch seasons for males and females with increasing growth rates from autumn to spring, and for each season male squid had slower growth rates than females (Figure 1-6; Appendix I).

Reproductive condition and the ratio mature to immature individuals varied temporally for males and females. Most male squid caught were mature; whereas females showed greater seasonal variability in maturity stages. In the GAB less immature male squid were caught during summer and autumn and more in winter, whereas relatively more immature female squid were found during summer and fewer in autumn (Figure 1-9; Appendix I). In Victoria maturity stages represented in the catch were more complex. Greater numbers of immature males were found during summer and less in autumn and winter. Whereas for females, a greater number of immature and fewer mature squid were found during summer; whereas during spring this observation was reversed with more mature and fewer immature squid found. Temporal trends in reproductive condition for individuals captured from the Victorian fishery appeared cyclic, with individuals in better reproductive condition during summer and poorer condition during winter. Such temporal trends were not as evident for squid collected in the GAB. Spatial comparisons showed that female reproductive condition was better in the GAB during winter 2008; however, better in Victoria during summer 2008/09.

Independent of season of capture, on average, reproductively mature individuals were older but the exact pattern of differences was dependent on sex and location. For both male and female squid in each maturity stage, Victorian squid were on average older than individuals from the GAB, indicating that Victorian squid matured later in life compared with individuals caught in the GAB.

More than fifty percent of the variation in weight-at-catch of male and female squid caught in Victoria or the GAB was explained by sea surface temperature (SST) and age. For both males and females caught in Victoria, weight-at-catch was heaviest for those individuals that hatched during warmer SSTs (Figure 1-14; Appendix I), whereas squid caught in the GAB experiencing warmer SSTs during the first 30-day post-hatching were lighter on the day of capture for their age (Figure 1-15; Appendix I). Analyses of the relationship between SST and growth using additive models confirm this pattern, suggesting that individuals hatching in warmer SST were larger for their age when caught in Victoria; but smaller for their age when caught in the GAB (Figure 1-16; Appendix I). Consequently, modelling temporal and spatial variability in arrow squid population dynamics in relation to environmental or oceanographic factors like SST is likely to remain complex.

3.2 Temporal and spatial recruitment variability

Distribution of hatch dates determined from back calculated statolith age estimates suggest that arrow squid collected in Victoria and the GAB during 2007 – 2009, hatch year round (Figure 2-5; Appendix II). Hatch date frequency distributions of individuals collected in Victoria from 2007 – 2009 and in the GAB in 2008 – 2009 suggest continual recruitment into the existing population, with temporal and spatial variability in cohort structure evident (Figure 2-6, Figure 2-7; Appendix II). Approximately four cohorts were identified and evenly distributed during a period of one year from Victoria and the GAB using Bayesian Information Criterion (Figure 2-8 to Figure 2-11; Appendix II), indicating a degree of periodicity in hatch frequency shared among locations. In the GAB, the greatest number of squid hatched during February 2008; whereas periods of increased hatching were not as distinct in Victoria.

3.3 Differences in catch composition between inshore jigging and offshore trawling fisheries

Squid caught inshore and offshore using jigs and trawls respectively showed significant differences in biological parameters for size, maturity, age, sex ratio, and growth rate (see Appendix III). Sex ratio differed significantly with greater numbers of male squid found inshore for all season and collection year combinations. In contrast, all offshore collections showed similar sex ratios (except during winter 2007). Male and female squid caught inshore were significantly larger (Figure 3-3, Figure 3-4; Appendix III), and of an older age class (Figure 3-9; Appendix III), than those caught offshore.

Overall a greater percentage of mature females were found inshore compared with offshore, whereas mature males were found at both regions (Table 3-3 and Table 3-4; Appendix III). However a comparison of maturity stage frequency distributions between seasons showed that a greater proportion of mature males were found inshore, whereas significantly more mature females found offshore during autumn as oppose to winter (Figure 3-10; Appendix III)..

Temporal variation in reproductive condition (testis or ovary weight) was observed among location, year, and season with squid caught during autumn 2007 in better reproductive condition compared to all remaining collections (Figure 3-6, Figure 3-7; Appendix III). Males collected by jig inshore were in poorer reproductive condition than those collected offshore by trawl, whereas no difference in female reproductive condition was evident between inshore and offshore collections.

Statolith elemental composition analysis showed no significant difference among location of capture. However, differences were found between the three different ablation regions (core, mid-point, edge). Element concentration of the statolith core was different from both the mid-point and edge concentrations (Figure 3-11, Table 3-7; Appendix III) indicating that ontogenetic changes (e.g. habitat shift or physiological processors) in arrow squid occur.

3.4 Diet and feeding ecology

The diet of arrow squid as indicated by stomach content and digestive gland lipid and FA profiles varied markedly over the one year sampling period (see Appendix IV). Stomach contents provide clear evidence for dietary shift in prey groups consumed by arrow squid. Multivariate comparisons of lipid and FA profiles also indicated seasonal differences in the diet of squid, with evidence of diet variation between gender, two size-classes (< or > 300mm ML) and life-history (maturation) stages of females.

Individuals collected feed on a diverse range of fish, cephalopods and crustaceans, with a total of 1365 prey items identified from stomach contents (Table 4-1; Appendix IV). During the one year sampling period, arrow squid fed largely on fish and supplemented its diet with a range of other prey. During the summer months, the myctophid, *Lampanyctodes hectoris*, dominated while during the winter period, cephalopods (as a prey group) and the pearlside (*Maurolicus muelleri*) dominated. Cephalopods were the second most important prey group, followed by the crustaceans. Arrow squid were found to consume a large range of prey sizes with some individuals consuming large fish relative to their mantle length. However, most fish consumed were small, including the two dominant prey species *L. hectoris* and *M. muelleri*. A high occurrence of parasitic loadings of the larval nematode *Anisakis* spp was noteworthy.

Lipid class and FA profiles of the digestive gland of arrow squid demonstrated dietary variability which was explained by seasonal, gender and size-related differences (Table 4-2 to Table 4-4, Figure 4-1; Appendix IV). Analysis of the digestive gland revealed an average total lipid content of 16.4 ± 8.4% (wet weight), composed primarily of triacylglycerols, and twenty nine different fatty acids in greater than trace levels (>0.5%). Differences in lipid class composition were significantly affected by season, with total lipid content higher during the summer collection months.

As shown in the stomach contents analysis, multidimensional scaling analysis of FA profiles highlighted that myctophids, squid and certain non-myctophid fish are strongly represented in the diet of arrow squid, and that crustaceans are unlikely to be more than supplementary components.

3.5 Temporal and spatial population structure using statolith elemental composition and shape

Analysing shape and element composition of statoliths from arrow squid in unison provided a mechanism to analyse stock structure and migration characteristics. Fourier shape analysis indicated that statoliths significantly varied among sexes, squid size and location (Figure 5-3 and Figure 5-4; Appendix V), suggested that shape needed to be analysed on sexes separately. Independent of sex and location of capture, statolith shape was significantly different when comparing small and large statoliths indicating that shape changes over time with increasing age. Greatest difference between small and large statoliths was from female squid caught in the GAB. A comparison between GAB and Victoria found that both male and female squid had significant differences in the shape of their statoliths when an equal size distribution of statolith areas was selected for analysis (Table 5-3; Appendix V).

The composition of statoliths, particularly elements Mg, Mn, Sr and Ba, varied temporally and spatially (Appendix V). Analysing individual elements on the pre-edge region of statoliths revealed significant interaction between location and season of capture for Mg, Mn and Sr; however, no interaction was evident for Ba concentrations. Barium showed significant difference between locations with concentrations greater in Victoria compared with GAB. Season effects on Ba were also found with concentration lowest during winter and greatest during summer. For Mg, the lowest average concentration on the statolith pre-edge was found in squid collected in the GAB during autumn 2009 and greatest in Victoria during summer. Concentrations of Mn in the pre-edge of the statolith was lowest for squid collected in Victoria during winter 2008 and greatest for squid collected in Victoria during summer 2008/09. Post hoc tests showed that variation in Mn concentration between seasons was less variable than for squid caught in the GAB. For Sr, average element concentration was highest for squid collected in the GAB during summer 2008/09 and lowest in the GAB during winter 2008.

In summary Fourier shape analysis indicated significant phenotypic heterogeneity among squid from the two locations, while statolith element composition indicates that squid caught from either Victoria or the GAB originally hatched in various regions of their distribution.

3.6 Ecosystem model based approaches to squid stock assessment

Model results for arrow squid show that both the GAB and southeast Tasmanian ecosystems were fairly robust to high levels of fishing on arrow squid populations. Current fishing rates in the GAB had little effects on the overall biomass of arrow squid, largely because the contribution from predation mortality rates (per year) was higher than that of fishing (Table 6-3; Appendix VI). Fishing effort would need to be increased by a factor of at least x500 in the GAB system before noticeable changes occur to their populations (Figure 6-5; Appendix VI). Increased fishing levels, and thus increased depletion of arrow squid, would however cause direct positive effects on principal prey groups like Jack mackerel, sardines and mesopelagic fish, and negative effects on predators like New Zealand fur seals (Figure 6-4; Appendix VI).

The Atlantis model found that a high removal of squid from the southeast Tasmanian ecosystem will have a minimal effect on the biomass of most trophic groups and the broad system structure, largely due to the generalist nature of arrow squid as both predator and prey (Figure 6-7; Appendix VI). As oppose to the myctophids, the squid population here appeared considerably robust to high fishing pressure, with model results suggesting that up to 85% of the squid biomass could be fished before the population became unviable. A reduction in squid biomass resulted in a proportional response in respect to predator and prey relationships, with some restructuring of trophic linkages expected. Increased fishing levels were found to cause direct positive effects on principal prey groups (mainly myctophids), negative effects on predatory species (e.g. demersal sharks) and indirect negative effects on others (e.g. pipefish, gobies, damselfish etc). These indirect effects occurred due to the switching behaviour of some of the piscivorous fish like barracouta, pike, morwong and sharks; meaning that these predators consumed a larger proportion of these small fish due to the lack of squid prey available.

4. DISCUSSION

Using a range of methods, we investigated stock structure and the spatial and temporal distribution of arrow squid *Nototodarus gouldi* collected from Victoria and the Great Australian Bight (GAB). The catch composition from both the inshore jig fishery and the offshore trawl fishery in these two regions was determined. Arrow squid dietary analysis gave us an insight into the condition of squid. Using squid predator-prey relationships an ecosystem model was developed to understand the effects of fishing pressure on arrow squid.

Seasonal patterns in growth rates were similar for male and female arrow squid caught in both locations. Squid grew faster and larger in cooler waters off Victoria compared with individuals caught in the warmer waters of the GAB. Victorian caught squid however matured later in life compared with squid caught in the GAB. Temporal trends in reproductive condition for male and female squid from Victoria appeared cyclic, with individuals in better reproductive condition during summer and poorer condition during winter. Such temporal trends were not as evident for squid collected in the GAB. Temporal and spatial growth variability is likely driven by environmental and oceanographic conditions experienced during early life and less influenced by conditions experienced as adults. Squid hatchlings that experienced warmer sea surface temperature were larger for their age when caught in Victoria; but smaller for their age when caught in the GAB. However, the smallest adults caught in Victorian waters occurred when hatchlings experienced water temperatures typical found in spring and autumn. For effective assessment of cephalopod fisheries, greatest benefit in modelling population dynamics is achieved when life history and population characteristics are determined annually. However, as it is difficult to predict future growth variability using SST alone, analysis of other factors contributing to the dynamic population structure such as food availability may contribute to assessment and management of the fishery (see Appendix I).

To determine spatial and temporal recruitment in arrow squid, hatch dates were back calculated from statolith age estimates. Results using this method suggest that arrow squid collected in Victoria and the Great Australian Bight hatch year round resulting in all ontogenetic stages being represented in the population at any one point of time. The timing of spawning influences future biomass of arrow squid. Embryo and juvenile growth, and survival rates are a product of the environmental and oceanographic factors they experience, which results in spatial and temporal variability of the population structure. The strength of recruitment success and future biomass is a reflection of inter- and intra-annual variability of environmental conditions that affects all ontogenetic life stages.

Although year round spawning characteristics showed peaks in hatch date frequency distributions, large overlaps in cohort structure coupled with their rapid response to changes in oceanographic and environment factors suggests that forecasting recruitment will remain challenging. However, given their fast growth and rapid turnover, it is unlikely that future stocks of arrow squid will be affected by overfishing as long as there are sufficient recruits entering the fishery (see Appendix II).

The selective nature of fishing gear on arrow squid catch composition was investigated using both traditional morphometric methods and statolith elemental analyses. Squid caught inshore by jiggers were larger, consisted of a greater M:F ratio and had a greater percentage of mature females that those caught in offshore trawls. These results suggest that spawning is occurring inshore and that the jig fishery may have greater influence on recruitment than the trawl fishery. Squid caught by both fishing methods had similar statolith elemental composition which was probably a reflection of uniform ocean water chemistry throughout the region, rather than inshore / offshore population intermixing. There was significant selectivity for adult squid, as juveniles were not represented in the catch of either the jig or trawl fishery. The observed differences in catch between the jig and trawl fishery are likely to be influenced by squid ontogenetic characteristics and behavioural response to fishing gear, as well as selectivity of fishing methods (see Appendix III).

Analysis of the diet of arrow squid showed clear evidence of seasonal dietary shifts in prey composition. Teleost fish and two mesopelagic planktivorous lightfish, (*Lampanyctodes hectoris* and *Maurolicus mulleri*) dominated the diet. Cephalopods and crustaceans made up a smaller proportion of the diet however, an increased representation of these were found over the winter months. Digestive gland lipid content was rich in triacylglycerol and monounsaturated fatty acids. Seasonal differences in lipid content were related to summer upwelling events and local changes in productivity. Fatty acid analyses demonstrated dietary differences associated with sex, size and female maturation. As arrow squid display sexual dimorphism this result is not surprising as smaller males would be more likely to consume smaller prey species. Such relationships demonstrate that the diet of arrow squid is closely linked to prey size, abundance and availability and possibly also, to key life-history stages (see Appendix IV).

Arrow squid populations caught in Victoria and the Great Australian Bight were investigated with the view to determine if they were a single population or separate stocks. The method used involved describing the shape of the statolith from individual squid and comparing them. Comparing statolith shape from squid collected in Victoria to squid collected in the Great Australian Bight suggested significant phenotypic heterogeneity in stocks. These results conflicted with results from statolith elemental composition. Statolith element concentrations were used to determine where individual squid are likely to have hatched, and suggested that squid caught at either location hatched throughout their distribution. Although squid caught in the Great Australia Bight and Victoria appear as separate stocks, squid from both regions are important for recruitment (see Appendix V).

Ecosystem models are increasingly providing theoretical frameworks in which to assess the ecological impacts of fishing and other perturbations allowing more strategic and sustainable fisheries management. In this study we utilise two recently developed ecosystem-based models to investigate the ecological impact of increased fishing pressure on arrow squid in the Great Australian Bight and in south east Tasmania. Model results show that both ecosystems were fairly robust to high levels of fishing on arrow squid populations. We found that the current fishing effort on arrow squid would need to be increased substantially (i.e. by a factor of at least x500 in the GAB system) before noticeable changes occur to their populations. Increased fishing levels would however cause a direct positive effects on principal prey groups (mainly sardines and myctophids) and negative effects on predators (New Zealand fur seals and predatory fish). Cascading effects of arrow squid removal include those from a changing demographic structure and increased feeding competition on important lower-trophic groups such as zooplankton. The abundance and feeding behaviour of arrow squid, influences the population size and distribution of its predators and the energy flow patterns of the pelagic ecosystem (see Appendix VI).

5. BENEFITS

Results from this research constitute a major contribution to the knowledge base of arrow squid biology and ecology, and the relationship between squid biology/ecology and environmental conditions. This research was conducted for the benefit the squid fishing industry as stated in the original application. The extension of these results through to management policy should be considered by AFMA via SquidMAC and SquidRAG. This final report submitted to FRDC, will then be distributed to relevant industry participants and managers. Results of the research have been presented at relevant national and international scientific conferences. In addition, the results from this research are being published as a series of papers in high profile international journals for the benefit of the scientific community.

6. FURTHER DEVELOPMENTS

Determining the environmental and oceanographic parameters that influence recruit will allow better forecasts of future biomass and hence allow informed management of cephalopod fisheries. Temporal and spatial recruitment variability of *N. gouldi* was determined using collections from selective fishing methods (jig and trawl) that influenced the size of the population sampled; and can bias estimates of growth, recruitment, and ontogenetic migration characteristics (Caddy, 1991; Rodhouse, 2001). Analyses of recruitment and ontogenetic migration characteristics really require a sampling method (such as using fine mesh cod-ends) that would ensure all individuals in the population have equal chance of capture. Trawl selectivity of commercial gear can bias the size distribution of *N. gouldi* caught therefore routine sampling (e.g. monthly) using fine mesh trawl nets will increase the likelihood to catch squid of a greater range of sizes. Such data will increase confidence in accurately describing the spatial and temporal patterns of *N. gouldi*, including accessibility by the fishers, as well as growth and distribution information of juveniles (e.g. <100 d) which is presently lacking. Although recruitment variability of *N. gouldi* could be analysed using back calculated hatch dates, a preliminary investigation to estimate future recruitment strength based on data about the juveniles was attempted using light traps. Traps were deployed at three depths within the water column (surface, middle and bottom) off the coast of Victoria in February, April, and June 2009; however, no *N. gouldi* were collected. Either juvenile *N. gouldi* were not attracted to the lights in the traps, or juveniles were not present in the sampling area.

Statoliths provide much information on stock structure and lifecycle characteristics however, greater information from statoliths can be gained. For example, age-at-capture was used to calculate average lifetime growth rates of individuals in this research. However, differences in increment width can be reflective of changes in growth rates that can be modelled with environmental factors. This information will provide greater explanation on the effect environment variability has on all ontogenetic stages of growth used to forecast variability in population biomass.

During the past 10 years research has provided fisheries managers with biological and stock structure information used to manage the *N. gouldi* resource and is hoped this research will go toward their assessment. Based on previous and current research (Jackson and McGrath-Steer, 2004), the implementation of harvest strategies is an effective and suitable approach to manage this resource with relatively low cost; however, modeling the effect each fishing method (jig and trawl) has on future availability of stocks would greatly increase confidence in ongoing management.

This research successfully used current analytical and statistical methods to describe population stock structure, recruitment variability, migratory characteristics, catch composition, and ecosystem importance of *N. gouldi* collected in southern Australia from 2007–2009. Results suggest that current management restrictions and effort applied to the fishery will not increase the likelihood of overfishing.

All data from this project will be available in public access publications.

7. PLANNED OUTCOMES

Results from this research have led to considerable progress towards achieving the stated outcomes originally proposed. The original planned outcomes of the project were to develop:

1. a better understanding of arrow squid and their environment through detailed modelling using both biological and oceanographic/environmental parameters
2. optimal use of the arrow squid resource by enhancing our knowledge on how squid respond to the environment and providing the potential to predict times of squid abundance and a model that estimates stock levels
3. sustainable use of the arrow squid resource by providing needed information to management for ecologically sustainable management of the fishery.

Results from these outcomes have been communicated through this final report submitted to FRDC, and distributed to relevant industry participants and managers. Results of the research have been presented at relevant national and international scientific conferences. In addition the results are being published as a series of scientific papers in high profile international journals.

8. CONCLUSION

This research included a range of approaches to investigate stocks of arrow squid. We investigated the influence environmental and oceanographic variables had on arrow squid growth and recruitment using satellite derived data. We compared the biological composition of jig and trawl-caught squid to understand both spatial and temporal distribution of squid, and the effects of the fishing method on the composition. Statolith shape was used to investigate stock discrimination, and lipid and fatty acid analysis gave us an insight into the condition and diet of squid. We development an ecosystem model based on squid predator-prey relationships to determine the effects of fishing pressure on both arrow squid and the ecosystem.

In order to manage a sustainable fishery, estimates of biomass and information on stock structure are used to calculate catch. Arrow squid are managed as a single stock, although in this study differences in stock structure were found between squid caught in the GAB and Victoria. However they were not considered unique stocks because individuals have the capacity to migrate throughout their geographic distribution. Differences in statolith shape and growth rates supported the hypothesis of different stocks; however, statolith element composition and recruitment analyses suggest that arrow squid were not restricted to either the GAB or Victoria. Squid in the GAB and Victoria are likely to have hatched from various locations within their distribution making estimates of biomass for a particular region and season, difficult. Without annual estimates of biomass, analysing the migration, growth, reproduction, and recruitment of arrow squid provided information to help manage the fishery.

Results from this research suggest that squid undertake mesoscale (1–100 km) migration parallel to the continental shelf, as well as between inshore and offshore locations. Arrow squid don’t need to migrate great distances as their main prey species (*Lampanyctodes hectoris* and *Maurolicus mulleri*) have a common distribution along and on the continental shelf. A localized depletion of stocks could result if intensive fishing were to occur over short time scales, although no evidence of within season depletion has been documented possibly due to low fishing effort. However, migration of squid from adjacent areas and constant recruitment would allow depleted populations to recover relatively quickly (< 1 yr). This is supported by the ecosystem model results, where a simulated population of arrow squid recovered within 2 – 3 years after increases in fishing effort reduced the biomass to 10% of its original estimate. Current fishing effort in the GAB would need to be increase substantially, at least 500 times, before changes in population size would occur. Arrow squid population biomass is likely to be more responsive to biomass changes of their dominant predators (seals).

Year round recruitment of arrow squid is likely as spawning occurs multiple times throughout life resulting in individuals hatching throughout the year. Continuous recruitment into the fishery suggests that juveniles hatch during both favourable and unfavourable conditions which can influence the size distribution of cohorts. Consequently, estimating or forecasting the size and time that cohorts will be available to fishers will be dependent on predicting the number of cohorts during a fishing season, the characteristics of the cohort based on when they hatched, and how fast they grow. Temporal variability in cohort structure is a function of seasonal oceanographic and environmental factors, which can be used to predict recruitment strength. Back calculated hatch dates provided a better tool to analyse stock structure and recruitment variability of stocks, given that variation in individual growth rates obscures the identification of cohorts derived from length frequency distributions. Greatest spawning success occurs during summer and autumn in inshore fishing grounds, a time when fishing conditions are favourable. Fishing during summer and autumn maximizes catch weight because squid are largest during this period.

The population structure, growth and reproductive characteristics of arrow squid are diverse due to the substantial differences seasonal environmental conditions. Warmer sea surface temperatures in Victoria were more influential than cooler temperatures in maximizing growth of individuals and therefore population biomass because of greater spawning activity and larger size-at-age. To manage this fishery, this temporal and spatial variability in biomass requires a regular stock assessment. The use of ecosystem modelling is also a powerful tool for managers to determine the environmental and oceanographic parameters that most influence recruitment and hence able to forecast future biomass estimates.

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APPENDICES

1. APPENDIX I

Growth and reproductive variability of arrow squid Nototodarus gouldi from southern Australian waters in relation to environmental and oceanographic factors

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1. ABSTRACT

This study aimed to compare growth and reproductive data from *Nototodarus gouldi* collected from Victoria and the Great Australian Bight (GAB) with respect to oceanographic and environmental factors. Size, age and reproductive data of *N. gouldi* from Victoria was collected monthly from March 2007 – June 2009, and from the GAB bimonthly from June 2008 – June 2009. Seasonal patterns in the changes in growth rates were similar for male and female squid caught in both locations. *Nototodarus gouldi* grew faster and larger in cooler waters off Victoria compared with individuals caught in the warmer waters of the GAB. Victorian *N. gouldi* matured later in life compared with *N. gouldi* caught in the GAB. Most male *N. gouldi* caught were mature; whereas females showed greater seasonal variability in maturity stages. Temporal trends in reproductive condition for male and female *N. gouldi* from Victoria appeared cyclic, with individuals in better reproductive condition during summer and poorer condition during winter. Such temporal trends were not as evident for *N. gouldi* collected in the GAB. Spatial comparisons showed that female reproductive condition was better in the GAB during winter 2008; however, better in Victoria during summer 2008/09. Temporal and spatial growth variability in *N. gouldi* is likely driven by environmental and oceanographic conditions experienced during early life and less influenced by conditions experienced during adult stages. Analysing the relationship between sea surface temperature (SST) and growth using additive models suggest that *N. gouldi* hatchlings that experienced warmer SST were larger for their age when caught in Victoria; but smaller for their age when caught in the GAB. However, the smallest adults caught in Victorian waters occurred when hatchlings experienced water temperatures typical of spring and

autumn. Consequently, modelling temporal and spatial variability in *N. gouldi* population dynamics in relation to environmental and oceanographic factors is likely to remain complex.

2. INTRODUCTION

Life history characteristics such as reproductive strategies and rates of growth, recruitment, mortality, and migration, coupled with environmental and anthropogenic factors all contribute to variation in population structure and biomass size of marine biota (Myers 1998, Law 2000, Agnew *et al.* 2002, Conover & Munch 2002, King 2007). For longer lived species, the effect of environmental and oceanographic influences on biological characteristics (e.g. growth) is often difficult to detect as these characteristics are a result of lifetime influences averaged over several years. However, for species with short generation times, such as anchovy, changes in their population structure is more likely detected over relatively short periods as many of the biological processes and population characteristics occur on scales of days and weeks rather than months and years (Cury & Roy 1989, Jacobson *et al.* 2001, Taylor *et al.* 2002, Chavez *et al.* 2003, Hobday *et al.* 2006, Takasuka *et al.* 2007). For short-lived species such as cephalopods, assessment and management is difficult as the biomass being estimated can change rapidly in response to variation in environmental and oceanographic factors (Cushing 1982, Caddy 1983, Boyle & Boletzky 1996, Nigmatullin 2004, FAO 2005). Determining short term effects of environmental and oceanographic factors on lifecycle and population structure of squid provides an increased opportunity to forecast the response of other short-lived species to variable ambient conditions (Dawe *et al.* 2001, Jackson & O'Dor 2001). Then the changes in environmental and oceanographic conditions occurring over longer time frames can be explored and modelled to forecast fisheries population biomass and structure (Boyle & Boletzky 1996, Agnew *et al.* 2002, Moltschaniwskyj 2004, Boyle & Rodhouse 2005, Harley *et al.* 2006).

Biological growth parameters of squid derived from size-at-age data varies in relation to water temperatures (Forsythe 2003, Jackson & Domeier 2003b). Warmer temperatures typically promote faster growth rates for many wild caught loliginids and ommastrephids (Dawe & Beck 1997, Jackson *et al.* 1997, Arkhipkin *et al.* 2000a, Arguelles *et al.* 2001, Jackson & Moltschaniwskyj 2001b); however, for *Loligo opalescens* increased water temperature decreased average growth rates (Jackson & Domeier 2003b, Reiss *et al.* 2005). Similarly, slower growth rates for *Sepioteuthis lessoniana* and *Loliolus noctiluca* were a result of warmer temperatures and reduced food availability that provided sub-optimal growth conditions (Jackson & Moltschaniwskyj 2001b, a). However, the critical period of the life that appears to determine adult size-at-age is the embryo and juvenile phases. For many squid, increased water temperature speeds up embryo developmental rates, but as a consequence often results in smaller hatchlings (Boletzky 1994, Villanueva 2000, Vidal *et al.* 2002, Steer *et al.* 2003). Although smaller hatchlings experiencing warmer conditions does not necessarily result in faster growth rates and larger size-at-age. Hatchling size has a cascading effect that influences adult size due to the exponential growth pattern during the first few months of life. The difference in adult body weight between an individual that grows at a rate 10% per day versus 7% per day is substantial; this is further compounded if the starting weight of the squid is also substantial (Pecl *et al.* 2004b, Pecl & Jackson 2007). Therefore, it is essential that models describing the relationship between adult size-at-age and environmental data need to include conditions during the juvenile phase.

Large and valuable ommastrephid fisheries, such as *Todarodes pacificus*, *Illex illecebrocus* and *I. argentinus*, appear to be associated with oceanographic systems, that includes major western boundary currents (e.g. Kuroshio, Gulf stream and the Brazil current) which carry deep, narrow, and fast flowing equatorial water poleward (Mann & Lazier 1991, Anderson & Rodhouse 2001b, Dawe *et al.* 2007). In southern Australia, oceanic squid populations in the Great Australian Bight (GAB) and Victoria (Bonney coast) are associated with ocean current systems that are influenced by upwelling, topography, wind and El Niño cycles (Li & Clarke 2004, Middleton *et al.* 2006, Middleton & Bye 2007). Winter ocean flow consists of an eastwards shelf current at approximately 20 cm sec^{-1}, and the deeper (600 m) westerly Flinders current (10 – 15 cm sec^{-1}) (Middleton & Bye 2007). During summer, the Flinders current slows and becomes shallower (5 – 10 cm sec^{-1} at 300 – 400 m) and the shelf currents move west (Middleton & Bye 2007). High fishing effort regions in Victoria and the GAB are approximately 1,200 km apart, with sea surface temperature approximately 2.3°C warmer in the GAB (Modis satellite data from 31/12/2006 – 01/01/2010). Summer and autumn upwelling plumes in the GAB and off the Bonney coast result in fluctuating temperature and productivity (Butler *et al.* 2002a, Ward *et al.* 2006, Middleton & Bye 2007, Willis & Hobday 2007). Greater concentrations of chlorophyll *a* (Chl *a*), indicative of very productive upwelling events, propagate through the food webs supporting higher trophic levels influencing squid growth (Ichii *et al.* 2002, Jackson *et al.* 2003, Ichii *et al.* 2009). For some cephalopod fisheries, inter- and intra-annual variability in environmental and oceanographic systems alter food webs that in turn defines population dynamics and recruitment characteristics of near-shore larvae, juvenile and adult squid (Gonzalez *et al.* 1997, Golub 2001, Otero *et al.* 2009). Given *Nototodarus gouldi* (McCoy 1888) populations are subjected to large variations in environmental conditions at small and large temporal and spatial scales, the capacity to predict biological and population characteristics based on real-time environmental data is a powerful tool for the management of short-lived species composed of a single generation (Jackson *et al.* 2003).

Spatial differences in growth and reproductive maturity of *N. gouldi* at different locations of southern Australia indicate that the population structure of this species is complex and dynamic (Jackson *et al.* 2003). *Nototodarus gouldi* are capable of spawning multiple times, releasing eggs in small batches during their life without compromising growth and oocyte production (McGrath & Jackson 2002). Such characteristics, coupled with protracted hatch dates, suggest year round spawning (Chapter 4; Uozumi 1998, McGrath & Jackson 2002, Jackson *et al.* 2003, Jackson *et al.* 2005). Consequently, hatchlings experience inter- and intra-annual variation in environmental conditions which in turn affect individual growth and reproductive characteristics. Seasonal variability in growth correlates with water temperature with *N. gouldi* hatching during summer/autumn having faster lifetime growth. However, inter-annual differences in growth rates between years are currently unexplained (Jackson *et al.* 2003). Spatial variability in growth has been analysed seasonally using parametric generalized linear models (GLM, Jackson *et al.* 2003), but this approach is limited as data normality is required and constrained to fit linear functions. Semi-parametric models such as generalized additive models (GAM, Hastie & Tibshirani 1990), provide more flexibility in fitting response curves, and provide a reasonable ecological explanation that suite analysing the affect environmental factors have on growth (Austin 2002, Stark 2008).

Squid landed in the southern and eastern scalefish and shark fishery (SESSF) is almost entirely comprised of *N. gouldi* and are subjected to variable environmental and oceanographic systems. Within the SESSF, squid are targeted by jig vessels on the continental shelf (< 120 m depth) that form the southern squid jig fishery (SSJF); whereas demersal trawlers operating on the continental slope (>250 m depth) retain squid as bycatch within the Commonwealth trawl sector (CTS; Larcombe & Begg 2008). Like other cephalopod fisheries, the *N. gouldi* fishery exhibits large temporal variation in catch weight and catch rates (Lynch 2005). In 2008 and 2009, jig fishing resulted in 179 t (883 jigging hours) and 308 t (1229 jigging hours) of squid caught; whereas the CTS caught 3.5 and 1.8 times more respectively (Wilson *et al.* 2010). Greatest effort in the SSJF is concentrated off the Bonney coast near the Victorian and South Australian border; however, *N. gouldi* are also landed in the Great Australian Bight trawl sector (GABTS; Wilson *et al.* 2010). In 2006, 262t of *N. gouldi* was landed in the GABTS; however, in 2008 and 2009, only 62 t and 29 t were caught respectively.

As climate change is expected to have considerable impact on marine ecosystems (IPCC 2002, Hobday *et al.* 2006), the need to define and compare current inter- and intra-annual growth and reproductive characteristics of *N. gouldi* caught in key fishing areas is required for future assessment and management of the resource. Despite decreasing interest in catching and retaining *N. gouldi* as a bycatch in the GABTS in recent years (possibly attributed to poor domestic prices), there is developing interest in using mid-water trawlers to target *N. gouldi* within the GABTS. However, compared with *N. gouldi* collected in the SSJF and CTS, little is known about the GAB population stock structure (Wilson *et al.* 2010). In the present study, comparisons of growth and reproductive characteristics of squid from the GAB and Victoria will be analysed using GLMs; whereas the influence of water temperature and water productivity on growth will be analysed using GAMs. Results will provide fisheries managers with additional information on current population dynamics used to refine current and future management strategies.

3. METHODS

3.1. Samples

Nototodarus gouldi were collected opportunistically from commercial trawl fishers operating in two regions of southern Australia. Off the coast of Victoria, up to 242 *N. gouldi* were collected at approximately monthly intervals from March 2007 – June 2009, whereas up to 354 *N. gouldi* were collected from the GAB bimonthly from June 2008 to June 2009 (Figure 1-1, Table 1-1). After freezing, squid were shipped to the University of Tasmania or the Marine and Freshwater Fisheries Research Institute in Victoria for dissection.

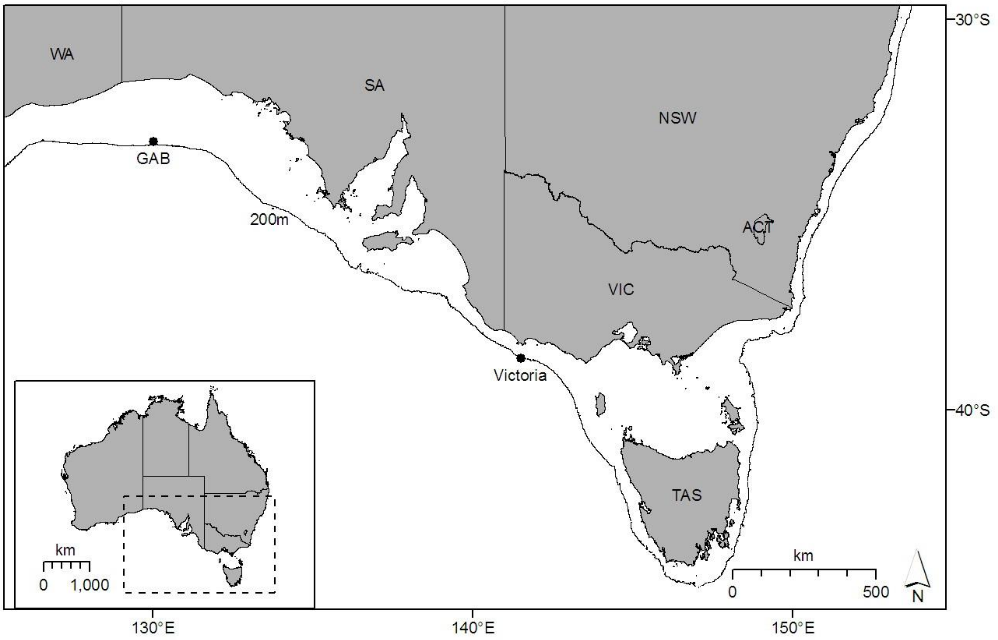

Figure 1-1 Map of southern Australia detailing the general location where N. gouldi were caught along the continental shelf from the Great Australian Bight (GAB) and Victoria.

Table 1-1 Details of *N. gouldi* collected from the Great Australian Bight (GAB) and Victoria, Australia from March 2007 – June 2009.

Date of collection	Season	Number collected in the GAB	Number collected in Victoria	Latitude	Longitude	Mean (s.d.) SST at date and location of collection
01-Mar-2007	Autumn		100	38.72	141.37	18.5 (0.08)
27-Mar-2007	Autumn		100	38.77	141.60	18.2 (0.08)
07-Jun-2007	Winter		100	38.30	140.52	15.9 (0.20)
06-Aug-2007	Winter		101	38.58	141.17	14.6 (0.20)
19-Sep-2007	Spring		100	38.30	140.55	13.7 (0.10)
15-Oct-2007	Spring		100	37.93	139.98	13.8 (0.08)
Total collections in 2007			601			
19-Feb-2008	Summer		100	38.75	141.53	19.3 (0.08)
14-Mar-2008	Autumn		101	38.93	142.18	18.5 (0.08)
14-May-2008	Autumn		142	38.33	140.63	16.5 (0.08)
25-May-2008	Autumn		100	38.23	140.42	16.8 (0.08)
22-Jun-2008	Winter		100	38.58	141.17	15.8 (0.20)
23-Jun-2008	Winter	112		33.25	130.03	18.3(0.08)
25-Jul-2008	Winter		100	37.55	139.28	15.6 (0.09)
01-Aug-2008	Winter		100	38.62	139.27	14.6 (0.10)
15-Aug-2008	Winter	80		33.22	126.48	15.9 (0.20)
16-Aug-2008	Winter	90		33.28	128.58	15.9 (0.20)
17-Aug-2008	Winter	85		33.30	130.13	16.0 (0.20)
19-Aug-2008	Winter	99		33.28	130.13	16.0 (0.20)
28-Aug-2008	Winter		137	38.32	140.53	13.1 (0.09)
30-Sep-2008	Spring		121	38.68	141.27	13.6 (0.08)
06-Nov-2008	Spring	158		33.22	131.25	17.8 (0.09)
14-Nov-2008	Spring		140	38.62	141.20	17.0 (0.09)
11-Dec-2008	Summer		156	33.75	141.47	15.9 (0.20)
22-Dec-2008	Summer	83		33.35	132.10	19.0 (0.09)
Total collections in 2008		707	1297			
21-Jan-2009	Summer		130	38.70	141.30	17.6 (0.09)
12-Feb-2009	Summer	135		33.18	128.39	21.3 (0.08)
19-Feb-2009	Summer		175	38.68	141.32	18.2 (0.09)
25-Mar-2009	Autumn		105	38.75	141.35	17.7 (0.09)
02-Apr-2009	Autumn	96		33.16	128.30	19.9 (0.08)
30-Apr-2009	Autumn		81	38.73	141.37	16.8 (0.10)
02-Jun-2009	Winter		78	38.77	141.57	16.4 (0.09)
10-Jun-2009	Winter	204		33.19	129.00	18.0 (0.09)
Total collections in 2009		435	569			
Total collections combined years		1142	2467			

4.1. Age and reproductive parameters

Defrosted squid were dissected to collect biological information including mantle weight (Mwt, g), sex, ovary weight (g) and testis weight (g). Maturity stages were assigned from I–V using descriptive scales described by Borges and Wallace (1993); Stages I–III were classified as immature; whereas stages IV–V were mature.

Age was determined from individual statoliths by counting incremental structure from the hatch mark near the statolith core, to the edge of the dorsal dome using a modified method described by Jackson (2005). Statolith pairs were located within the statocyst of the squid cranium and removed with fine-tipped forceps, rinsed with distilled water, and stored dry in cavity trays. One statolith was randomly removed from the cavity tray and attached to a glass microscope slide with the anterior side closest to the slide using heated thermoplastic glue (Crystalbond™). Once cooled, the posterior side of the statolith was ground down with 30 µm lapping film (3M™) until the core was visible just under the ground surface. A finer grade of lapping film (5 µm) was used to grind closer to the core and remove larger surface scratches. For preparations where increments were not clear, the slide was reheated, the statolith ground on the anterior side and flipped back to the posterior side. Preparations were polished using Leco Clothe® and aluminum oxide powder (0.005 µm) and water to remove finer scratches. A small amount of immersion oil was smeared on the statolith to improve increment clarity. Statoliths were observed at up to 400x magnification using a compound microscope integrated with the image analysis system Optimas™. Daily age was determined by counting incremental structure from the presence of the hatch mark to the edge of the dorsal dome, along the apex of increment formation and was assumed that the periodicity of increment formation in *N. gouldi* statoliths occurs daily (Jackson *et al.* 2005). Due to the subjective nature of increment interpretation, some preparations were aged up to three times with the average count recorded.

4.2. Analysis of growth and reproduction characteristics

Spatial and temporal comparisons of *N. gouldi* growth rates were analysed using weight-at-age data. Mantle weight was used as the dependant variable rather than total weight as this removes the variability in the mass of stomach and gonad. Patterns of growth were fitted with two regression models; power law and exponential, where log age and age were used as predictor variables respectively. Akaike's Information Criterion (AIC) was used to assess the goodness of fit of the two regression models.

To determine if growth rates of *N. gouldi* differed among fisheries and hatch season an ANCOVA was used to analyse the relationship between log mantle weight-at-age and log age (covariate) of *N. gouldi* collected in the GAB and Victoria. Back-calculated hatch dates were grouped according to season of hatch (Table 1-2). Given growth rates of *N. gouldi* differ between sexes (Jackson *et al.* 2005) all analyses were conducted for males and females separately. Hatch season of *N. gouldi* collected in

Victoria ranged from autumn 2006 – summer 2008/09 which generates 12 seasons-year combinations, whereas for the GAB hatch season ranged from spring 2007 – summer 2008/09 generating six seasons-year combinations. Consequently, growth comparisons between the GAB and Victoria were limited to the five hatch seasons they had in common (spring 2007 – spring 2008). *Nototodarus gouldi* that hatched during summer 2008/09 were excluded from analysis due to small numbers. Seasonal and yearly comparisons were made within each fishery by fitting models that allowed intercepts and slopes to vary with both year and season; years and seasons were treated as fixed factors. Where there was evidence of a difference in slopes, pairwise comparisons of slopes were performed by the methods of Hothorn *et al.* (2008).

Table 1-2 Number of N. gouldi hatching from each season and year with corresponding mean SST.

Hatch season and year	Number hatched in the GAB	Mean (s.d) SST for the GAB	Number hatched in Victoria	Mean (s.d) SST for Victoria
Aut 06			5	
Win 06			161	
Spr 06			138	
Sum 06/07			149	18.1 (0.09)
Aut 07			58	17.5 (0.14)
Win 07			195	14.6 (0.20)
Spr 07	80	16.7 (0.09)	327	14.3 (0.13)
Sum 07/08	295	20.1 (0.08)	344	17.5 (0.16)
Aut 08	221	20.0 (0.10)	335	17.1 (0.13)
Win 08	210	17.2 (0.11)	345	14.9 (0.18)
Spr 08	152	17.0 (0.08)	117	13.9 (0.15)
Sum 08/09	35	20.4 (0.09)	3	16.9 (0.13
Total	993		2177	

Differences in the reproductive status (i.e. immature or mature) of *N. gouldi* collections were made by comparing the proportion of immature to mature *N. gouldi* as a function of season of capture for each sex and fishery using a Pearson’s chi-square test. Where significant chi-square tests occurred, standardized residuals were used to determine for which catch season significant differences occurred between observed and expected frequencies. The variability in the age of squid caught was explained as a function of location, sex, and maturity (immature or mature) using 3-way factorial ANOVAs with all factors fixed.

Spatial and temporal differences in reproductive condition were analysed for each sex by calculating and comparing the standardized residuals from the regression between Mwt (independent variable) and testis weight for mature males (stage IV and V); and Mwt and ovary weight for mature females (stage IV and V). Individuals whose reproductive organs are lighter than predicted (i.e. negative standardized residuals) were in poorer reproductive condition than individuals whose organs that were heavier than the predicted weight (i.e. positive standardized residuals) (Moltschaniwskyj & Semmens 2000). Due to the nature of the fishing activity the structure of the dataset was not fully orthogonal; collections from Victoria were made from all 10 combinations of season and year, but collections from GAB were only from five of the year-season combinations (Table 1-1). Therefore, it was not possible to use a three factor (location, year and season) ANOVA, instead separate analyses were undertaken to explore spatial and temporal variability in reproductive condition of individuals. Spatial variation in reproductive condition was analysed using a one-way ANOVA to compare mean standardized residuals between *N. gouldi* collected in the GAB and Victoria for five seasons of collection from winter 2008 – autumn 2009 for females, and winter 2009 for males. Following a significant ANOVA, planned contrasts were used to determine if there was a difference between the two fisheries for each season. Temporal variation in reproductive condition for each sex from the two locations was analysed separately, in this analysis the mean standardized residuals were compared among the season-year combinations (10 combinations for Victorian collections and five for GAB collections) using a one-way ANOVA, followed by Tukey's post-hoc test to determine the pattern of differences among the year-season combinations occurring in each location.

4.3. Modelling of growth with environmental and oceanographic factors

Generalized additive models (Hastie & Tibshirani 1990) provide a semi-parametric extension to the standard GLM regression model. The standard regression model assumes the response y is normally distributed about its expected value μ

$$y_i \sim N(\mu_i, \sigma^2) \qquad \text{Eq 1.1}$$

which in turn can be expressed as a linear combination of predictors $x_1, x_2, \cdots, x_m$

$$\mu_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \cdots + \beta_m x_{mi} \qquad \text{Eq 1.2}$$

where $\beta_0, \beta_1, \cdots, \beta_m$ are regression coefficients to be estimated. In an additive model, the expected value is modeled as

$$\mu_i = \beta_0 + f_1(x_{1i}) + f_2(x_{2i}) + \cdots + f_m(x_{mi}) \qquad \text{Eq 1.3}$$

where $f_1, f_2, \cdots, f_m$ are arbitrary smooth functions to be estimated. That is, where the standard regression model estimates the regression coefficients $\beta_0, \beta_1, \cdots, \beta_m$, and the fitted model is summarized by tables of coefficients, an additive model estimates smooth functions $f_1, f_2, \cdots, f_m$, and the fitted model is presented as plots of the smooth $f_1, f_2, \cdots, f_m$. The advantage of an additive model is that it can model very complex functional relationships between single predictors and the response. The disadvantage of an additive model is that it is difficult to model interactions between predictors. Interactions between predictors can be modeled to a limited degree by incorporating smooth terms in multiple predictors $f_{ab}(x_{ai}, x_{bi})$ (Wood 2006). Unfortunately, such terms need substantially more data to be reliably estimated.

To determine the relationship between growth and sea surface temperature (SST), additive models were fitted to log mantle weight-at-catch data, as a function of the individual's age-at-catch, and mean SST that the individual experienced in the first 30 days post-hatching i.e. log Mwt = $f_{(\text{age})} + f_{(\text{SST})}$. To determine the relationship between growth and sea surface colour (SSC; a measure of water productivity) additive models were fitted to log mantle weight-at-catch data, as a function of the individual's age-at-catch, and SSC that the individual experienced in the first 30 days post-hatching i.e. log Mwt = $f_{(\text{age})} + f_{(\text{SSC})}$. Weekly mean SST was calculated for GAB (33°15'0.00"S, 130° 1'48.00"E) and Victoria (38°42'0.00"S, 141°18'0.00"E) over the period that *N. gouldi* hatched (Figure 1-2). Temperature data was acquired from the NOAA-CIRES Climate Diagnostic Centre (http://www.cdc.noaa.gov/) (Reynolds *et al.* 2002). The productivity data were derived from the SeaWiFS (Sea-Viewing Wide Field-of-view Sensor) 8 d 9 km chlorophyll *a* product, and average values for each 8 d period for a 1° box at each location were obtained using customized Matlab programs. Productivity estimates measured by SeaWiFS provided quantitative data on the optical properties of the ocean between the hatch period January 2007 and December 2008 (Figure 1-3). This data was used to calculated the standing stock of chlorophyll *a* which was considered to be a measure of phytoplankton (Joint & Groom 2000). It was assumed that the measured standing stock (colour) is proportional to productivity; consequently, sea surface colour (SSC) was used as a proxy for chlorophyll *a* productivity (Joint & Groom 2000). SeaWiFS measures the ocean colour from the upper 20 – 30 m of the ocean surface; however, SSC may not represent actual concentration. Factors that may influence SSC including depth, turbidity, dissolved organic matter, and suspended sediments particularly at coastal regions (Joint & Groom 2000). This study assumed that the SSC data was correlated with productivity estimates calculated at their respective locations (Jackson *et al.* 2005).

Sea surface temperature and SSC time series were interpolated to fill in any missing values to produce daily time series for each of Victoria and the GAB. Additive models were fitted to the individuals from each of the four combinations of location and sex. However, additive models fitted to model log mantle weight-at-catch, in terms of the individual's age-at-catch and SSC was too difficult to interpret biologically and provided little explanation of the variation in growth of *N. gouldi*. Consequently modelling *N. gouldi* growth using mantle weight-at-age and SSC was excluded from further analyses.

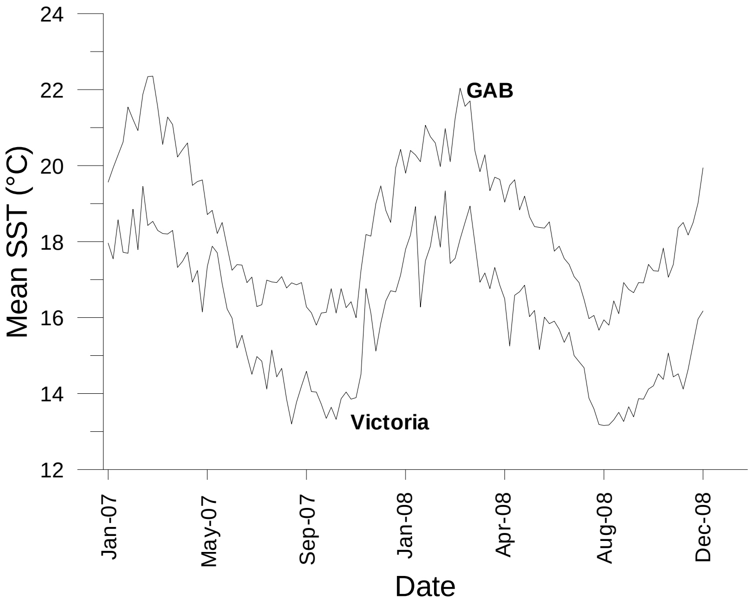

Figure 1-2 Weekly mean sea surface temperature (SST) calculated from 1-degree boxes for Great Australian Bight (33°15'0.00"S, 130° 1'48.00"E) and Victoria (38°42'0.00"S, 141°18'0.00"E) over the hatch duration.

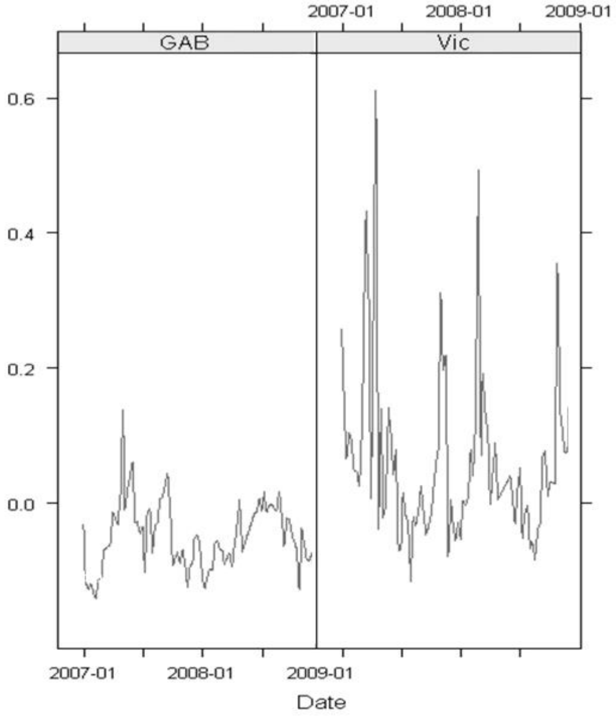

Figure 1-3 Weekly mean sea surface colour (productivity) anomaly calculated from 1-degree boxes for Great Australian Bight (33°15'0.00"S, 130° 1'48.00"E) and Victoria (38°42'0.00"S, 141°18'0.00"E) over the hatch duration.

5. RESULTS

Nototodarus gouldi collected from March 2007 – June 2009 in Victoria and the GAB had variable mantle weight frequency distributions over approximately monthly and bimonthly time scales (Figure 1-4). The size range of *N. gouldi* collected was 25 – 525 g and 50 – 400 g in the GAB and Victoria respectively. Of the 1142 and 2467 *N. gouldi* collected in the GAB and Victoria, 993 and 2177 age estimates were determined and used in calculating hatch date. Failure in obtaining age estimates from statoliths was attributed to over grinding, fractures and poor increment clarity. The minimum age of *N. gouldi* collected in the GAB was 140 d and 145 d for females and males respectively; whereas in Victoria, the minimum age was 145 d and 166 d for females and males respectively.

Comparing power law (log-Mwt against log-age) and exponential (log-Mwt against age) growth models showed little difference in their adequacy to fit the data. However, the exponential model did not fit as well as the power law model as the youngest and oldest individuals lie below the fitted line, suggesting there is some residual curvature that is unexplained by the model. This was confirmed with the AIC favoring the power law model (AIC = 793.9) over the exponential model (AIC = 855.3). Although both models yield near identical results, growth analyses based on the power law model were presented.

For both males and females the growth rates of *N. gouldi* that hatched from spring 2007 – spring 2008 differed between the GAB and Victoria but the nature of the difference depended on the hatch season (Males $F_{location*season\ year}$= 10.59, df 4,1810, p<0.001; Females $F_{location*season\ year}$= 7.02, df 4,1242, p<0.001). Female *N. gouldi* caught in the Victorian fishery grew faster than GAB squid for all hatch seasons except autumn 2008 (Figure 1-5). For example, during spring 2008, fastest growth rate was observed in *N. gouldi* from Victoria (4.76 g day^{-1} ±0.38 se) compared with *N. gouldi* from the GAB (1.93 g day^{-1} ±0.51se). Similarly, males caught in Victoria grew faster except during spring 2007 and autumn 2008 hatch seasons (Figure 1-5). Female *N. gouldi* caught in Victoria that hatched during autumn 2008 had the slowest growth rate; however, this same cohort of animal caught in the GAB grew the fastest (Figure 1-5).

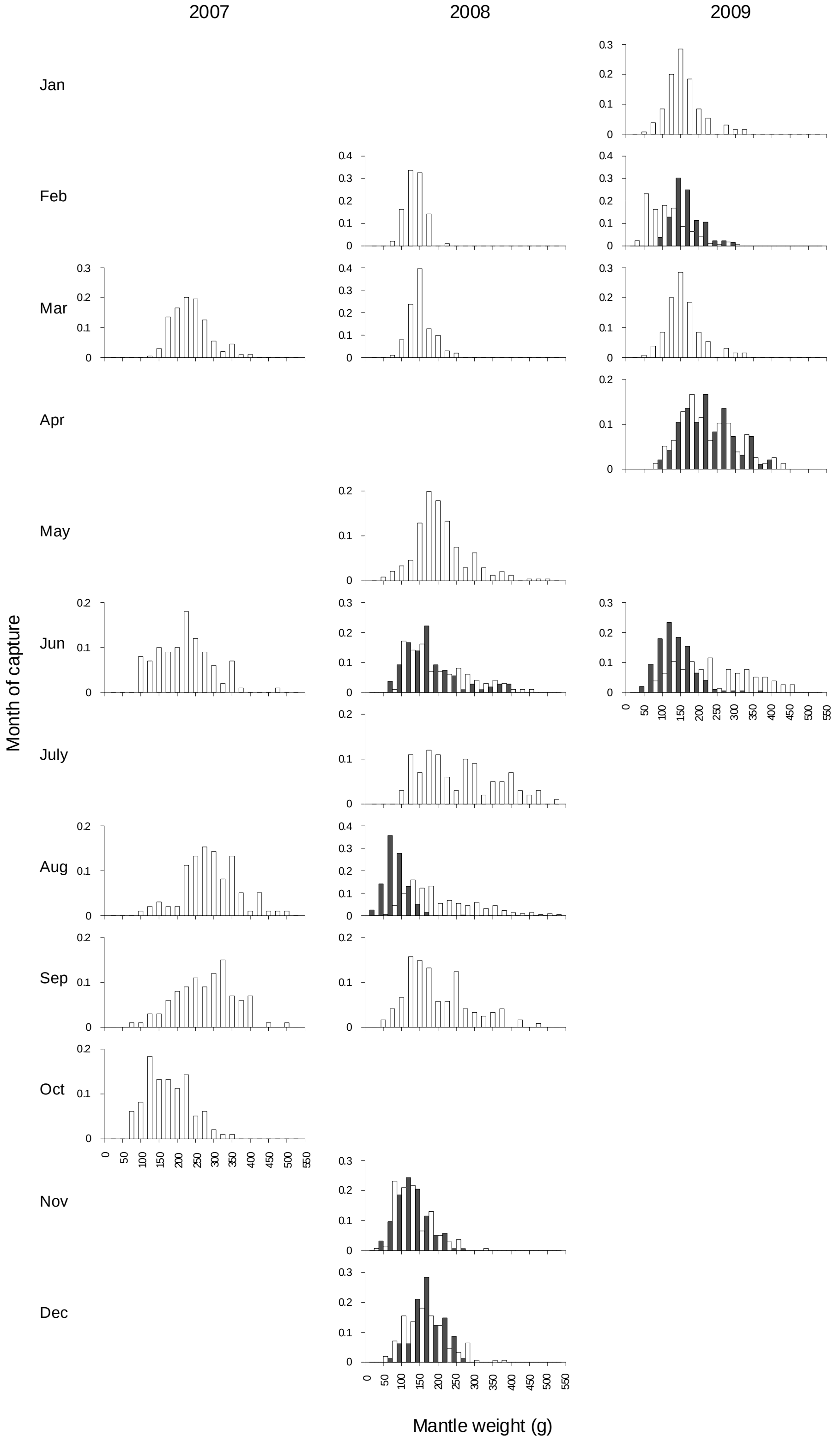

Figure 1-4 Relative frequency distribution of N. gouldi mantle weight (g) at each month and year of collections between March 2007 and June 2009; GAB (solid bars) and Victoria (open bars).

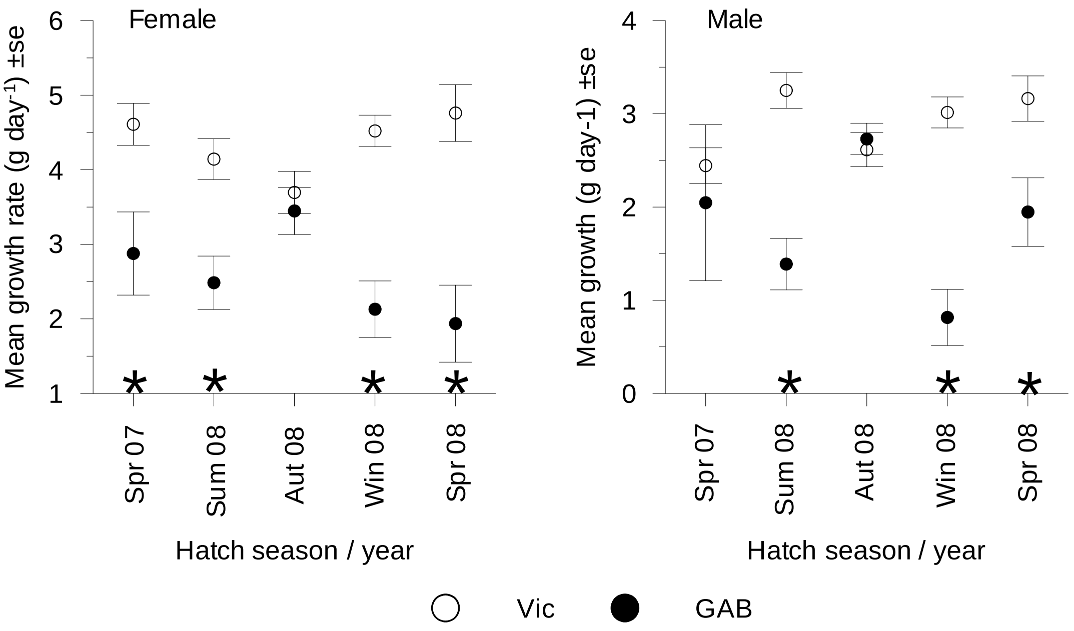

Figure 1-5 Growth rates of female (left) and male (right) N. gouldi that hatched in the GAB and Victoria. Asterisk on x-axis indicates where significant difference in growth rate between fisheries occurred for each season and year.

Temporal variation in growth rates of female *N. gouldi* from Victoria was evident among hatch years (F_{year}= 12.37, df 2,827, p<0.001) and hatch seasons (F_{season}= 10.84, df 3,827, p<0.001); and seasonal patterns were the same in each year ($F_{year*season}$ = 1.89, df 4, 827, p=0.109). Female squid caught in Victoria that hatched in spring grew fastest compared with all other hatch seasons (Figure 1-6). Male *N. gouldi* caught in Victoria had a similar pattern of differences in growth rates with differences among hatch year (F_{year}= 12.37, df 2,827, p<0.001) and hatch season (F_{season}= 10.84, df 3,827, p<0.001) being independent of one another ($F_{year*season}$ = 1.31, df 4, 1263, p=0.264). Growth rates of Victorian male *N. gouldi* hatching in autumn were significantly slower than *N. gouldi* hatching in spring by 0.64 g day^{-1}. Trends in seasonal growth rates were similar for males and females caught in Victoria with increasing growth rates from autumn to spring, and for each season male *N. gouldi* had slower growth rates than females (Figure 1-6). Comparing growth rates among hatch year for female *N. gouldi* caught in Victoria show significant differences between years 2006 – 2008; with males hatching in 2008 having greatest growth (3.03 g day^{-1} ±0.09 se) compared with males hatching in 2006 and 2007 (Figure 1-7).

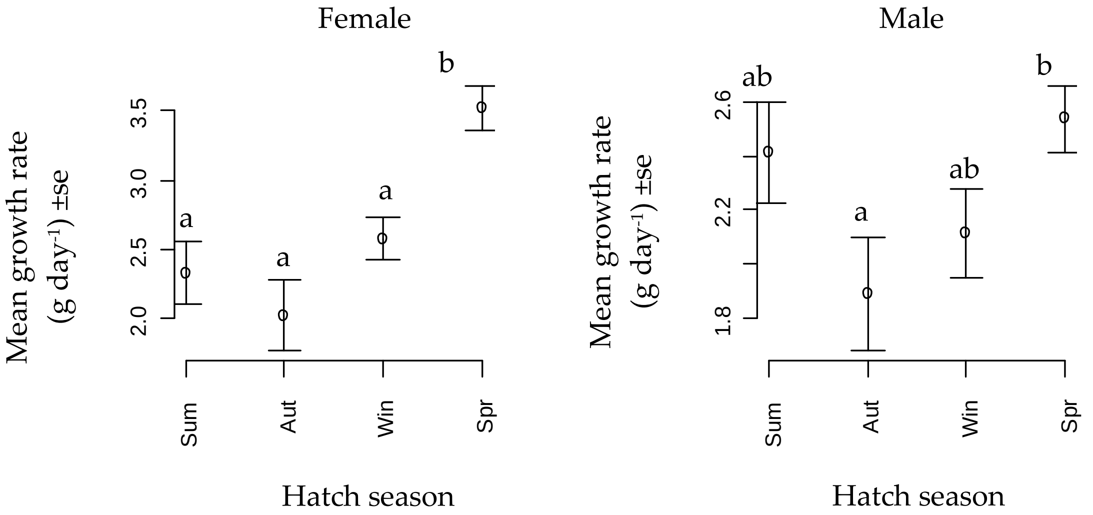

Figure 1-6 Growth rate by hatch season and sex for female (left) and male (right) N. gouldi collected in Victoria. Like letters indicate similar means within each sex.

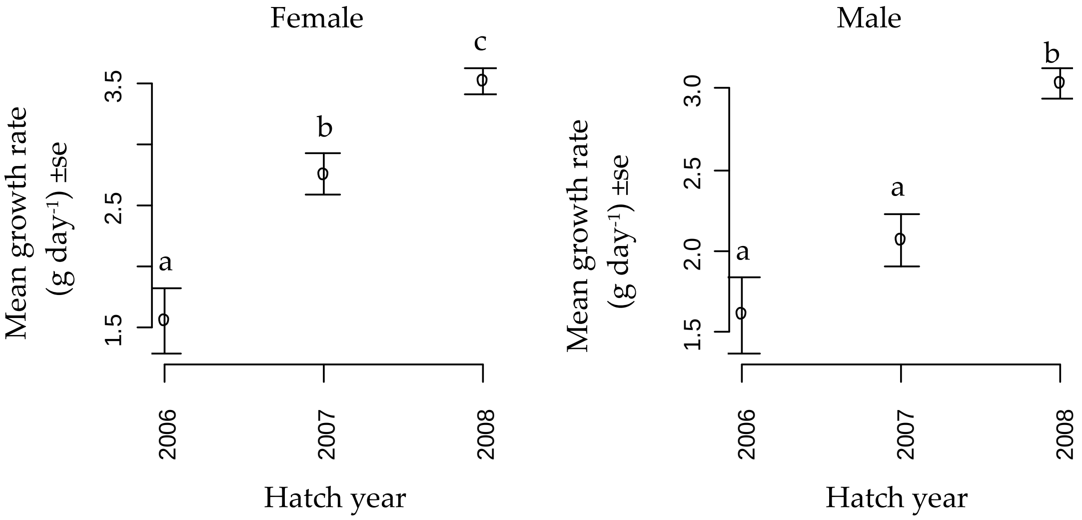

Figure 1-7 Growth rate by hatch year and sex for female (left) and male (right) N. gouldi collected in Victoria. Like letters indicate similar means within each sex.

For *N. gouldi* collected in the GAB, differences in growth rates among hatch season were found for females ($F_{\text{hatch season}}$= 4.35, df 5,412, p<0.001) and males ($F_{\text{hatch season}}$= 9.66, df 5,44, p<0.001). During summer 2008/09, growth rate variation was greatest for both male and female *N. gouldi* caught in the GAB (Figure 1-8)

Figure 1-8; and likely attributed to the relatively low number of female (n = 12) and male (n = 23) replicates; and were consequently removed from growth analyses. Comparing growth rates across five hatch seasons for *N. gouldi* collected in the GAB suggest that females hatching in autumn 2008 grew significantly faster than females that hatched in winter and spring 2008 by 1.42 g d^{-1} and 1.78 g d^{-1} respectively (Figure 1-8). Growth in males caught in the GAB show a similar trend to females with *N. gouldi* hatching in autumn 2008 growing significantly faster to those hatching in summer 2007/08 and winter 2008 by 1.34 g d^{-1} and 1.91 g d^{-1} respectively (Figure 1-8). Fastest growth rates from *N. gouldi* collected in the GAB were found when squid hatched during autumn for females (3.1 g d^{-1} ±0.24 se) and males (2.7 g d^{-1} ±0.15 se). No seasonal differences in growth rate were evident for either sex.

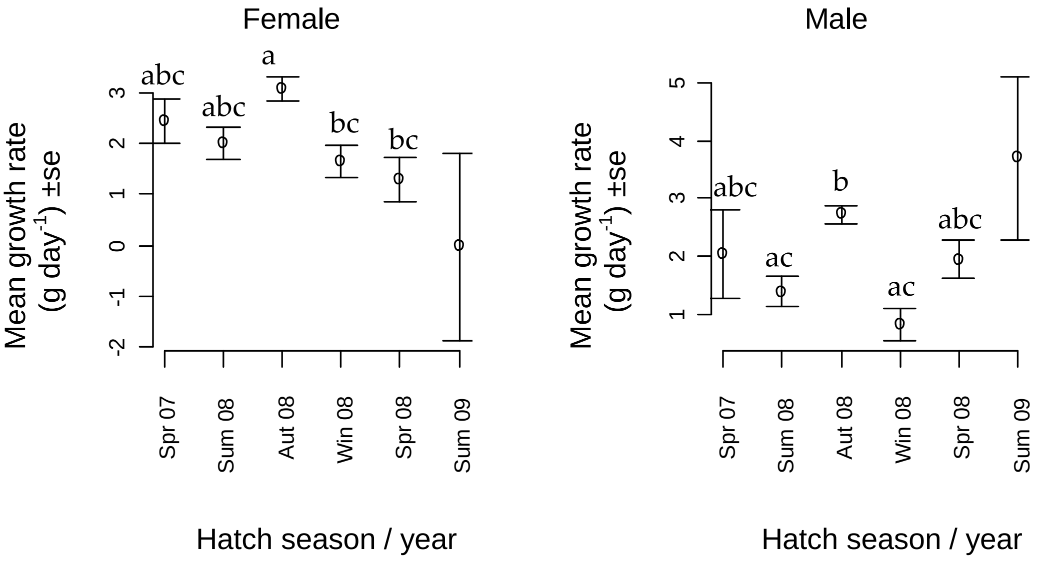

Figure 1-8 Growth rate by hatch year and season for female (left) and male (right) *N. gouldi* collected in the GAB. Like letters indicate similar means within each sex. Pairwise comparisons were not made for *N. gouldi* hatching in summer 2009 due to low numbers.

5.1. Maturity and reproductive condition

The relative number of mature male *N. gouldi* caught in the GAB was dependent upon their season of capture (χ^2=17.15, df 3, p<0.001), with fewer than expected immature squid caught during summer and autumn (Figure 1-9) and more than expected in winter. Similarly, the proportion of mature females caught in the GAB was dependent upon their season of capture (χ^2=33.79, df 3, p<0.001) with relatively more immature squid found during summer and fewer in autumn. For *N. gouldi* collected in Victoria maturity stages represented in the catch was more complex as most seasons showed more or less than expected numbers of mature and immature compared with squid caught in the GAB (Figure 1-9). For *N. gouldi* caught in the GAB, the relative number of mature squid was dependent on season of capture for females (χ^2=50.32, df 3, p<0.001) and males (χ^2=64.5, df 3, p<0.001). For Victorian males, fewer mature and more immature *N. gouldi* were found during summer; whereas, fewer immature *N. gouldi* were found in autumn and winter. For females caught in Victoria, a greater number of immature and fewer mature squid were found during summer; whereas during spring this observation was reversed with more mature and fewer immature squid found (Figure 1-9).

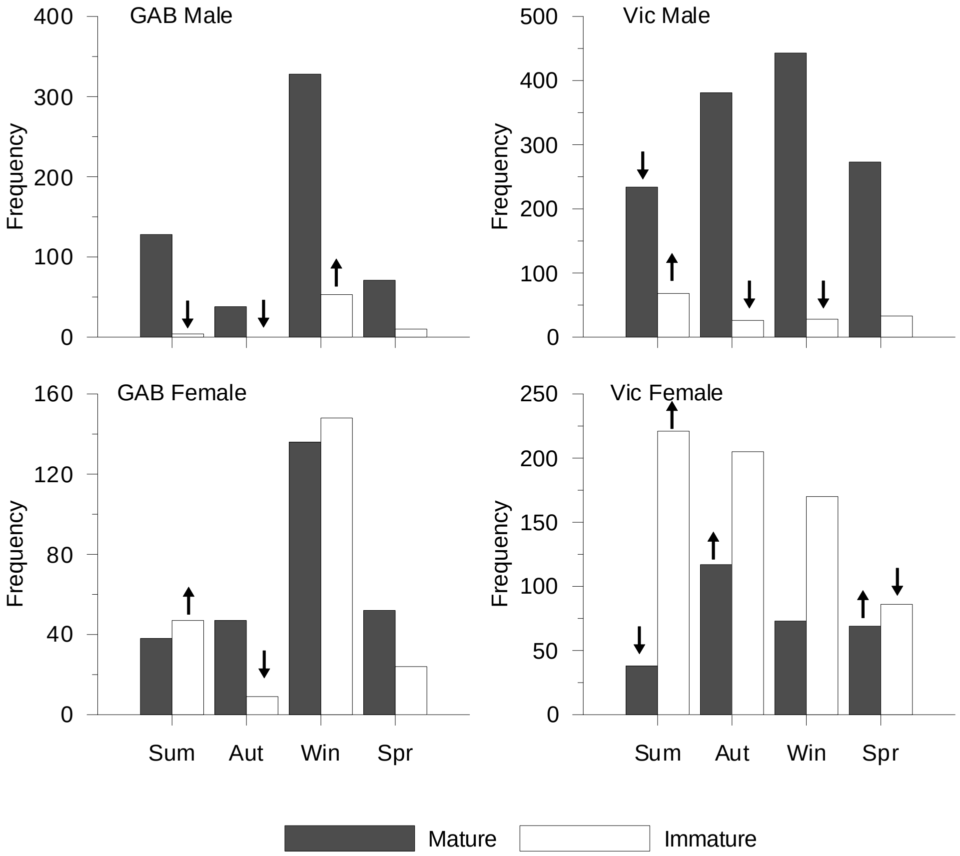

Figure 1-9 Number of mature and immature N. gouldi represented in the catch by season, location and sex. Arrows indicate the direction of differences between observed and expected frequencies based on the $\chi 2$ test.

Independent of season of capture, on average, reproductively mature individuals were older but the exact pattern of differences was dependent on sex and location ($F_{maturity*sex*location}$ = 2.64, df 4,3136, p=0.032). For both male and female squid in each maturity stage, Victorian squid were on average older that individuals from the GAB (Figure 1-10).

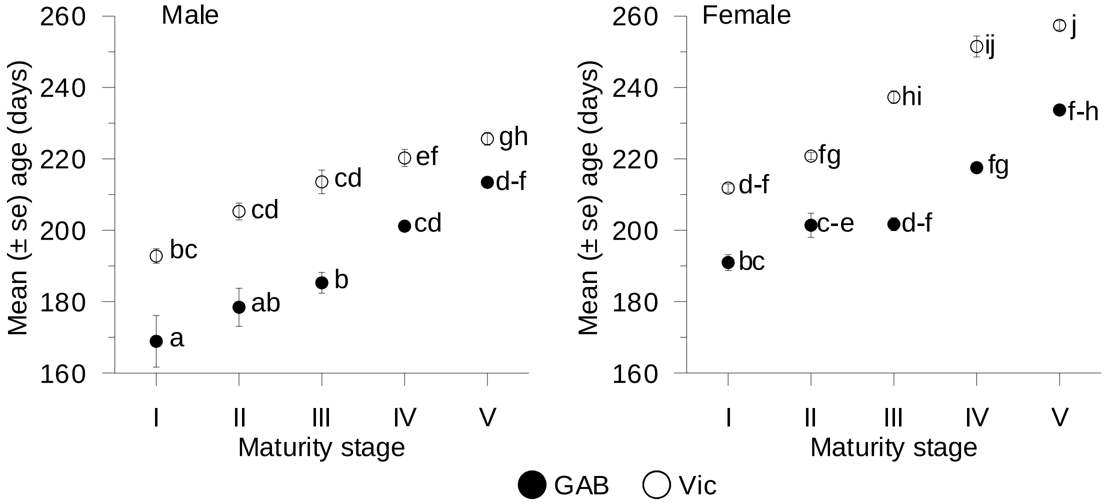

Figure 1-10 Mean daily age at each maturity stage for males (left) and females (right) N. gouldi collected in the GAB and Victoria. Like letters indicate similar means.

The average reproductive condition of female *N. gouldi* significantly differed among each location/season/year combination ($F_{location*season*year}$= 22.88, df 7,336, p<0.001). Female *N. gouldi* collected during winter 2008 in the GAB were in better reproductive condition than *N. gouldi* collected in Victoria during the same period (Figure 1-11). However, this spatial pattern changed in summer 2009, with females caught in Victoria in better reproductive condition than females caught in the GAB (Figure 1-11). For male *N. gouldi*, reproductive condition differed among location, season and year combinations ($F_{location*season*year}$ = 22.82, df 9,1264, p<0.001). *N. gouldi* collected in Victoria during summer and autumn 2009 were in better reproductive condition to those caught in the GAB (Figure 1-12).

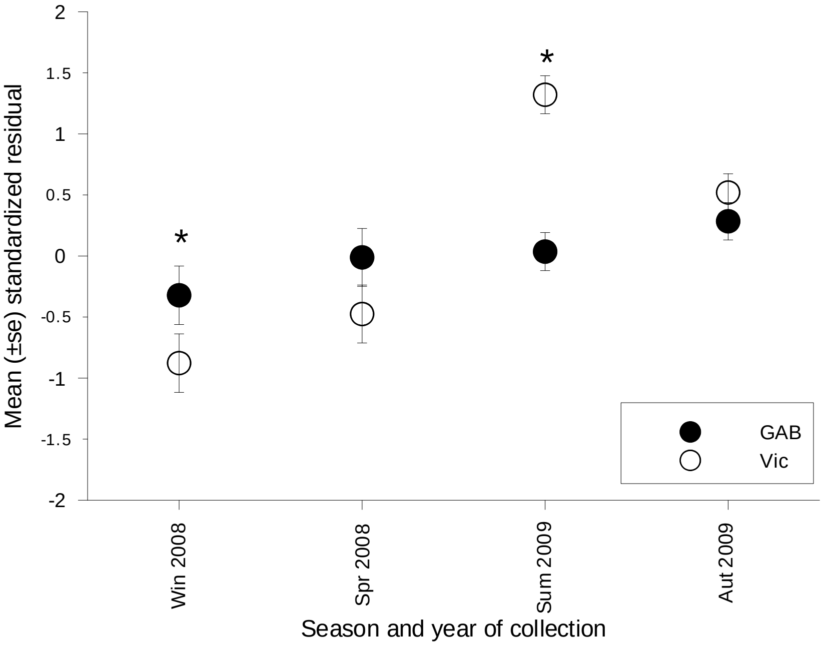

Figure 1-11 Spatial variation in female reproductive condition by season and year of collection. Mean standardized residuals of the relationship between dorsal mantle weight and ovary weight presented. Asterisk denotes significant difference between locations for a given season of collection.

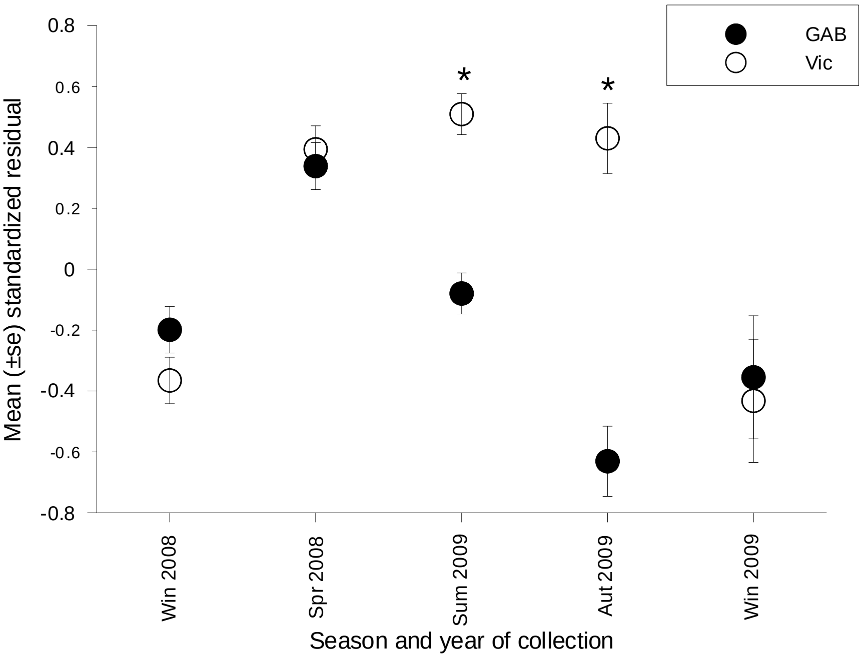

Figure 1-12 Spatial variation in male reproductive condition by season and year of collection. Mean standarised residuals of the relationship between dorsal mantle weight and testis weight presented. Asterisk denotes significant difference between locations for a given season of collection.

Reproductive condition of *N. gouldi* caught in the GAB (Figure 1-13) varied temporally for males ($F_{season\ year}$=33.57, df 9,1291, p<0.001) and females ($F_{season\ year}$=12.14, df 9,275, p<0.001). Male reproductive condition increased from winter – spring 2008, and then decreased until autumn 2007; whereas a trend in reproductive condition for females was less apparent with winter 2008 and autumn 2009 showing poorest and greatest condition respectively. For *N. gouldi* caught in Victoria, reproductive condition varied temporally for males ($F_{season\ year}$=33.57, df 9,1291, p<0.001) and females ($F_{season\ year}$=12.14, df 9,275, p<0.001). Males showed a cyclic pattern with better reproductive condition during summer and poorer condition during winter collection periods (Figure 1-13). For females collected in Victoria, temporal trends in reproductive condition was not as evident; however, increased from spring 2008 – summer 2009 before decreasing in winter 2009.

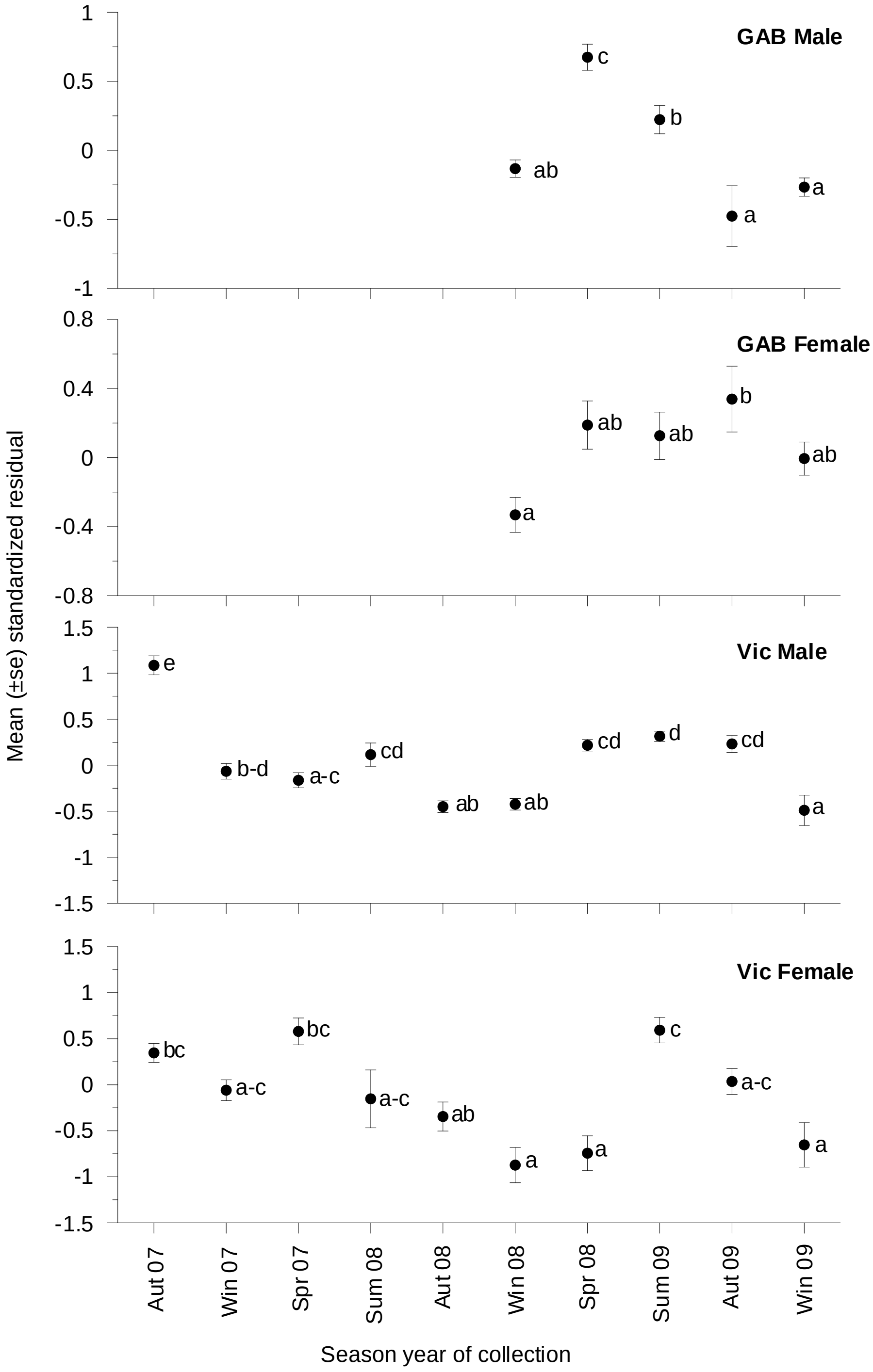

Figure 1-13 Temporal variation in reproductive condition by season of collection for each sex and location. Mean standardized residuals of the relationship between dorsal mantle weight and gonad weight presented. Like letters indicate similar mean residuals for each sex and location combination.

5.2. Modelling of growth with sea surface temperature

Fifty-four percent of the variation in weight-at-catch of male *N. gouldi* caught in Victoria was explained by SST (F=7.8, df 7.9, p<0.001) and age (F=196.6, df 4.7, p<0.001). The response of weigh-at-catch with SST (that has been adjusted for the effects of age) suggested by additive models was not consistent when SST increased from 13 – 19°C. For both males and females caught in Victoria, weight-at-catch was heaviest for those individuals that hatched during warmer SSTs. Male *N. gouldi* caught in Victoria that experienced SST of 16 – 17°C during the first 30 days post-hatch resulted in a lighter weight-at-catch compared with *N. gouldi* hatchlings experiencing approximately either 15°C or 18°C post-hatch. Sixty-five percent of the variation in weight-at-catch of female *N. gouldi* caught in Victoria was explained by SST (F=4.4, df 6.5, p<0.001) and age (F=158.3, df 6.7, p<0.001), when fitted to log mantle weight-at-catch. Adjusting for the effects of age, female hatchlings that experienced SST 15 – 17°C post-hatch had the lightest weight-at-catch; however, greatest weight-at-catch was found when hatchlings experience water temperatures of >17°C (Figure 1-14).

For male *N. gouldi* caught in the GAB, 54% of the variation in weight-at-catch was explained by SST (F=29.1, df 4.9, p<0.001) and age (F=38.2, df 5.7, p<0.001) when fitted to log mantle weight-at-catch. Similarly, for female *N. gouldi* caught in the GAB, 54% of the variation in weight-at-catch was explained by SST (F=6.8, df 5.8, p<0.001) and age (F=24.8, df 5.8, p<0.001) when fitted to log mantle weight-at-catch. The relationship between mantle weight-at-catch and SST (that has been adjusted for the effects of age) was more consistent for *N. gouldi* caught in the GAB compared with *N. gouldi* caught in Victoria. Male and female *N. gouldi* caught in the GAB experiencing warmer SSTs during the first 30-day post were lighter on the day of capture (Figure 1-15).

Individual functions of SST and age fitted to log mantle weight-at-catch were consistent with using combined functions of SST and age used to analyse the interactive effects. For *N. gouldi* from Victoria, weight-at-catch increased when hatchlings experienced increasing SST; however, the effects of age were more influential in predicting mantle weight-at-catch when individuals hatched during cooler periods. For Victorian caught *N. gouldi* that hatched during warmer sea surface temperatures, age was not strongly related to weight-at-catch. For *N. gouldi* caught in the GAB, the explanatory capacity of SST and age-at-catch for mantle weight-at-catch differed for males and females (Figure 1-16). Male *N. gouldi* caught in the GAB that hatched during cooler temperatures had greatest weight-at-catch, with warmer SSTs experienced resulting in lighter weight-at-catch. Like males from the GAB, female weight-at-catch was smaller when *N. gouldi* experienced warmer SSTs post hatch; however, for female *N. gouldi*, SST had less capacity to predict weight-at-catch compared to their age-at-catch.

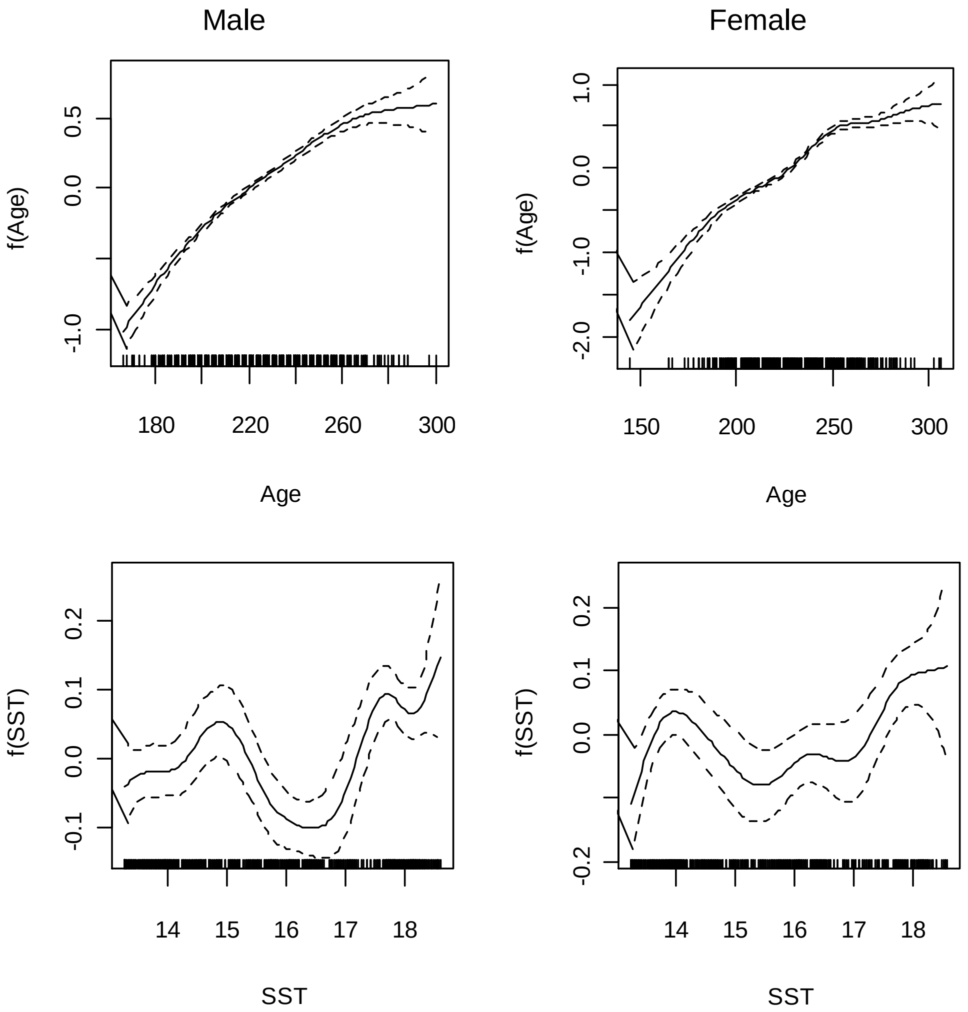

Figure 1-14 Smoothed functions of sea surface temperature (f(SST) expressed in °C) and age (f(age) expressed in days) for male and female N. gouldi Victoria as estimated by additive models. Dashed lines represent 95% confidence intervals.

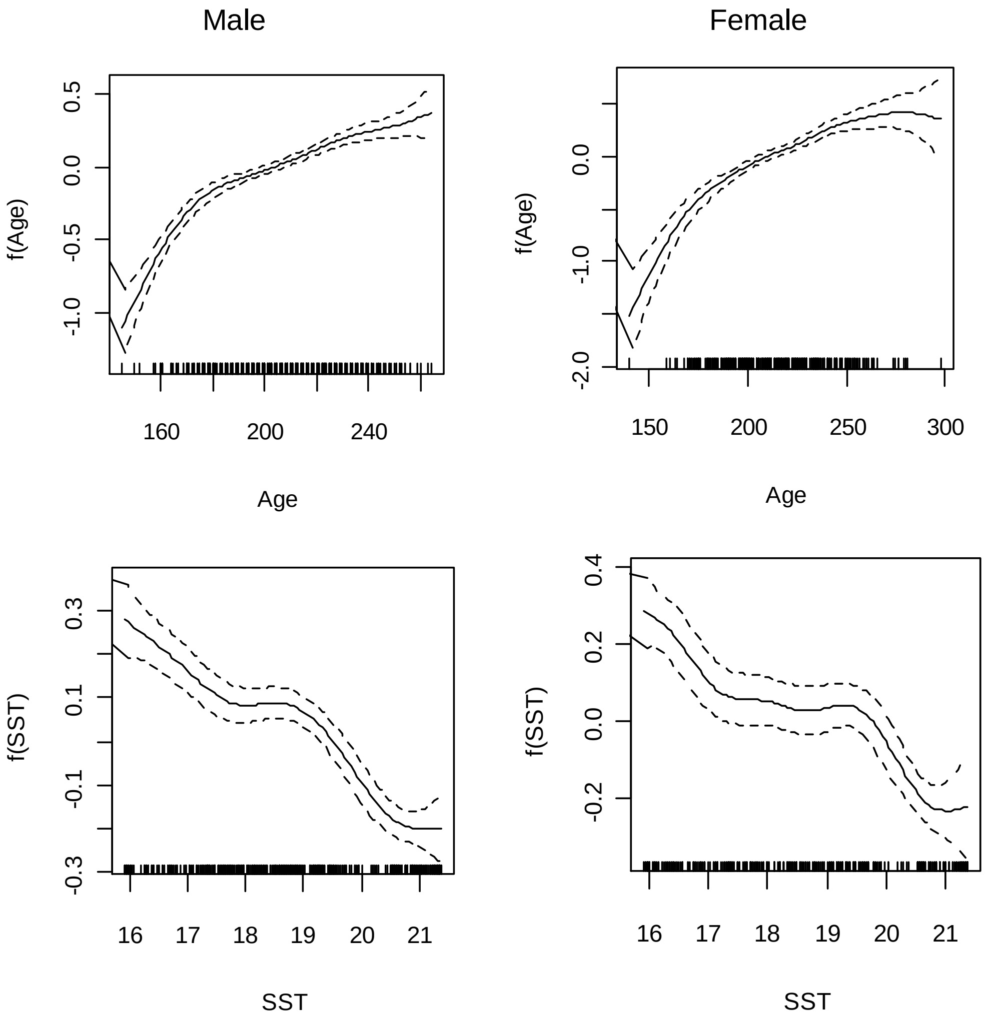

Figure 1-15 Smoothed functions of sea surface temperature ($f_{(SST)}$ expressed in °C) and age ($f_{(age)}$ expressed in days) for male and female *N. gouldi* from the GAB as estimate by additive models. Dashed lines represent 95% confidence intervals.

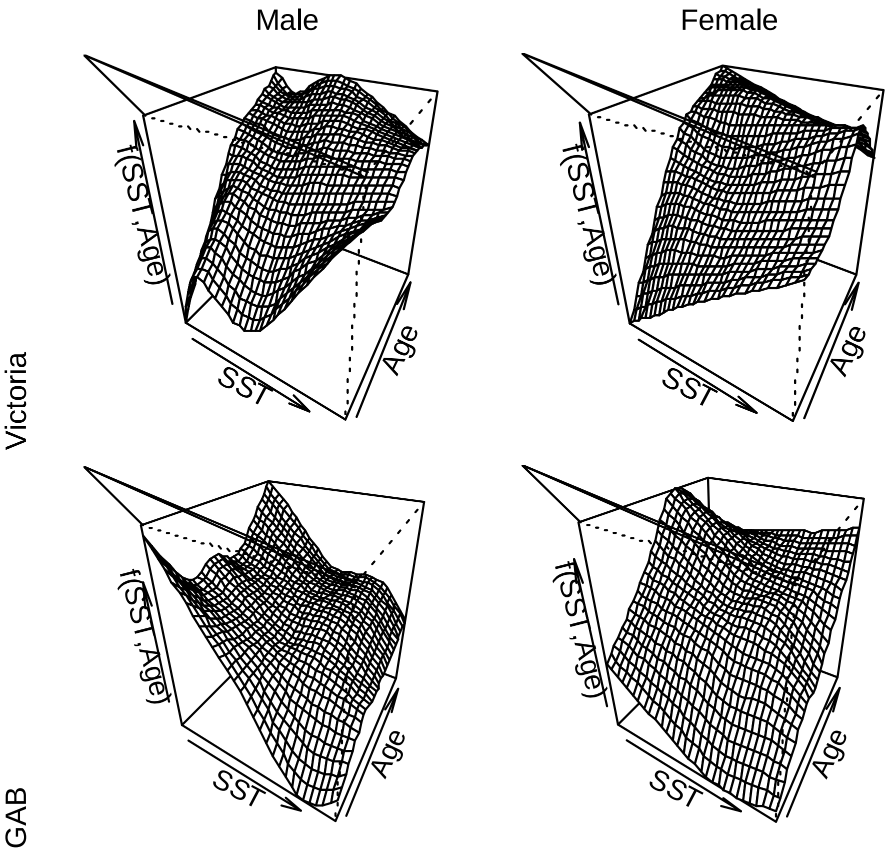

Figure 1-16 Transformation of age and sea surface temperature estimated by additive models fitted to *N. gouldi* caught in Victoria and the GAB for both sexes.

6. DISCUSSION

Spatial and temporal variability in size-at-age, maturity and size-at-capture was found for *N. gouldi* collected in southern Australia from 2007 – 2009, and was explained by environmental conditions experienced early in life. Lifetime adult growth rates were fastest for male and female *N. gouldi* in cooler waters off Victoria which resulted in larger body sizes, compared with populations in warmer waters of the GAB. Environmental conditions experienced during early life are likely to have greater influence on growth compared with conditions experienced during adult stages. In particular, hatchlings that experienced warmer SST were heavier as adults when caught in Victoria than the adults caught in the GAB. Mature *N. gouldi* caught in Victoria were older than *N. gouldi* caught in the GAB, as a result the relative proportion of mature squid caught in each location depended on time of year. However, seasonal trends in reproductive condition were similar in both locations with individuals in poorest reproductive condition present during winter. Due to the spatial and temporal plasticity in biological characteristics, modelling and forecasting the affects of environmental conditions on *N. gouldi* population dynamics is complex. As the influence of variable climatic conditions on ecosystems and resources is of high priority (Hays *et al.* 2005, Harley *et al.* 2006), current research has provided vital information in the study of growth and population dynamics of cephalopods and other short-lived marine species in relation to environmental conditions.

When allowing for the effect of age, squid caught in the GAB that experienced warmer water during juvenile stages are likely to have contributed to a lighter mantle weight-at-catch compared to juveniles experiencing cooler temperatures. However, in Victoria the influence of SST at hatching on the mantle weight-at-catch was different compared to the GAB. Although *N. gouldi* hatchlings that experienced warmest SSTs resulted in greatest mantle weight-at-catch, lightest weight-at-catch was observed when squid hatch during mid-range (e.g. 16 – 17°C) sea surface temperatures. *Nototodarus gouldi* hatching in mid-range temperatures hatched during autumn and spring; therefore the effect of increasing or decreasing SST on juvenile growth is unlikely to have contributed to lower weight-at-catch. Therefore it is hypothesized that other factors are contributing to low weight-at-catch during autumn and spring hatch periods. Independent of the affect SST has on weight-at-catch, *N. gouldi* require food to increase mass; therefore the quantity of food for juveniles may be not be sufficient during autumn and spring to produce heavier individuals. Similarly, the cost of increasing reproductive condition and migration may be affecting weight-at-catch (Chapter 5; McGrath Steer & Jackson 2004).

Although differences in water temperature during juvenile stages may explain differences in *N. gouldi* size in the GAB and Victoria, food availability may have more influence on adult weight-at-catch. *Nototodarus gouldi* prey on teleosts, crustaceans, and other cephalopods (Appendix 1; Machida 1983, O'Sullivan & Cullen 1983b, Uozumi 1998), and although the interactive effect of temperature coupled with food availability is unknown for *N. gouldi*, food availability does effect growth rates of many species including *Sepioteuthis lessoniana* and *Illex illecebrosus* (Hirtle & O'Dor 1981, Jackson & Moltschaniwskyj 2001a). Although Chl *a* is a good proxy for food availability, time is required for blooms to propagate resulting in increased growth and biomass throughout the food web. The effect of food availability and SST may provide greater insight in explaining differences in *N. gouldi* growth compared to SSC and SST alone.

Results of modelling the effect of SSC and age on weight-at-catch was too difficult to interpret biologically and provided little in explaining variation. However, other factors not analysed during this research such as food availability and density-dependency effects, may be influencing trends in growth. Embryo, paralarval, and juvenile growth periods are thought to be the most influential in determining adult size of squid (Villanueva 2000, Steer *et al.* 2003, Pecl *et al.* 2004b, Pecl & Jackson 2007). However, this relationship is not straightforward; warmer water temperatures accelerate development times of embryos but not growth, therefore faster development times gained from warmer water produces smaller hatchings (Villanueva 2000, Vidal *et al.* 2002, Steer *et al.* 2003, Pecl & Jackson 2007). Under experimental conditions, post hatchlings incubated in warm water grow faster, such that when 90 days old they are threefold heavier than hatchlings grown in cooler water (Forsythe & Hanlon 1989). In the present study, growth of *N. gouldi* was calculated based on the relationship between mantle weight and age-at-capture (average lifetime growth), therefore calculating instantaneous growth at different stages of life was not possible as the youngest and smallest *N. gouldi* collected was 140 d and 27 g Mwt respectively. Given the sensitivity of growth rates during the pre-adult phase, it is likely that the ambient water temperatures *N. gouldi* experience as adults will be less important. Therefore it is becoming increasingly critical that cephalopod fisheries scientists need to obtain better estimates of early life history parameters of individuals.

Growth rate and size of *N. gouldi* caught in the GAB and Victoria appeared to be influenced by latitudinal differences; individuals that experienced warmer water temperatures within 30 d post hatch in the GAB were smaller for their age and achieved smaller adult size. Latitudinal differences in size of individuals in populations of *Illex illecebrocus*, *Todarodes sagittatus*, *Dosidicus gigas*, and *Nototodarus sloanii* is likely a function of relative oxygen uptake and gill surface area (Rosenberg *et al.* 1981, Ehrhardt *et al.* 1983, Roper *et al.* 1984, Pauly 1997, 1998, Arkhipkin *et al.* 1999). Such latitudinal patterns are not restricted to ommastrephids with loliginids like *Loliolus noctiluca*, *Loligo opalescens*, *Sepioteuthis lessoniana* and *S. australis* showing similar growth and size variation (Jackson & Moltschaniwskyj 2001b, Pecl 2001, Jackson & Domeier 2003b, Reiss *et al.* 2005). As such, variation in growth characteristics in relation to environmental and oceanographic variables is likely to affect most major cephalopod fisheries. *Nototodarus gouldi* collected in 2008 – 2009 in the GAB were smaller compared with Victorian caught squid. This data was similar to that found for *N. gouldi* caught in lower latitudes (Ulladulla, New South Wales) of Australia during 2001 compared to those from higher latitudes regions like Victoria and Tasmania (Jackson *et al.* 2003).

Greater proportions of mature male *N. gouldi* in the population are likely to be a function of the selective nature of fishing gear. Trawl gear only catches individuals above a certain size (i.e. > 130 mm DML); hence the dominance of mature males is most probably due to males maturing at a size smaller. Therefore, smaller and immature males may be present in the population, but were not captured by trawls. Consequently, it is not possible to make conclusions about male maturity stage with respect to seasonal variability, except that mature males are present all year. Females mature at a larger size; therefore, both immature and mature individuals were caught by the trawl. As such, the number of mature females caught was dependent on season of capture with relatively more immature females caught during summer compared to other seasons. Such seasonal variability in maturity is also evident and advantageous in species such as *Sepioteuthis australis*. A variation in the number of mature and immature animals in a population provides a way to distribute mortality risk through time. This information provides evidence for managers to provide protection to spawning biomass through the introduction of seasonal closures during peak spawning activity (O'Dor 1998a, Moltschaniwskyj *et* *al.* 2002, Moltschaniwskyj & Steer 2004). Similarly, implementing size selectivity gear restrictions can be used to maintain the number of mature animals in a population.

Mean age at each maturity stage is greater for females compared with males; and possibly attributed to maximising reproductive success and oocyte production (Mangold 1983, McGrath & Jackson 2002). Sexual dimorphism of mean age at each maturity stage is temporally and spatially consistent with findings from other *N. gouldi* populations; however, individual and population reproductive strategies can fluctuate in response to temperature, food availability and length of day (Boyle 1987, Jackson 1993, Uozumi 1998, Arkhipkin *et al.* 2000a, Jackson *et al.* 2003). For both females and males, the mean age at first maturity (stage IV) was older in squid from Victoria, which is likely to be in response to their different environments (Jackson *et al.* 1997). In the present study, males and females from Victoria and females from the GAB had similar reproductive trends with individuals in better reproductive condition found during warmer seasons. Similar characteristics are found in other species including *Sepioteuthis australis* and *S. lessoniana*, where reproductive index was approximately doubled in squid caught in summer compared with winter (Pecl 2001, Jackson & Moltschaniwskyj 2002). Such variability in maturity and reproductive condition indicates forecasting intra-annual trends in reproductive strategies difficult; however, greater consistency in inter-annual trends is useful for future assessment of *N. gouldi* populations.

Mean sea surface temperature is expected to rise over the next century causing an increase in thermal stratification and subsequent decrease in ocean productivity that is likely to impact population dynamics of squid (Seibel & Fabry 2003, Pecl & Jackson 2007). Aside from food availability, *N. gouldi* hatchlings that experience increased temperature due to extremes in climate variability may experience greater growth in Victoria but reduced growth in the GAB. As the effects of climate change on species population dynamics and ecosystems are likely to be difficult to model, forecasting growth and biomass of cephalopods will be complicated due to their plasticity in biological characteristics. Grainger (1992) estimated that the effect of increasing temperate is likely to be most evident on marine populations, such as squid, and intertidal animals. In a global warming scenario, *Todarodes pacificus* gets smaller with increased water temperature as growth optimums are exceeded (Kishi *et al.* 2009). Such response could occur in *N. gouldi* populations as temperatures may limit growth due to competition for food and/or oxygen (Challier *et al.* 2005).

As life history characteristics of *N. gouldi*, including reproductive strategies and rates of growth, are influenced by environmental factors, temporal and spatial variation in their population structure is likely to continue. Although seasonal differences in growth were found in this study, annual differences could not be explained. Consequently, for effective assessment of cephalopod fisheries, greatest benefit in modelling population dynamics is achieved when life history and population characteristics are determined annually. However, as it is difficult to predict future growth variability using SST alone, analysis of other factors contributing to the dynamic population structure such as food availability may contribute to assessment and management of the fishery. However, where such research is cost prohibitive, alternative assessment and management strategies may be more suited. For example, ecosystem-based approaches may provide a more cost effective method to analyse variation in population size in relation to changes in predator-prey relationships, fishing effort, and biomass estimate fluctuations at various locations over time (Sainsbury & Sumaila 2003).

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2. APPENDIX II

Temporal and spatial recruitment variability of arrow squid Nototodarus gouldi from southern Australian waters

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1. ABSTRACT

For cephalopods, periods conducive to high recruitment provides evidence for fisheries managers to consider imposing harvest strategies that promotes maintaining biomass and future sustainability. As peak periods of population recruitment are unknown for the Australian arrow squid *Nototodarus gouldi* fishery, the aim of the study was to determine spatial and temporal recruitment variability. Distribution of hatch dates determined from back calculated statolith age estimates suggest that *N. gouldi* collected in Victoria and the Great Australian Bight (GAB) during 2007 – 2009, hatch year round. Aseasonal hatching events suggests that all ontogenetic stages are represented in the population at any one point of time, which is conducive to the succession of future generations; however, this feature obscures the identification distinct cohorts particularly as hatch frequency is a function of spawning events, developmental rates, and mortality. Using Bayesian Information Criterion, at least five cohorts were identified from Victoria and the GAB from mid 2007 to late 2008, indicating that squid hatching during different months have a level of periodicity in hatch frequency. The timing of spawning influences future biomass of *N. gouldi* because embryo and juvenile growth and survival rates are a product of the environmental and oceanographic factors they experience, which results in spatial and temporal variability of the population structure. Consequently, the strength of recruitment success and future biomass is a reflection of inter- and intra-annual variability of environmental conditions that affects all ontogenetic life stages. Based on spawning characteristics and recruitment variability in the *N. gouldi* fishery, current fishing effort is unlikely to jeopardize the sustainability of the resource.

2. INTRODUCTION

Inter- and intra-annual variability in catch weight and biomass is common to most commercial teleost and cephalopod fisheries and is due to the response of recruitment rates to environmental stochasticity and reproductive life history strategies (Cury & Roy 1989, Fogarty *et al.* 1991, Hilborn & Walters 1992, Hatfield & Des Clers 1998, Myers 1998). Recruitment is the renewal of individuals within a population (Jennings *et al.* 2001, Waluda *et al.* 2001, Challier *et al.* 2005). For cephalopod and short-lived teleost fisheries, recruitment success is entirely reliant on the capacity of the current standing stock to produce offspring (Boyle & Boletzky 1996, Myers 2002). However, population dynamics and recruitment success are influenced by environmental and anthropogenic factors. Excessive fishing effort when the biomass of the stock is small increases the risk of recruitment failure which in turn affects related ecosystems and economic viability of commercial fisheries (Boyle & Boletzky 1996, Myers *et al.* 1997, King 2007). Although recruitment failure and stock collapses are rare, management strategies can facilitate recruitment success through knowledge of reproductive characteristics. Therefore, forecasting periods conducive to high recruitment based on lifecycle characteristics provides information on future stock biomass and the time that harvesting can occur. Such information gives evidence for mangers to consider implementing harvest strategies that maintain spawning biomass, promote population succession, and avoid risks of overfishing (Hatfield & Des Clers 1998, Moltschaniwskyj *et al.* 2002).

Teleost fisheries managers commonly rely on numerical (or mass) estimates of population size to calculate harvesting quota used to set catch or effort restrictions that will ensure sufficient spawning biomass is retained for successful recruitment. However, limiting factors such as food availability and environmental carrying capacity can affect growth, reproduction and population size (Wootton 1990, Christensen & Pauly 1998, Agnew *et al.* 2000a, Sánchez Lizaso *et al.* 2000, Challier *et al.* 2006). For cephalopods such as *Loligo gahi*, *L. duvauceli* and *Todarodes pacificus*, density-dependent recruitment is limited by available resources (Okutani & Watanabe 1983, Mohamed & Rao 1997, Agnew *et al.* 2000a, Agnew *et al.* 2002). However, unlike most teleost fisheries where estimates of individual age are in excess of one year, the short lifespan of cephalopods means that stock biomass estimates are no longer accurate within a short period of time (Jackson 1990, Arkhipkin 1993, Dawe & Hendrickson 1998, Arguelles *et al.* 2001, Bettencourt & Guerra 2001, Leporati *et al.* 2008). Periods of naturally low abundance coupled with relatively high fishing pressure has resulted in recruitment overfishing in *Illex illecebrosus* and *Loligo bleekeri* fisheries (O'Dor 1998b, Tian 2009). Consequently, fisheries managers would like to implement policies to ensure future sustainability based on robust biological indicators and information that will allow forecasts of biomass. Although establishing cephalopod management strategies using traditional teleost models (e.g. Ricker model) are unsuitable, alternative models such as depletion analyses successfully estimates abundance within the current fishing season (Caddy 1983). For example, Leslie-DeLury models are used for assessment and management of the *Loligo pealei* fishery in the north western Atlantic (Brodziak & Rosenberg 1993).

In major squid fisheries such as *Todarodes pacificus, Illex argentinus* and *Dosidicus gigas*, recruitment variability is most likely driven by abiotic and biotic factors (Rodhouse 2001a, Waluda *et al.* 2001, Isoda *et al.* 2005), rather than fluctuating fishing effort (Beddington *et al.* 1990). Many factors affect recruitment variability, with ambient environmental conditions such as sea surface temperature considered most influential (Bakun & Csirke 1998, Dawe *et al.* 1998, Roberts 1998, Dawe *et al.* 2000, Agnew *et al.* 2002). Sea surface temperature greatly influences squid recruitment in species such as *Illex argentinus*, *Loligo forbesi* and *L. vulgaris* (Pierce 1999, Robin & Denis 1999, Waluda *et al.* 2001, Challier *et al.* 2005, Chen *et al.* 2007), as it affects spawning and growth characteristics at an individual and population level (Hatfield 1998, Agnew *et al.* 2000a, Arkhipkin *et al.* 2000a, Villanueva 2000, Isoda *et al.* 2005). Although aseasonal spawning occurs in cephalopods such as *N. gouldi* (McGrath & Jackson 2002, Jackson *et al.* 2005), periods of increased spawning activity can result in the ‘match-mismatch’ of hatchings with planktonic food, thus affecting future recruitment and future biomass (Cushing 1982, Grahame 1987, Boyle & Rodhouse 2005).

When estimates of biomass are unavailable, it may be possible to use knowledge about reproductive ecology and biology (e.g. single or repeated mating, protracted or seasonal spawning events) to protect the spawning biomass and maintain recruitment. Reproductive and spawning characteristics vary within loliginids (Boyle *et al.* 1995, Pecl *et al.* 2002), and ommastrephids (Sakurai *et al.* 2003, Ibáñez & Cubillos 2007) with single spawning and multiple spawning strategies employed. Patterns of spawning activity at population level varies among species and can be seasonal, extended seasonality with or without peaks of spawning activity, or year-round with or without peaks of spawning activity are some of the spawning strategies recorded (Mangold 1987). Fisheries managers utilise different spawning strategies to develop management options. For example, aggregated spawning behaviour identified in the *Sepioteuthis australis* fishery in Tasmania, Australia, highlight the need to implement temporal closures (Moltschaniwskyj *et al.* 2002) to protect the spawning biomass required for recruitment; whereas fishing effort in the *Illex argentinus* fishery is regulated to ensure that 40% of the pre-fishing spawning biomass is retained (Beddington *et al.* 1990, Basson & Beddington 1993).

Temporal and spatial recruitment variability is commonly estimated by determining the number of cohorts present in a fishery using hatch date, length- or age-at-catch based models, where discrete cohorts represent a period of peak recruitment. Length frequency is used to analyse intra-annual variability in growth and cohort identity in squids such as *Loligo duvauceli* (Supongpan *et al.* 1993). However, substantial variability in growth rate among individuals and continual input of recruits into the population has meant that for some species length based methods have not been useful in cohort analyses (Hilborn & Walters 1992, Pierce & Guerra 1994, Jackson *et al.* 1997, Jackson *et al.* 2000). Despite the limited value of length frequencies for analysing squid population dynamics, age frequency distributions can assist in the identification of cohorts and the age which squid are susceptible to fishing or sampling gear. However, care does need to be taken as presumed cohorts can be a result of sampling artifacts such as sampling periodicity which reduces confidence in estimating periods of high recruitment (Boyle & Boletzky 1996, Jackson *et al.* 2005). For example, growth was not determined in *Loligo vulgaris reynaudii* using length frequency distributions from monthly sampling as modal progression in length frequency was not observed (Augustyn 1990). In comparison, when spawning is highly seasonal and undertaken by large numbers of individuals over a short period of time it may be possible to obtain sensible estimates of the timing of cohorts recruit into a fishery, e.g. *Dosidicus gigas* (Tafur *et al.* 2001), *Loligo gahi* (Arkhipkin *et al.* 2004b), and *Todarodes pacificus* (Kidokoro & Sakurai 2008). However, it is possible that inter-annual variability in the timing and extent of the spawning period and growth rates can obscure cohort identification (Pecl *et al.* 2004a). However, where it is possible to identify seasons of high recruitment, it provides the option for managers to develop policies that protect future spawning biomass, thereby reducing the risk of recruitment failure as well as indicating periods conducive to greater catches.

Given that cephalopods display substantial growth plasticity, squid of similar size may be very different in age, as much as months (Jackson & Choat 1992, Pierce & Guerra 1994, Brodziak & Macy 1996). Due to size selectivity of fishing gears (Lipinski 1994, Koronkiewicz 1995, Hastie 1996), a single normal length frequency distribution may comprise of squid that hatched over an extended period of time. Although size selectivity of individuals by fishing gears is unavoidable, regular intra-annual (e.g. monthly) sampling coupled with the calculation of hatch date frequency distributions, derived from age estimates, reduces biases associated with cohort identification compared with length frequency analyses. However, determining the size of squid caught provides an indication of when squid are susceptible to fishing gear. Age estimates of squid is determined by counting incremental structure within their statoliths, and as long as the increment structure is clear and obtained with some degree of confidence, then hatch date is easily calculated (Jackson 1994).

Within the southern and eastern scalefish and shark fishery (SESSF), *N. gouldi* are targeted by jig vessels on the continental shelf (< 110 m depth) that form the southern squid jig fishery (SSJF). They are also caught and retained as by-catch by demersal trawlers operating on the continental slope (>250 m depth) within the Commonwealth trawl sector (CTS) and the Great Australian Bight trawl sector (GABTS) (Larcombe & Begg 2008). In all sectors, large seasonal variation in stock abundance exists, which has been attributed to rapid stock regeneration during periods of favourable environmental conditions (Wilson *et al.* 2009). Catches of *N. gouldi* in the GABTS was greatest in 2006 with 262 t landed; however, this has since declined possibly due to a reduction in stock size. Despite this, interest is developing in the use of mid-water trawlers to target *N. gouldi* stocks within the Great Australian Bight (GAB) as it is likely to be more economically viable compared to the jig-fishery.

Unlike *N. gouldi* collected in southeastern Australia from the SSJF and CTS fisheries, recruitment variability of *N. gouldi* in the GAB is unknown.

Nototodarus gouldi (McCoy 1888) spawn multiple times, releasing eggs in small batches without compromising somatic growth and oocyte production (Uozumi 1998, McGrath & Jackson 2002, Jackson *et al.* 2003, Jackson *et al.* 2005). Reproductive output is unknown for *N. gouldi;* however a similar species, *Todarodes sagittatus*, is capable of producing 523,500 eggs (Lordan *et al.* 2001). Paralarvae (0.8 – 1.0 mm dorsal mantle length, DML) are distributed in most areas of their adult geographical distribution with squid 9 – 10 mm DML captured during summer months and abundant in depths of 50 – 200 m (Dunning 1985). Reproductive and spawning characteristics of *N. gouldi* provided much needed detail required for fisheries assessment and management of this fishery; however, greater clarification of cohort structure and temporal patterns of recruitment are required. Jackson *et al.* (2005) identified four cohorts of *N. gouldi* in the population off Portland, Victoria during 2001; however, the sampling regime missed the early-hatched squid in the first cohort and the later-hatched squid in the last cohort. Lengthening the time series of collections will clarify periods conducive to high recruitment as well as identifying the periodicity of cohorts moving through a fishing area that is subject to fishing (Jackson *et al.* 2005). This study aimed to identify periods of high recruitment and compare recruitment variability from an existing fishery in Victoria, with a developing fishery in the GAB based on back calculated hatch dates of *N. gouldi*. Such information will expand our knowledge of the *N. gouldi* resource off southern Australia for the purpose of improving management practices promoting sustainability.

3. METHODS

Nototodarus gouldi were collected from commercial trawl fishers operating in two spatially separated regions of southern Australia. Off the coast of Victoria, up to 242 *N. gouldi* were collected at approximately monthly intervals from March 2007 – June 2009, whereas up to 354 *N. gouldi* were collected from the GAB bimonthly from June 2008 – June 2009 (Figure 2-1, Table 2-1). Frozen squid were shipped to the University of Tasmania or the Marine and Freshwater Fisheries Research Institute in Victoria for dissection. Sex and dorsal mantle length (DML) was recorded for each individual. Statolith pairs were located within the statocyst of the squid cranium and removed with fine-tipped forceps, rinsed with distilled water, and stored dry in cavity trays. Age was determined by counting incremental structure from the hatch mark near the statolith core, to the edge of the dorsal dome using a modified method described by Jackson (2005). One statolith was randomly removed from the cavity tray and attached to a glass microscope slide with the anterior side closest to the slide using heated thermoplastic glue (Crystalbond™). Once cooled, the posterior side of the statolith was ground down with 30 µm lapping film (3M™) until the core was visible just under the ground surface. A finer grade of lapping film (5 µm) was used to grind closer to the core and remove larger surface scratches.

For preparations where increments were not clear, the slide was reheated, the statolith ground on the anterior side and flipped back to the posterior side. Preparations were polished using Leco Clothe® and aluminum oxide powder (0.005 µm) and water to remove finer scratches. A small amount of immersion oil was smeared on the statolith to improve increment clarity. Statoliths were observed at up to 400x magnification using a compound microscope integrated with the image analysis system Optimas™. Daily age was determined by counting incremental structure from the presence of the hatch mark to the edge of the dorsal dome, along the apex of increment formation and was assumed that the periodicity of increment formation in *N. gouldi* statoliths occurs daily (Jackson *et al.* 2005). Due to the subjective nature of increment interpretation, some preparations were aged up to three times with the average count recorded.

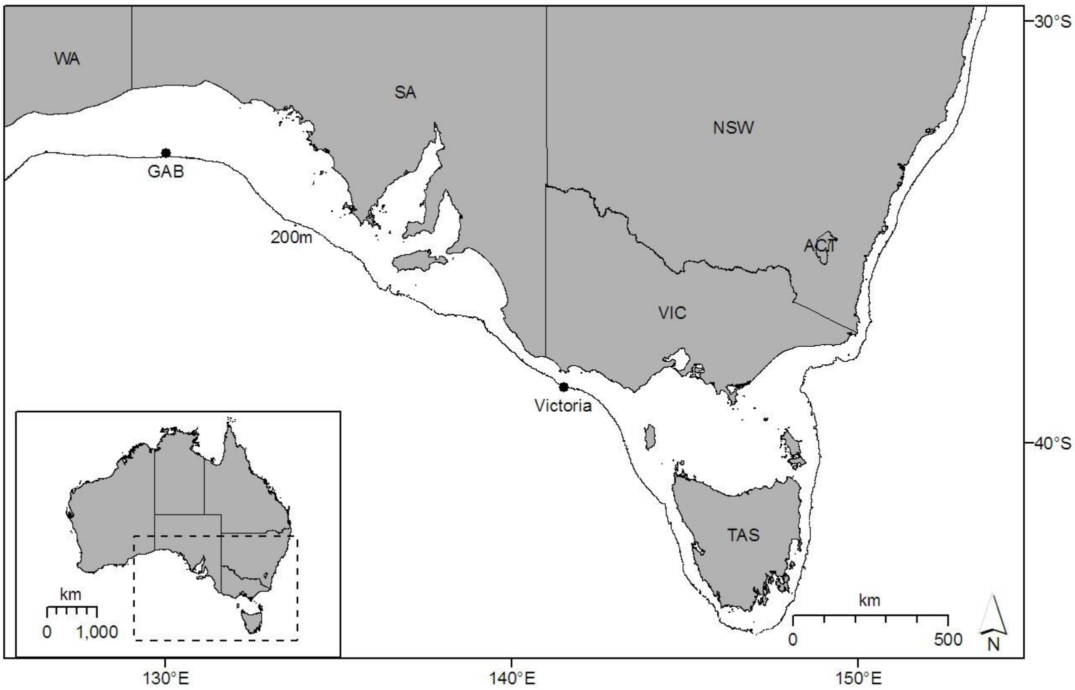

Figure 2-1 Map of southern Australia detailing the general location where *N. gouldi* were caught along the continental shelf in the Great Australian Bight (GAB) and Victoria.

Table 2-1 Details of *N. gouldi* collected from the Great Australian Bight (GAB) and Victoria, Australia from March 2007 - June 2009.

Date of collection	Numbers collected in GAB	Numbers collected in Victoria	Latitude	Longitude
01-Mar-2007		100	38.72	141.37
27-Mar-2007		100	38.77	141.60
07-Jun-2007		100	38.30	140.52
06-Aug-2007		101	38.58	141.17
19-Sep-2007		100	38.30	140.55
15-Oct-2007		100	37.93	139.98
Total collections in 2007		601		
19-Feb-2008		100	38.75	141.53
14-Mar-2008		101	38.93	142.18
14-May-2008		142	38.33	140.63
25-May-2008		100	38.23	140.42
22-Jun-2008		100	38.58	141.17
23-Jun-2008	112		33.25	130.03
25-Jul-2008		100	37.55	139.28
01-Aug-2008		100	38.62	139.27
15-Aug-2008	80		33.22	126.48
16-Aug-2008	90		33.28	128.58
17-Aug-2008	85		33.30	130.13
19-Aug-2008	99		33.28	130.13
28-Aug-2008		137	38.32	140.53
30-Sep-2008		121	38.68	141.27
06-Nov-2008	158		33.22	131.25
14-Nov-2008		140	38.62	141.20
11-Dec-2008		156	33.75	141.47
22-Dec-2008	83		33.35	132.10
Total collections in 2008	707	1297		
21-Jan-2009		130	38.70	141.30
12-Feb-2009	135		33.18	128.39
19-Feb-2009		175	38.68	141.32
25-Mar-2009		105	38.75	141.35
02-Apr-2009	96		33.16	128.30
30-Apr-2009		81	38.73	141.37
02-Jun-2009		78	38.77	141.57
10-Jun-2009	204		33.19	129.00
Total collections in 2009	435	569		
Total collections combined years	1142	2467		

Minimum age estimates were determined to indicate the age which *N. gouldi* are susceptible to trawling gear; whereas mantle length and age frequency distributions were used to visually identify cohorts as well as indicating if the size and age structure progressively gets larger and older over time. Hatch date was determined by subtracting the estimated age from the date of capture. To illustrate if hatching occurs throughout time, individual hatch date was plotted against capture date for each location of capture, fitted with a loess smoothed line (Simonoff 1996). To analyse the existence of cohorts within a collection, a normal mixture model was fitted to the distribution of hatch dates for the GAB and Victorian collections separately. Where a normal mixture model did not provide a realistic presentation of cohort structure, a log-normal mixture model was used. It was assumed that the frequency distribution of hatch dates was consistent for female and male *N. gouldi* at each location (Figure 2-2).

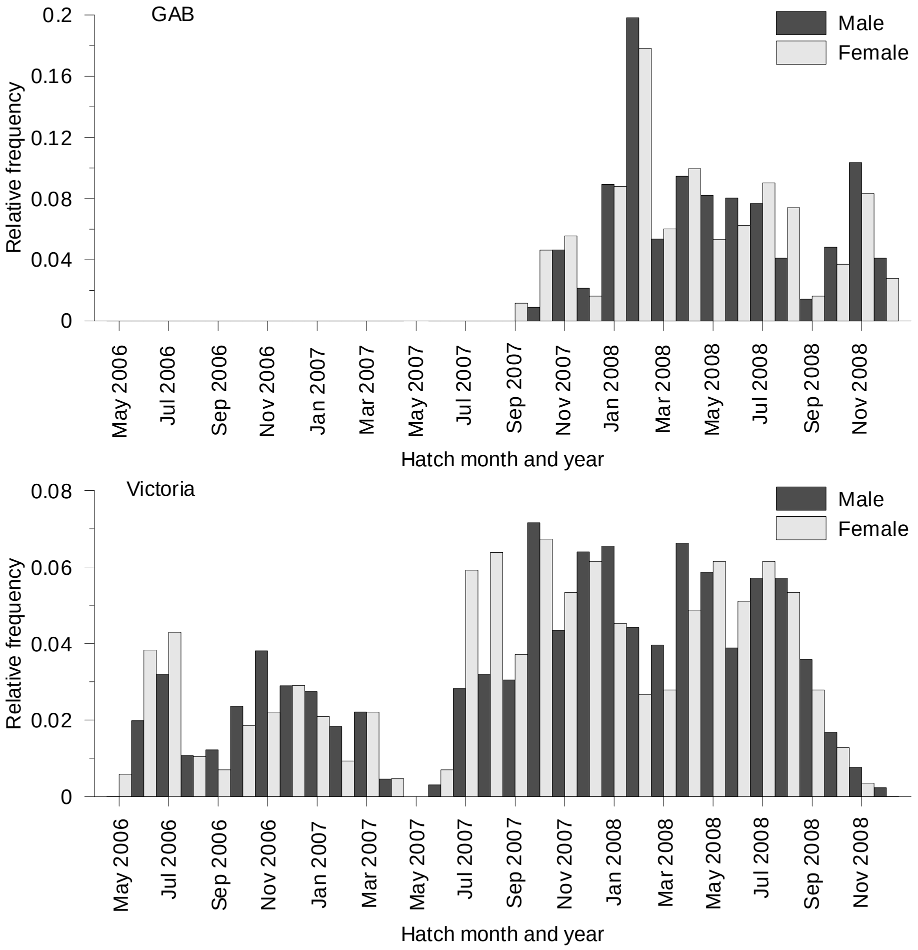

Figure 2-2 Relative frequency distribution of hatch dates for male and female *N. gouldi* collected in the GAB (top) and Victoria (bottom).

Based on the central limit theory, within each cohort it was assumed that hatch dates are approximately normally distributed (Jackson *et al.* 2005), with density and mean μ and variance σ^2 Eq 2.1.

$$f_i(x;\mu,\sigma)=\frac{1}{\sqrt{2\pi\sigma^2}}e^{-(x-\mu)^2/(2\sigma^2)} \qquad \text{Eq 2.1}$$

The distribution of hatch dates was estimated using a normal kernel density estimator (Simonoff 1996). The bandwidth of the kernel was used to determine the level of smoothness of the estimated density, with several choices of bandwidth made to accentuate possible peaks in the hatch date distribution. The distribution of the hatch date was a mixture of normal components, one component for each cohort, with the multipliers λ_i representing the portion that each cohort makes up of the entire distribution Eq 2.2,

$$f(x) = \sum_{i=1}^{k} \lambda_i f_i(x; \mu_i, \sigma_i) \qquad \text{Eq 2.2}$$

The mixture model was fitted to the distribution of hatch dates using the mclust library in R statistics. Mclust fits several different models and then automatically chooses the best based on Bayesian Information Criterion (BIC, Shwartz 1978). The model determines the number of normal components (cohorts) that it can fit from the hatch frequency distribution. Two models were run, the first where the normal components were constrained to have a common variance (estimated by the model); the second where the variance was allowed to differ from component to component. We aimed to estimate the number of cohorts including the mean and variance of the hatch dates for each cohort determined from the GAB and Victoria.

4. RESULTS

Of the 1142 and 2467 *N. gouldi* collected in the GAB and Victoria, 993 and 2177 age estimates were determined respectively. Failure in obtaining age estimates from statoliths was attributed to over grinding, fractures and poor increment clarity. The minimum age of *N. gouldi* collected in the GAB was 140 d and 145 d for females and males respectively; whereas in Victoria, the minimum age was 145 d and 166 d for females and males respectively.

Nototodarus gouldi collected from March 2007 – June 2009 in Victoria and the GAB appeared to have unimodal and multimodal mantle length frequency distributions over approximately monthly and bimonthly time scales (Figure 2-3). A progression of increasing size over time was not visually identified as modal length increased and decreased temporally over the collection duration. The size range of *N. gouldi* collected in Victoria was 130 – 380 mm and 140 – 410 mm in the GAB and Victoria respectively. Compared to length frequency distributions, age frequency distributions of *N. gouldi* collected at both locations appeared smoother between each binned frequency class over their age range (Figure 2-4). Independent of location of capture, identifying the presence of either unimodal or multimodal distribution in age was not viable. Consequently, detecting modal progression in age frequency was not possible over successive collection months. For example, the modal age of *N. gouldi* in Victoria for each monthly collection period from Feb 2008 – March 2009 was either 210 or 220 days (Figure 2-4).

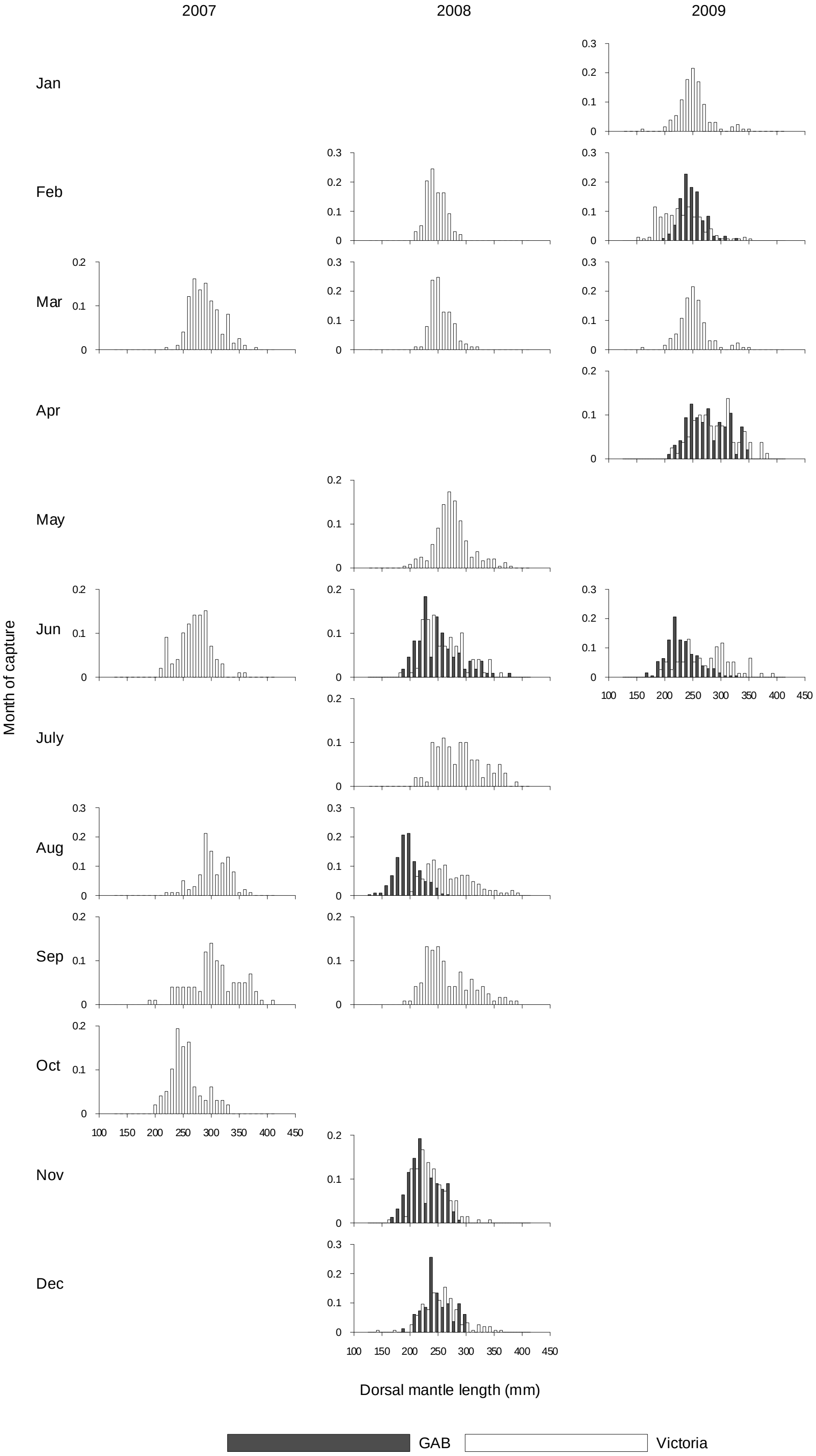

Figure 2-3 Relative dorsal mantle length frequency distribution *N. gouldi* collected in Victoria from March 2007 – June 2009 (open bars) and in the GAB from March 2007 – June 2009 (shaded bars). Empty spaces indicate where no *N. gouldi* were collected. Frequency binned at 10 mm DML intervals.

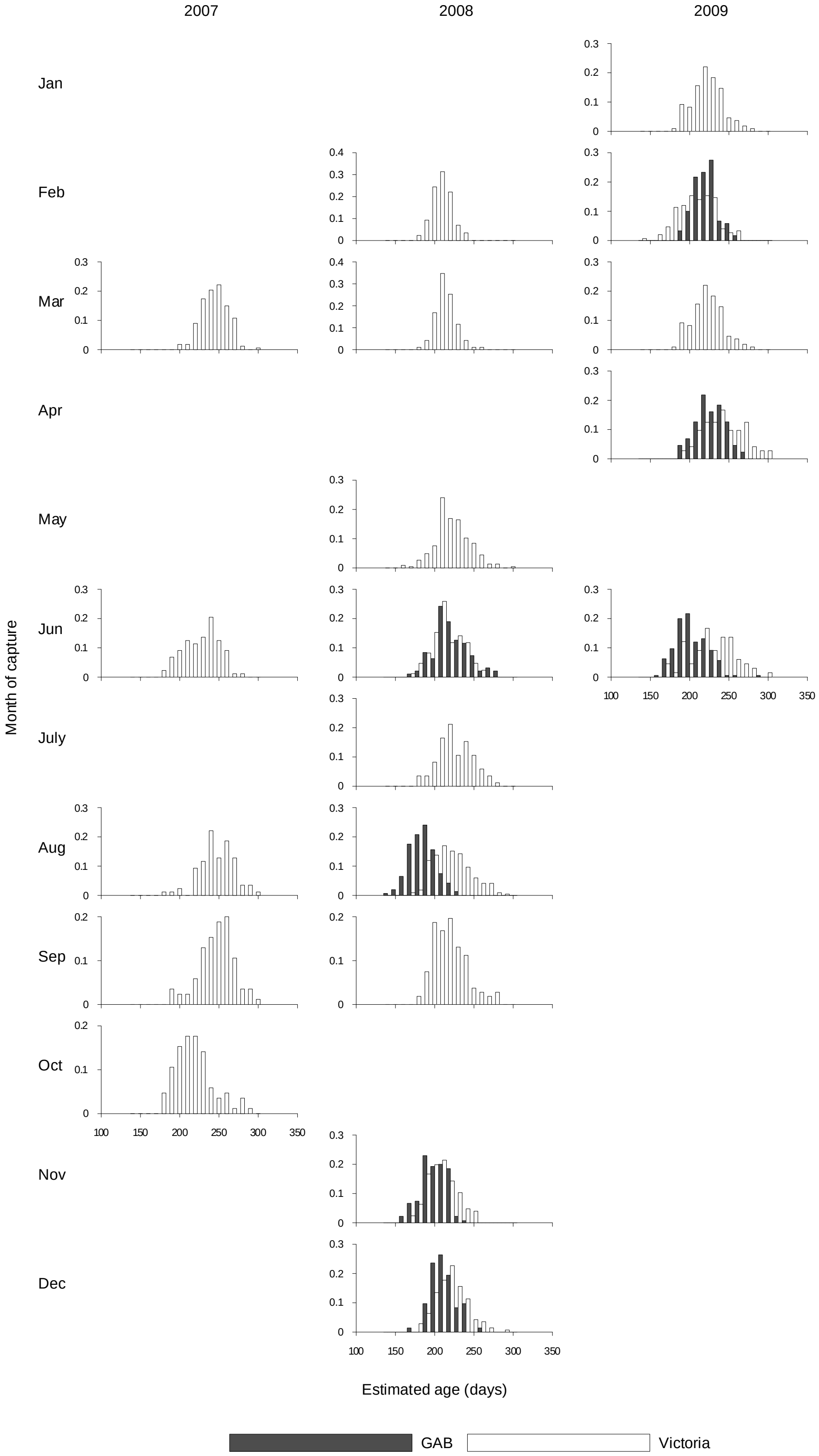

Figure 2-4 Relative age frequency distribution *N. gouldi* collected in Victoria (open bars) from March 2007 – June 2009 and in the GAB (shaded bars) from March 2007 – June 2009. Empty spaces indicate where no *N. gouldi* were collected. Frequency binned at 10 day intervals.

For *N. gouldi* caught in Victoria, hatching ranged from May 2006 – December 2008. However, no collections in Victoria from November 2007 – January 2008 are likely to have contributed to zero hatching observed during May 2007. In the GAB, hatching was observed from September 2007 – December 2008. Differences in hatch date range among the two locations of capture were a reflection of different sampling periods. The correlation between hatch date distribution and collection date confirms that there is no strong cohort structure for squid populations from either the GAB or Victoria (Figure 2-5).

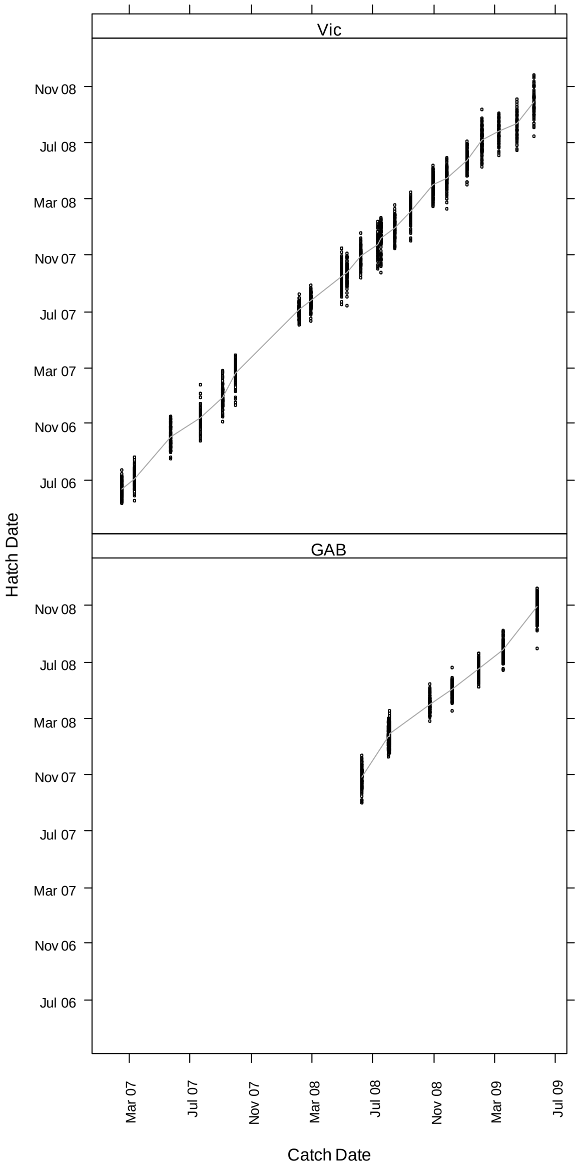

Figure 2-5 Scatter plot of hatch dates for each catch date of *N. gouldi* collected in Victoria (top) and the GAB (bottom). Each point represents one individual with the line representing a loess smoothed average.

Hatch date density distributions showed protracted hatching period for *N. gouldi* from approximately March 2006 – March 2009 in Victoria (Figure 2-6) and from June 2007 – March 2009 in the GAB (Figure 2-7), though the extent of the hatch period was largely attributed to sampling period. Despite protracted hatching, there were clearly identifiable peaks in the GAB samples, but not the Victoria samples. For *N. gouldi* collected at both locations, different bandwidths used to determine the level of smoothness in the hatch date density distributions indicated that smaller bandwidths provided greatest number of peaks and were considered to be more biologically plausible than using larger bandwidths.

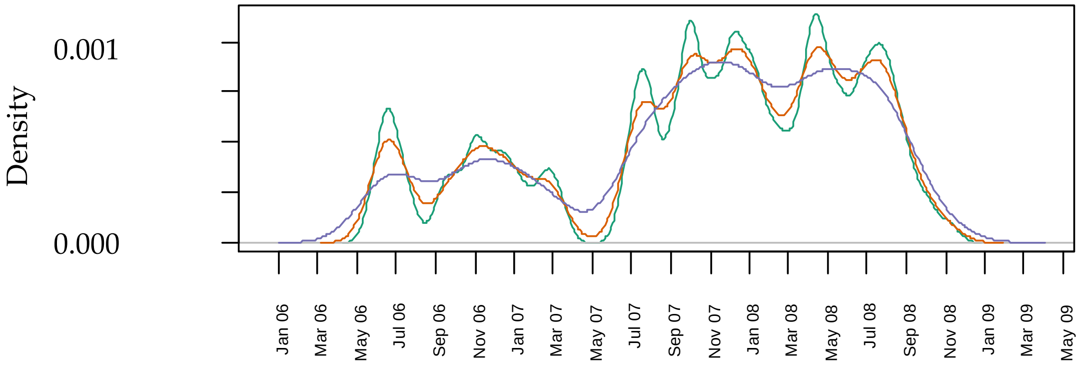

Figure 2-6 Smoothed density distribution of hatch dates for *N. gouldi* collected in Victoria. Coloured lines represent three kernel density estimates corresponding to successively smaller bandwidths. The green line (smallest bandwidth) represents the hatch date distribution with greatest peaks.

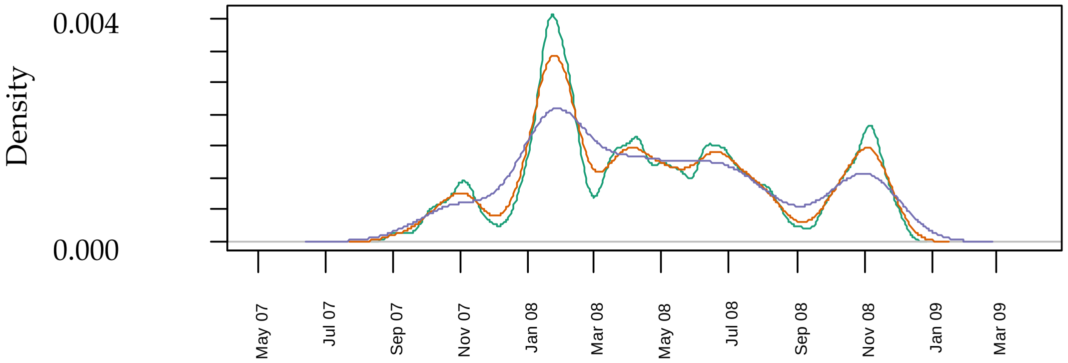

Figure 2-7 Smoothed density distribution of hatch dates for *N. gouldi* collected in the GAB. Coloured lines represent three kernel density estimates corresponding to successively smaller bandwidths. The green line (smallest bandwidth) represents the hatch date distribution with greatest peaks.

Using a bandwidth that achieved greatest peaks in hatch frequency distribution of *N. gouldi* caught in Victoria, 7 – 12 cohorts of unequal variance were estimated using a normal mixture model (Figure 2-8). Although the BIC predicted eight cohorts; considerable overlap in cohorts seven and eight (modes in July 08 and August 08) was evident and appeared biologically unrealistic. However, fitting a log-normal mixture model to seven cohorts provided a more biologically plausible cohort distribution (Figure 2-9). In Victoria from July 2007 – June 2008, four to five cohorts were estimate; however, from July 2006 – June 2007 two cohorts were estimated.

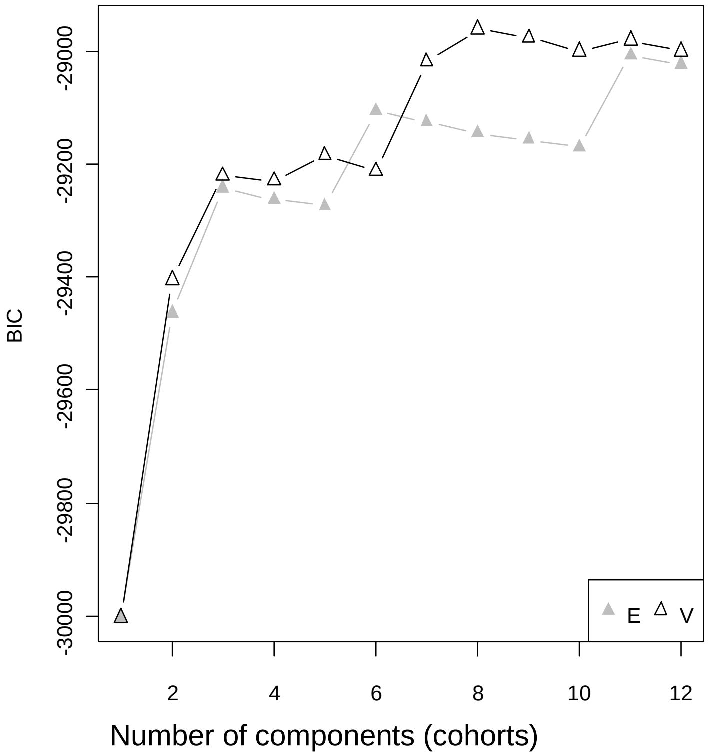

Figure 2-8 Diagnostic plot of a normal mixture model for *N. gouldi* collected in Victoria. Larger values of BIC indicate the preferred model. The "E" models have equal variance for each cohort, while for the "V" models, variance is allowed to vary. Number of components represents the number of normally distributed cohorts.

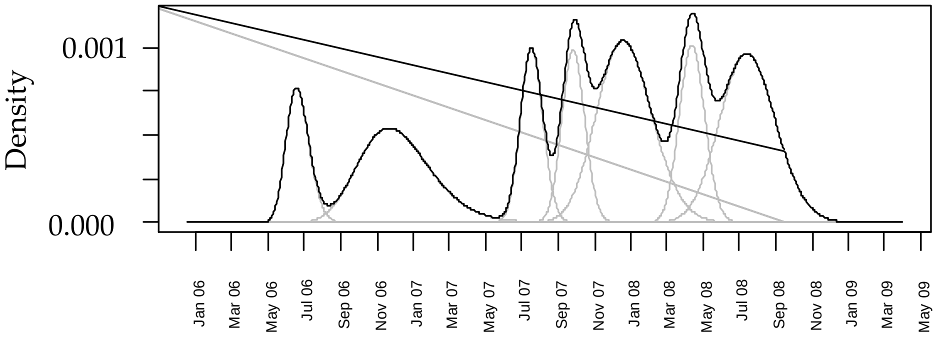

Figure 2-9 Estimated allocation of cohorts determined using a log-normal mixture model of the distribution of hatch dates for *N. gouldi* caught in Victoria. The individual cohorts are shown in grey, with the smoothed density distribution in black.

For *N. gouldi* caught in the GAB, a bandwidth that achieved greatest peaks in hatch frequency distribution of *N. gouldi* was used to estimate four to five cohorts of unequal variance using a normal mixture model (Figure 2-10). However, BIC values suggest that when equal variance of the normal distribution was used in the mixture model, six cohorts were identified. Both normal and log-normal mixture models suggested biologically plausible distribution of cohorts for squid that hatched in the GAB; however a log-normal mixture model is presented (Figure 2-11). Both models suggested that the greatest peak in hatch frequency occurred during February 2008.

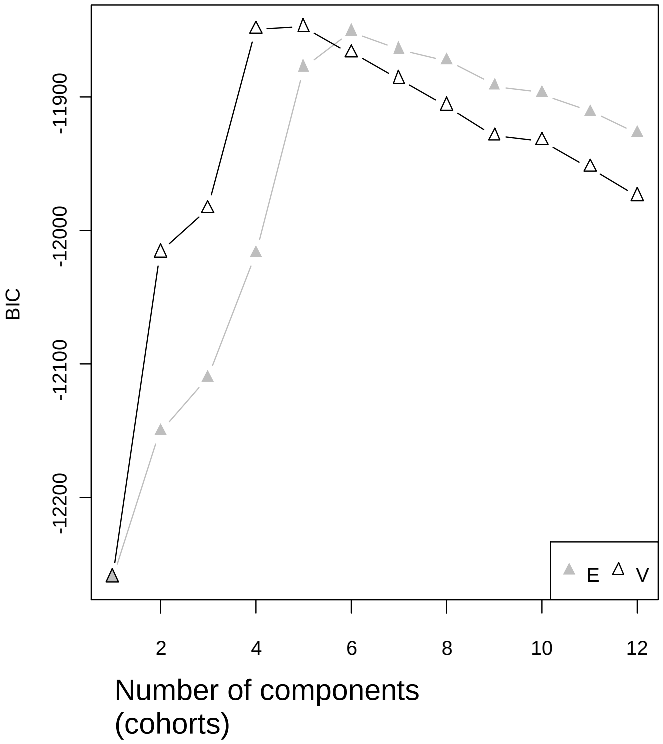

Figure 2-10 Diagnostic plot of a normal mixture model for *N. gouldi* collected in the GAB. Larger values of BIC indicate the preferred model. The "E" models have equal variance for each cohort, while for the "V" models, variance is allowed to vary. Number of components represents the number of normally distributed cohorts.

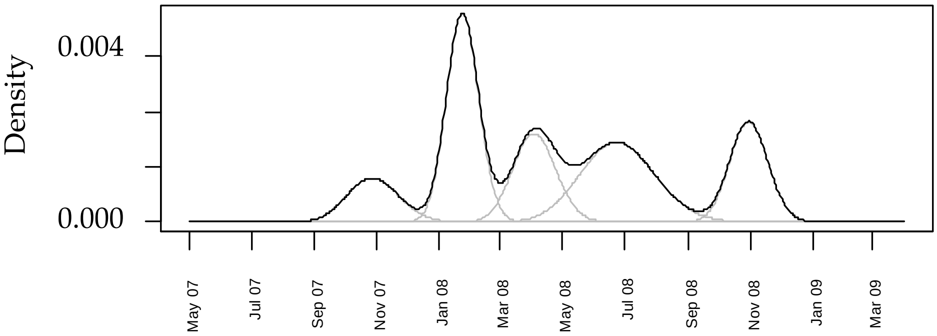

Figure 2-11 Estimated allocation of cohorts determined using a log-normal mixture model of the distribution of hatch dates for *N. gouldi* caught in the GAB. The individual cohorts are shown in grey, with the smoothed density distribution in black.

5. DISCUSSION

Hatch date frequency distribution of *N. gouldi* collected in Victoria from 2007 – 2009 and in the GAB in 2008 – 2009 suggest continual recruitment into the existing population, with temporal and spatial variability in cohort structure evident. Approximately four cohorts were identified and evenly distributed during a period of one year from Victoria and the GAB using BIC, indicating a degree of periodicity in hatch frequency shared among locations. In the GAB, greatest number of *N. gouldi* hatched during February 2008; whereas periods of increased hatching were not as distinct in Victoria. However, only two cohorts were identified in Victoria from May 2006 – May 2007 which is most probably due to a lack in samples collected during November 2007 – January 2008 rather than a period of poor recruitment. Hatch date distributions and subsequent cohort identification also showed four *N. gouldi* cohorts present during 2001 separated by similar time durations (Jackson *et al.* 2005) indicating that successive inter-annual hatch frequency cohorts are common for *N. gouldi* in Victoria. Similarly, *N. gouldi* caught off New Zealand hatch throughout the year with inter-annual variability in hatch intensity (Uozumi 1998). Protracted hatch distribution and the selective nature of trawl gears allowing escapement of younger animals suggests that the *N. gouldi* fishery in the GAB and Victoria is not likely to be subjected to recruitment overfishing. Forecasting periods conducive to greater numbers available to fishers appears difficult to predict as ontogenetic migration and ocean current systems imply that hatchings and juveniles may experience a greater level of spatial variation. For example, a peak in hatch frequency in one region may result in an increase of adult squid in a different region when, and where, fishing is in operation.

The detection of cohorts in the population was not possible using both length and age frequency distribution data of *N. gouldi* collected in the GAB and Victoria. There are various reasons suggested of this. The selective nature of trawl gear suggests that small squid are able to escape entrapment by moving through open mesh thereby biasing length and age frequency distributions. For example, Lipinski (1994) found that *Loligo vulgaris reynaudii* collected using three different fishing methods, jigging, mid-water trawling and purse seining, biased length frequency distributions. However, in the present study, the absence of small squid (<130 mm DML) in the catch may be a function of ontogenetic migration (Chapter 5) with smaller squid not being present at the bathymetries fished using trawl methods. Furthermore, substantial temporal and spatial variation in somatic growth in *N. gouldi* (Chapter 3; Jackson *et al.* 2003) support the evidence that length based frequency analysis is not a suitable method to identify periodicity of cohorts recruiting into the population. Such potential problems highlights the need for researchers to structure sampling that enables the collection of squid representing all ontogenetic stages and not to base sampling entirely on commercial catch.

Compared to length and age frequency distribution data, using hatch frequency distribution appeared to be a powerful approach to examine recruitment variability in *N. gouldi*. However, sampling methods and biological characteristics may still affect the interpretation of results. Considerable overlap of hatch date distributions suggested that identification of hatching modes can be obscured or possibly non-existent. However, what remains unclear is the contribution that fishing effort has on the structure of cohorts identified. If catch size varies from month to month, the periods of greater fishing effort would produce peaks in the distribution of hatch dates, while periods of little catch would result in troughs in the distribution of hatch dates. Similarly, the subjective nature of statolith age estimation could contribute to the high overlap in hatch data frequency distributions. The periodicity of increment formation in *N. gouldi* is assumed to be daily; but this assumption is untested due to poor growth increment structure in cultured animals (Jackson *et al.* 2005). However, daily increment periodicity in statoliths of *Loligo plei* (Jackson & Forsythe 2002), *L. vulgaris reynaudii* (Durholtz *et al.* 2002), *Illex illecebrosus* (Dawe *et al.* 1985) and *Todarodes pacificus* (Nakamura & Sakurai 1991) has been validated; supporting the assumption of one increment equals one day in *N. gouldi*. However, because when ageing squid, counts of the number of increments is typically from 100 – 250, there is potential for error. A 10% error in estimating age would equate to a 20 – 30 day difference in hatch date. This would increase the overlap in hatch date frequency distributions and limit the capacity to distinguish cohorts. A source of error when counting increments in the statoliths are the presence of very narrow increments, indicative of slower statolith growth rates during cooler months. These are difficult to resolve under the microscope and may be confused with sub-daily increments or not seen (Sakai *et al.* 2004).

In *Loligo vulgaris,* year round hatching is observed (González *et al.* 2010) with variability in biomass most probably due to the influence of seasonal oceanographic effects of growth and recruitment (Boyle & Boletzky 1996, Agnew *et al.* 2000a). For *N. gouldi*, analysis of back calculated hatch dates from animals collected at approximately monthly intervals showed that they continually recruit to the existing population; with recruitment strength varying over time. Ovaries of mature female *N. gouldi* continually produce and accumulate oocytes, suggesting the release of discrete batches of eggs over time (McGrath & Jackson 2002). However at a population level, reproductive condition varies throughout the year (McGrath Steer & Jackson 2004). Female *N. gouldi* collected off Portland during cooler months in 2001 showed less gonad investment and better somatic condition, than females caught during warmer periods (McGrath Steer & Jackson 2004). Males and females from the GAB and Victoria collected in 2007 – 2009, showed similar trends in reproductive condition with summer indicative of greatest reproductive potential (Chapter 2). Such characteristics may be reflected in the greater hatch date frequency distribution for the GAB occurring in February. Such temporal variability in reproductive characteristics that support continual year round hatching may maximise the survival of offspring in variable environmental conditions (McGrath Steer & Jackson 2004), particularly when environmental conditions are less conducive to increased recruitment.

Despite *N. gouldi* being capable of spawning throughout the year, it appears that in some seasons there is increased hatching and a greater reproductive potential at a population level. However, extended hatch date periods may also be attributed to early life history characteristics. For example, embryonic development time varies considerably within and between species, and due to environmental conditions and egg size (Boyle & Rodhouse 2005). For *Sepioteuthis australis* and *Loligo gahi*, embryo development time varies from 1 – 2 months (Steer *et al.* 2003) and 2 – 5 months respectively (Baron 2002). However, ommastrephids generally have relatively smaller eggs and shorter development times (Sakurai *et al.* 1996). For example, *Todarodes pacificus* embryos hatch after 96 hours from artificially fertilized eggs (Watanabe *et al.* 1996). Differences in ambient water temperature also significantly affect rates of embryo growth and development, with warmer water temperatures reducing development time (McMahon & Summers 1971, O'Dor *et al.* 1982, Sakurai *et al.* 1996). As the timing and duration of hatch date periods based on back calculated age estimates can only provide an approximate indication of peak spawning periods, imposing catch restrictions based on protecting spawning biomass and / or protecting new recruits should be considered.

As oceanographic and environmental characteristics effect recruitment strength in cephalopods (Bakun & Csirke 1998, Waluda *et al.* 1999, Dawe *et al.* 2000), density dependency is likely to contribute to recruitment variability (Agnew *et al.* 2000a). Density-dependency affects due to abiotic and biotic conditions on individual growth and survival appear to be specific to different ontogenetic stages. For example, ontogenetic migrations in squid species such as *Loligo gahi* are more likely to be caught inshore (Arkhipkin *et al.* 2004b); consequently the population size for a certain demographic may be regulated by environment factors (Caley *et al.* 1996) that in turn may affect growth and future recruitment. Continual spawning activity of *N. gouldi* (Chapter 3; Jackson *et al.* 2003) suggests that all ontogenetic stages are present throughout the year; however, migration characteristics suggest that juveniles inhabit different regions to adults (Chapter 5). Consequently, future recruitment success may be a function of many factors that can influence different ontogenetic stages both spatially and temporally. For example, a large spawning stock biomass is associated with a decline in recruitment for *Loligo gahi* from the Falkland Islands (Agnew *et al.* 2000a, Agnew *et al.* 2002), whereas the biomass of copepods upon which *Loligo vulgaris reynaudii* prey is positively associated with recruitment success (Roberts & van den Berg 2002). It is unknown if populations of *N. gouldi* are similarly influenced by resources biomass.

Oceanographic and environmental variables fluctuate in Victoria and the GAB due to variable current systems, ocean floor topography and upwelling events (Chapter 3, Butler *et al.* 2002a, Middleton & Bye 2007). Consequently, *N. gouldi* are likely to be subjected to spatially and temporally varying density-dependent factors at different ontogenetic stages within a population. Increased productivity (chlorophyll *a*) is likely to increase food availability and support a larger biomass and faster growth of paralarvae, thereby possibly reducing the risk of increased recruitment mortality. However, when paralarvae grow from juveniles to adults, they will be subjected to different biomass constraints that in turn will affect future recruitment. Such response to rapidly changing population dynamics may explain why recruitment success is difficult to quantify and forecast for *N. gouldi* from Victoria and the GAB.

Although year round spawning characteristics showed peaks in hatch date frequency distributions, large overlap in cohort structure coupled with their rapid response to changes in oceanographic and environment factors suggests that forecasting *N. gouldi* recruitment will remain challenging. However, given their fast growth and rapid turnover, it is unlikely that future stocks of *N. gouldi* will be affected by overfishing as long as there are sufficient recruits entering the fishery. Although the youngest squid caught were immature, most male squid caught were mature, with immature and mature females represented in the catch (Chapter 3). Therefore, the likelihood of recruitment overfishing is reduced due to the selective nature of fishing gears. Recruitment of *N. gouldi* is likely to continue to fluctuate and effect future biomass in Victoria and the GAB; however, current fishing effort in the trawl and jig sectors are unlikely to jeopardize the sustainability of the resource.

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3. APPENDIX III

Differences in catch composition of arrow squid Nototodarus gouldi caught from inshore jigging and offshore trawling fisheries using biological and statolith element composition analyses

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1. ABSTRACT

Cephalopod research and management commonly relies on commercial catches to gain information on biological and stock structure characteristics. However, the selective nature of different fishing gears coupled with squid lifecycle characteristics can bias the portion of the available stock caught. As arrow squid *Nototodarus gouldi* found in southern Australian are caught inshore using jigs and offshore using trawlers, direct comparison between fishing methods is unknown as ontogenetic characteristics may confound differences. The aim of this study is to compare the catch composition of *N. gouldi* caught inshore from the jig fishery to *N. gouldi* caught offshore from the trawl fishery using biological and statolith element analyses. Squid caught inshore by jiggers were larger, consisted of a greater M:F ratio and had a greater percentage of mature females that suggests inshore spawning. However, similar statolith element composition was likely to be driven by uniform ocean water chemistries and squid physiological processes, rather than inshore / offshore intermixing. Squid less than the 220 mm DML size class were not found indicating that juveniles were not represented in the present population or significant selectivity of *N. gouldi* is evident in both fishing methods. Despite trawlers catching greater tonnage compared jig fishers, it is unlikely trawling will affect spawning aggregations available to jiggers.

2. INTRODUCTION

Population structure of cephalopod resources can be influenced by many factors including spatial and temporal variability growth, productivity, density dependency, environmental variability, and lifecycle characteristics (Anderson & Rodhouse 2001b, Boyle & Rodhouse 2005). Coupled with short lifecycles makes assessment and management difficult compared to their teleost counterparts. Cephalopod research commonly relies on commercial catches of squid to gain information on biological and stock structure characteristics; however, due to the nature of the fishing gear, commercial trawling and jigging can selectively bias the portion of the available stock caught (Lipinski 1994). For species that exhibit inshore and offshore ontogenetic migratory characteristics biases can be compounded when different fishing practices are used in different habitats (Koronkiewicz 1995, Arkhipkin *et al.* 2004b). Research on arrow squid *Nototodarus gouldi* (McCoy 1888), has provided fisheries managers with vital information on biological and stock relationships (McGrath & Jackson 2002, Jackson *et al.* 2003, Jackson & McGrath-Steer 2004b, McGrath Steer & Jackson 2004, Triantafillos *et al.* 2004, Jackson *et al.* 2005); however, stock structure may be a function of sampling gear type and ontogenetic migratory characteristics.

In Australia, commercial catches of *N. gouldi*, are predominantly caught using two different methods: jigging and trawling (Kailola *et al.* 1993). Although fishing is conducted over vast regions off the southern coast, jigging commonly occurs at inshore locations on the continental shelf in waters <110 m depth; whereas demersal trawlers operate offshore near the continental slope where depths are >250 m (Larcombe & Begg 2008, Wilson *et al.* 2009). Factors such as feeding behavioral and lifestyle characteristics, ontogenetic migrations, and fishing method selectivity can influence what portion of squid are removed from the available resource and may impact future recruitment. For *Illex argentinus,* jigs catch larger squid than trawlers in similar regions (Koronkiewicz 1995); whereas diel distributions of *Illex illecebrosus* resulted in gear modifications to increase catch (Korotkov 1993). Similarly, larger *Loligo gahi* are more likely to be caught inshore in association with ontogenetic migration characteristics (Arkhipkin *et al.* 2004b). Although differences in catch composition using jig and trawl methods relating to gear selectivity and ontogenetic lifecycle characteristics are unknown, data will provide vital information to effectively manage this multidisciplinary fishery.

Nototodarus gouldi are fast growing ommastrephids capable of spawning multiple times a year and exhibit spatial and temporal variation in growth rates, reproduction, maturity and movement (McGrath & Jackson 2002, Jackson *et al.* 2003, McGrath Steer & Jackson 2004, Triantafillos *et al.* 2004, Jackson *et al.* 2005, Stark *et al.* 2005); however little is known whether different ontogenetic stages of *N. gouldi* are caught inshore by jiggers or offshore by trawlers. Inshore / offshore ontogenetic migration characteristics enable fishers to pre-empt and target aggregations to increase their catch-per-unit-effort. For example, juvenile *Loligo gahi* migrate from inshore (20 – 50 m) to offshore waters (200 – 350 m) where they grow, and upon maturation migrate back inshore to spawn where they are targeted by trawlers (Hatfield & Des Clers 1998, Arkhipkin *et al.* 2004b, Arkhipkin *et al.* 2004c). *Loligo vulgaris* and *Illex argentinus* follow similar ontogenetic migratory patterns are also targeted (Hatanaka 1988, Augustyn 1991). Differences in sex ratio occur during ontogenetic spawning migrations has the potential for a particular sex to be exploited more than other (Arkhipkin & Middleton 2002) and are commonly compared to 1:1 (Fisher's principle) based on the prediction that parental expenditure of males and females should be equal (Fisher 1930) The deepwater squid *Moroteuthis ingens,* shows sex specific migration patterns; mature females migrate to deeper water, whereas males do not show any clear pattern in their depth distribution (Jackson 1997). In *Sepioteuthis australis*, a greater portion of males caught during spawning can potentially modify sexual selection and mating behavior characteristics, as greater competition for males to mate favours squid that possess more attractive reproductive traits (Hanlon 1998, Hall & Hanlon 2002, Hibberd & Pecl 2007). Selectively biasing sex ratio in *S. australis* highlighted the need for management closures (Hibberd & Pecl 2007).

Catch selectivity of *N. gouldi* is unknown for jigging and trawling methods, but may influence the portion of the available stock caught. For example, squid caught in trawls will be dependent on mesh size. Jigging catches larger *Illex argentinus* than trawling in similar regions (Koronkiewicz 1995); whereas differences in length frequency distribution of *Loligo vulgaris reynaudii* are found between jigging, purse-seining, and mid-water trawling (Lipinski 1994). In the southern squid jig fishery (SSJF), most jigging effort occurs in waters off South Australia and Victoria where vessels are equipped with up to 12 automatic jig machines fitted with two spools of monofilament, each containing approximately 25 jigs (Larcombe & Begg 2008). High powered halogen lamps direct light downwards on the sea surface while casting a shadow underneath the hull to attracted squid that commonly aggregate in the shadowed area. Automatic lowering and retrieval of jigs prompts an attack of the illuminated jigs by squid, which is a behavioral response (Boyle & Rodhouse 2005). In contrast, demersal otter trawlers fish on the sea bed targeting species including pink ling *Genypterus blacodes* and morwong *Nemadactylus macropterus* (Kailola *et al.* 1993). Fish are herded to the net opening where they tire and fall back in to the codend (90 – 110 mesh size); *N. gouldi* is caught and retained as bycatch (Wilson *et al.* 2010).

In the SSJF, *N. gouldi* resources are managed beyond the 3 nm state jurisdiction using catch and effort restrictions implemented by the Australian Fisheries Management Authority (AFMA) where 560 (2011) standard machines are permitted. In 2008 and 2009, jig fishing resulted in 179 t (883 jigging hours) and 308 t (1229 jigging hours) of squid caught; whereas the Commonwealth trawl sector (CTS) caught 3.5 and 1.8 times more respectively (Wilson *et al.* 2010). Only 7% and 3% of the total *N. gouldi* catch in 2008 and 2009 respectively (trawl and jig sectors combined), were caught in the Great Australian Bight trawl sector (GABTS). Harvest strategies (HS) developed by AFMA provide safety measures in face of resource uncertainty and changing fishery dynamics (Dowling *et al.* 2007).

Harvest strategies are implemented in the *N. gouldi* resource where limits of catch, effort and catch per unit effort for all squid fishers (jig and trawl), signal the requirement for assessment and management review (Larcombe & Begg 2008) and were established prior to understanding what contribution each method has in removing different biological portions of the stock. Consequently HS may need to be assessed to determine if current measures provide an appropriate level of protection that promotes sustainability.

Unfortunately it is not possible to determine if differences in catch composition is in response to population and lifecycle characteristics, or selectivity in fishing gears as ommastrephids commonly possess inshore / offshore migratory characteristics and jiggers operate in different bathymetries to trawlers. Where biological characteristics including size and age distributions, sex ratio, and maturity status can be used to assess differences in catch composition, statolith (calcified balance organ) elemental composition can used to determine migration and life history characteristics. Used in conjunction, both approaches provide information used to explain biological and lifecycle differences between the two methods. Several studies have used elemental composition to identify ontogenetic migration characteristics in teleosts and cephalopods (Campana 1999, Elsdon & Gillanders 2003, Arkhipkin 2005, Elsdon & Gillanders 2006) in response to oceanographic and environmental differences (Arkhipkin *et al.* 2004a, Zumholz *et al.* 2007a), with their use in cephalopod research increasing (Semmens *et al.* 2007). The incorporation of different elements such as barium, strontium and magnesium into the hard parts of marine organisms changes as a function of environmental factors the concentration of these elements in seawater and water temperature. Therefore the concentration of the elements can be used to infer what environment an individual inhabited at a given time and location. For example, increases in barium (Ba) and strontium (Sr) concentrations in the statoliths of the squid *Gonatus fabrici* is suggested to be associated with movement to cooler waters (Zumholz *et al.* 2007b). The analysis of the elemental concentration of *N. gouldi* statoliths will be useful in elucidating inshore offshore mixing as well as ontogenetic migration characteristics.

The aim of this study is to compare the catch composition of *N. gouldi* caught inshore from the jig fishery to *N. gouldi* caught offshore from the trawl fishery using two approaches. Differences in size, sex, maturity, age, and growth rate will be used to explore biological differences among inshore and offshore collections; whereas statolith microchemistry will be used to determine whether *N. gouldi* caught in the two regions randomly mixed and explore the hypothesis about ontogenetic migrations between inshore and offshore waters. These findings have the potential to aid managers make informative decisions based on the biological characteristics of the animals caught by jiggers and trawlers.

3. METHODS

3.1. Samples

Nototodarus gouldi were collected at inshore and offshore locations off the western coast of Victoria, Australia between autumn and winter during 2007 and 2008. Squid samples (n=369) from inshore locations were caught by jig fishers operating on the continental shelf in waters < 110m depth sampled. Offshore samples (n=642) were collected by trawl fishers near the continental slope in waters > 250m depth. Jigged squid were caught approximately 25 km from trawled squid, perpendicular to the Australian coastline (Figure 3-1). Collections were grouped according to location of capture (inshore or offshore), year (2007 or 2008), and season of capture (autumn or winter) with details about exact location, dates of capture and biological summary statistics provided in Table 3-1.

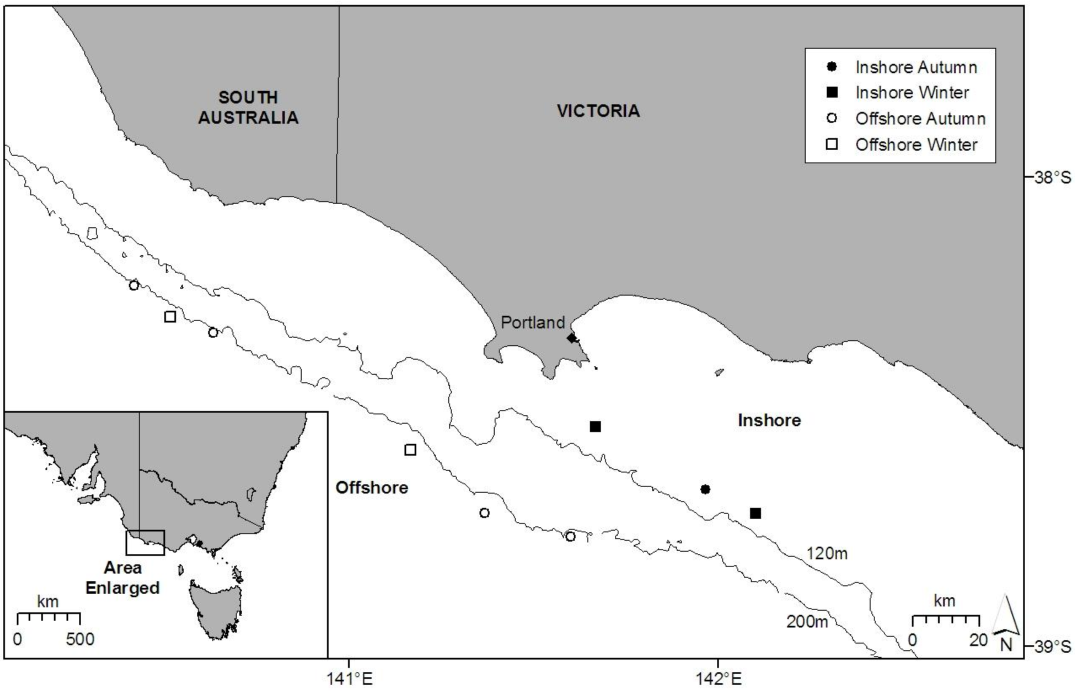

Figure 3-1 Map of south eastern Australia illustrating inshore (jig) and offshore (trawl) collection locations of *N. gouldi* during autumn and winter in 2007 and 2008

Table 3-1 Details of when and where *N. gouldi* were randomly selected from jiggers operating inshore and trawlers offshore off the south east coast of Australia. Average dorsal mantle length (DML) and standard deviation (s.d.) for males and females sampled on each collection day is provided.

Date of Capture	Location	Year	Season	Number	Depth (m)	Latitude	Longitude	Male Mean DML (±s.d.)	Female Mean DML (±s.d.)
14/03/2007	Inshore	2007	Autumn	95	75	38° 32`	141° 40`	280.9 (24.3)	316.6 (25.0)
17/06/2007	Inshore	2007	Winter	74	75	38° 32	141° 40`	301.8 (16.6)	338.9 (31.9)
14/05/2008	Inshore	2008	Autumn	100	80	38° 40`	141° 58`	285.0 (16.5)	325.5 (31.1)
02/06/2008	Inshore	2008	Winter	100	110	38° 43`	142° 06`	299.7 (19.5)	340.8 (25.0)
1/03/2007	Offshore	2007	Autumn	100	384	38° 43	141° 22`	279.0 (16.6)	305.1 (24.7)
27/03/2007	Offshore	2007	Autumn	100	250	38° 46	141° 36`	274.7 (17.3)	313.0 (26.9)
07/06/2007	Offshore	2007	Winter	100	293	38° 18`	140° 31`	271.6 (24.7)	279.7 (37.2)
14/05/2008	Offshore	2008	Autumn	142	290	38° 20`	140° 38`	266.9 (20.2)	291.7 (39.7)
25/05/2008	Offshore	2008	Autumn	100	366	38° 14`	140° 25`	275.2 (23.1)	291.6 (36.7)
22/06/2008	Offshore	2008	Winter	100	384	38° 35`	141° 10`	262.0 (32.2)	267.6 (42.8)

Standard commercial practices were used to catch squid. Jig vessels fished at night using up to 12 automatic jigging machines, each consisting of two monofilament spools equipped with approximately 25 jigs. Trawled squid were caught using demersal trawl nets fitted with 90 – 110 mm mesh size codends. Squid caught using both methods were chilled prior to unloading and subsequently frozen. Collections were transported to the University of Tasmania or the Marine and Freshwater Fisheries Research Institute in Victoria for storage prior to defrosting and dissection. Data acquired included total weight (TWt, g), dorsal mantle length (DML, mm), mantle weight (MWt, g), ovary weight (OWt, g), testis weight (TestWt, g), and sex. Maturity stage was determined based on methods developed by Borges and Wallace (1993) with stages I–III classified as reproductively immature and stages IV and V classified as reproductively mature. Within the cranium, the statocyst was located and both left and right statoliths removed, washed with distilled water, and stored dried in cavity trays. Age was determined by counting incremental structure from the hatch mark near the statolith core, to the edge of the dorsal dome using techniques described in Jackson (2005).

3.2. Biological comparisons

To determine whether size frequency distributions of *N. gouldi* caught from inshore and offshore locations differed at each combination of sex, year and season of collection, a Pearson's chi-square test of independence was used to compare DML frequency distributions. Dorsal mantle length was used as a measure of size as selectivity of codends is likely to be a function of lineal dimensions rather than weight. Where significant chi-square tests occurred, standardized residuals were used to determine for which size classes significant differences occurred between observed and expected frequencies. Reproductive maturity was shown in the size frequency distributions to give an indication of the proportion of reproductively mature animals (stages IV and V) at each 20 mm DML size class. A Pearson's chi-square test was used test whether the ratio of mature to immature at each size class differed from a ratio of 1:1. However, within a length class where 100% of the collection contained either immature or mature *N. gouldi*, a significant difference was allocated so long as assumptions of the test were met. Comparisons of DML frequency distributions between males and females were made using Pearson's chi-square test of independence for each combination of year, season and location. Sex ratio was used to indicate the relative contribution of males and females as a function of region, season, and year, with the number of males and females compared to a ratio of 1:1 using a chi-square test. Trends in sex ratio between inshore and offshore locations for autumn and winter collections were analysed to determine whether females have greater choice in male selection.

Reproductive condition was analysed for each sex by calculating and comparing the standardized residuals from the regression between MWt and testis weight for males; and MWt and ovary weight for females. Mantle weight was used as the independent variable rather than mantle length as weight is a more sensitive measure of size; using mantle weight rather than total body weight removed the influence of stomach mass and gonad mass. Individuals whose reproductive organs are lighter than a predicted (i.e. negative standardized residuals) organ weight are suggested to be in poorer reproductive condition than organs that are greater than the predicted weight (i.e. positive standardized residuals) (Moltschaniwskyj & Semmens 2000). Reproductive condition was analysed using a full three-way analysis of variance (ANOVA) to explore the effects of year, season and location, as well as their two and three way interaction on the mean standardized residuals calculated for each sex. Data transformation was not required as it met the assumption of the ANOVA. Tukey's post hoc tests were used to explore where significant differences were occurring. Only squid considered reproductively mature (stage IV and V) were used to calculate reproductive condition.

Growth rates of squid caught inshore and offshore were analysed by comparing the mean standardized residuals calculated from the relationship between MWt and estimated age. Individuals whose mantle weights are lighter than the predicted MWt are suggested to have growth slower than individuals whose MWt is greater than the predicted. Means standardized residuals were compared using a three-way ANOVA to explore the effects of year, season and location. Data transformation was not required as it met the assumption of the ANOVA. Tukey's post hoc tests were used to explore where significant differences were occurring. Age frequency was analysed between inshore and offshore collections for each sex, year and season using Pearson's chi-square test of independence.

To compare frequency of maturity stages between inshore and offshore animals a chi-square analysis was used. Due to few animals in some maturity stages years of collection were combined with comparisons made for each season and sex. For males, distributions were compared by combining stages I–III into a single category due to low numbers represented in stages I and II. Maturity stages I–V were used when analysing female maturity. Mean age at reproductive maturity (stage IV) was calculated and compared using an ANOVA to determine if differences between locations or seasons exist.

3.3. Statolith element composition analysis

To determine if inshore and offshore animals had a unique elemental signature in the statolith, the average concentration of elements representing approximately 10 days prior to death was compared between inshore and offshore collections. It was assumed that physiological and environmental processors from squid at both locations provide present unique signatures to begin with. Based on movement characteristics found by Stark (2005), 10 days was used to represent element concentrations within the statolith, as longer periods may increase the probability of movement between locations.

Different element concentrations among *N. gouldi* from inshore and offshore locations may infer that squid have been present at their location of capture for a period of 10 days without migration, provide that a unique elemental concentration exists at both locations. Ontogenetic migrations were examined by analysing element concentration at three different regions within the statolith; the 'core' representing the time when the squid hatched; the 'mid-point' at approximately 120 days of age; and 'edge' representing the time period prior to death (Figure 3-2). A total of 40 statoliths (20 inshore, 20 offshore) from *N. gouldi* caught during March 2007 were randomly selected and prepared to determine elemental concentrations at the three statolith regions. Using a 1 cm^2 silicone mould, statoliths were positioned on a thin layer of partially cured Aka Resin™ with the anterior side of the statolith tilted at approximately five degrees. Tilting was necessary so that the correct grinding plane could be achieved. Additional resin was used to completely embed each statolith; which were left to cure for approximately 12 hours at 30 °C. Resin blocks were removed from the silicone mould and excess resin trimmed using an Isomet™ saw until a small block containing the statolith was achieved. Resin blocks were attached to glass slides and ground using a series of increasingly finer grades of lapping film (3M®). A grade of 30 µm, followed by 10 µm lapping film was used to locate the statolith core slightly under the ground surface using a Leica compound microscope at magnification up to x400. When the area between the core and edge was exposed, statoliths were polished using 5 µm lapping film. Preparations were randomly sorted and renumbered prior to analysis. Five randomly selected statoliths were removed from their preparation slide and attached to a secondary glass microscope slide using the thermoplastic glue Crystalbond™. Statolith preparations were cleaned by sonication in MilliQ water for three minutes, triple rinsed, and let to air dry in a laminar flow cabinet for at least 12 hours.

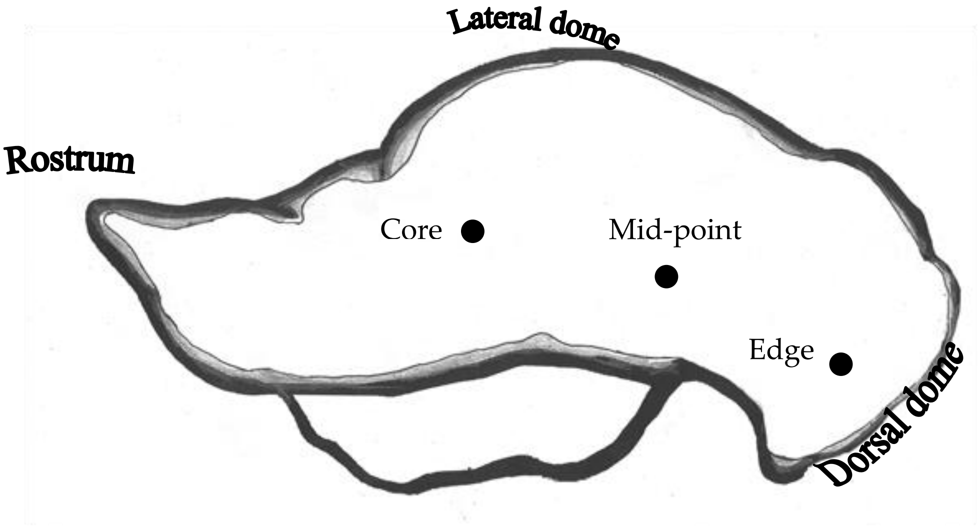

Figure 3-2 An example of a ground *N. gouldi* statolith with three ablation regions (black dot): core, mid-point, and edge.

Statolith element concentrations were determined using a Thermo Finnigan Element2 High Resolution Inductively Coupled Plasma Mass Spectrophotometer (HR-ICP-MS) in conjunction with a New Wave Research UP-213 Nd:YAG ultraviolet laser microprobe (Q-switched) based at the Marine and Freshwater Fisheries Research Institute, Queenscliff, Australia. Each ablation region (core, mid-point, and edge) was further decontaminated by pre-ablating the region of interest prior to element sampling using the laser at 80 µm spot size (pulse rate 6Hz, ≈9.5 J/cm^2) for 3 seconds. Each sample acquisition consisted of 15 blank seconds, followed by ablating the statolith for a period of 25 sec with the laser at 30 µm spot size (pulse rate 6Hz, ≈9.5 J/cm). The first 10 scans of the ablation period were excluded from analyses to allow for signal stabilization. The background concentration of elements in the instrument, determined from the blanks scans were subtracted from raw counts. Data was collected for the elements, ^{25}Mg, ^{44}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{138}Ba, and ^{208}Pb. Calcium was used as an internal standard to control for variation in the ablation yield with the concentration set at 388,000 µg g^{-1}. Element concentration was presented in µmol mol^{-1} Ca. Consistency between sample ablation was achieved using the National Institute of Standards (NIST) SRM 612 glass wafer was used after every five samples (Lahaye *et al.* 1997, Hamer *et al.* 2003). Detection limits of the instrument were calculated for each sample based on three standard deviations of the blank gas sample determined at the beginning and the analysis day. These data were used to highlight and reject data as a result of unwanted instrument noise. Average limit of detection based as a ratio of calcium (µmol mol^{-1}Ca) were Mg=2.26, Mn=0.17, Sr=1.92, Ba=0.39. Although levels of Li, Cu, Zn, Rb, and Pb were detected, most samples were below detection limits and were not used in analysis. Accuracy was calculated for individual elements as the mean percentage recovery of NIST SRM 612 (Mg=99.3 %, Ca=101.5 %, Mn=98.9 %, Sr=97.5 %, Ba=97.7 %). Precision, presented as the mean relative standard deviation of individual elements found in the NIST SRM 612 standard, was Mg=6.0 %, Mn=2.7 %, Sr=4.43 %, and Ba=2.5 %). After statolith ablation was complete, individual statoliths were viewed using a compound microscope to check whether ablation regions were accurately positioned. Data was not used where ablations were inaccurately aligned to their required ablation region.

To determine if elemental concentration present in the statolith edge differed between inshore and offshore collections and inferring separation, multivariate analysis of variance was used. Only Mg required a square root transformation to conform to assumptions of analyses. Using age estimates derived from interpreting incremental structure of statoliths indicated that squid caught in March 2007 (inshore and offshore collections combined) hatched from May – August 2006. Consequently the core and mid-point statolith regions represents elements that are incorporated at any time (sample dependent) over a four month period from squid that hatched and grew at unknown locations. As the concentration of elements can be reflective of physiological and environmental conditions (Arkhipkin 2005, Zumholz *et al.* 2007b); comparing elements in the core and mid-point region from samples collected in inshore and offshore regions has little relevance as the origin of where squid hatched and where they are located during the mid-point period is unknown.

The average concentration of elements at three different ablation regions was used to determine if ontogenetic migration from inshore and offshore regions (and vice versa) was occurring, and was analysed using multivariate analysis of variance (MANOVA). However element concentrations in the statolith core and mid-point may be subjected to greater variation in environmental and physiological factors as their location is unknown at both ablation regions. Consequently element concentrations were compared using research from species whether ontogenetic migrations exist.

4. RESULTS

For male and female squid, although there was no difference in the DML frequency distribution between inshore and offshore collections caught in autumn 2007, there were significant differences in the other year and season combinations (Table 3-2). For males, proportionally more larger squid were found inshore (Figure 3-3). For example, during winter 2007 and autumn 2008, relatively more large male squid (e.g. DML ≥300 mm and ≥280 mm respectively) and fewer small male squid (e.g. DML ≤240 mm) were found inshore compared with offshore locations. This trend was accentuated during winter 2008 with proportionally fewer smaller male squid (≤220 mm DML) and more large squid (300 mm DML) found inshore. For females caught inshore during autumn 2008, a significant lesser proportion of smaller squid (≤280 mm DML) and a significant greater proportion larger squid (≥320 mm DML) were represented in the catch compared with offshore collections (Figure 3-3). A similar trend in frequency distributions was observed in winter 2007 and 2008, where a significant lesser proportion small female squid (≤280 mm DML) were found inshore and more a significant greater proportion found offshore. This characteristic was reversed for larger squid ≥340 mm DML where significantly more squid were found inshore and less offshore during (Figure 3-4)

Table 3-2 Chi-square statistics comparing the dorsal mantle length distribution between inshore and offshore locations for each season, year and sex combination.

Season Year	Sex	χ^2	*df*	*p*
Autumn 2007	Male	3.11	4	0.540
Winter 2007	Male	21.89	3	<0.001
Autumn 2008	Male	20.31	3	<0.001
Winter 2008	Male	45.78	5	<0.001
Autumn 2007	Female	2.97	3	0.395
Winter 2007	Female	43.46	3	<0.001
Autumn 2008	Female	25.25	3	<0.001
Winter 2008	Female	39.65	3	<0.001

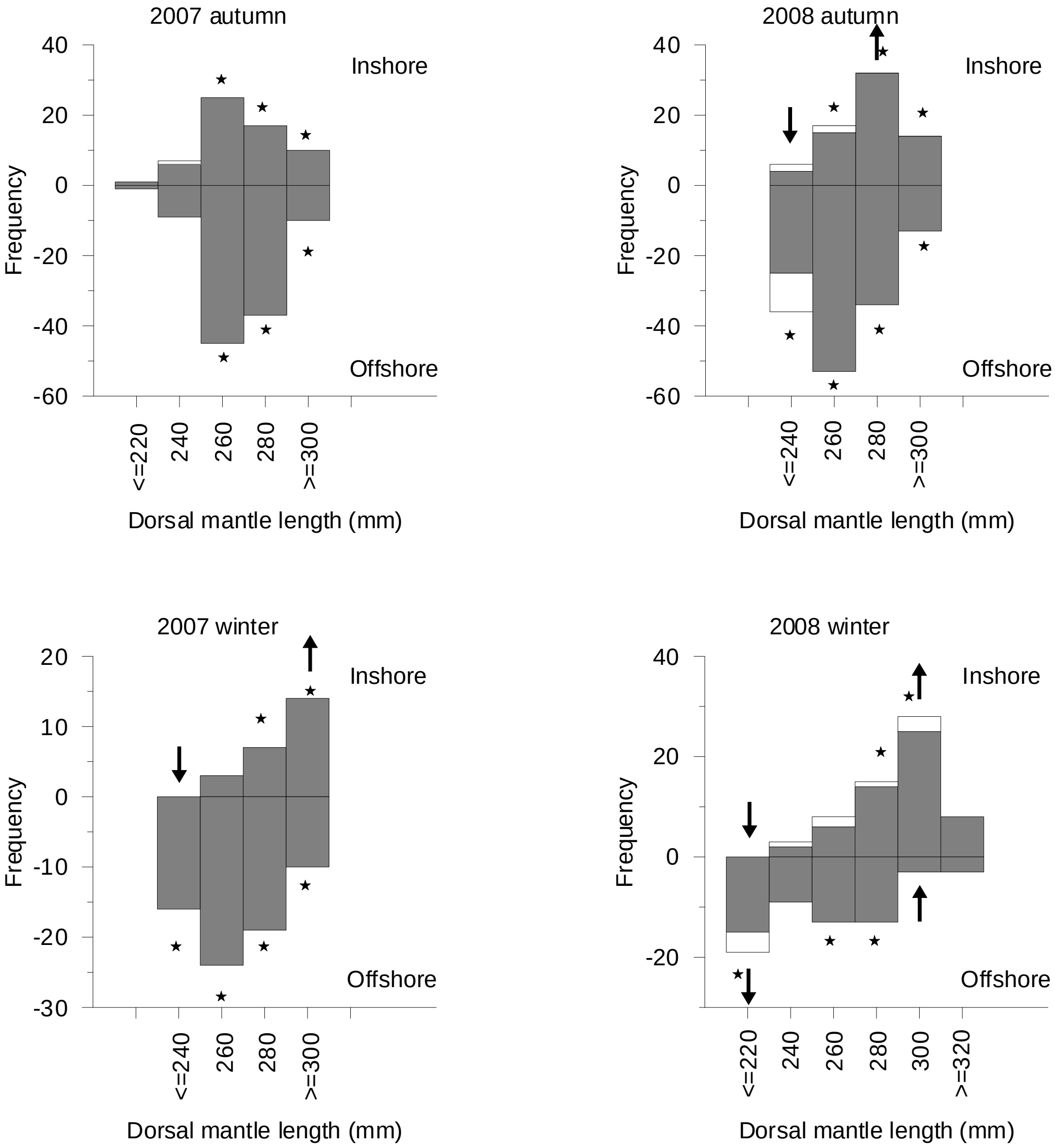

Figure 3-3 Dorsal mantle length frequency distribution of male *N. gouldi* collected from inshore and offshore locations off south eastern Australia by season and year of collection. Positive axis represent inshore collections, negative axis represents offshore collections. Shaded bars represent reproductively mature squid (stage IV–V), open bars represent reproductively immature squid (stages I–III). Arrows indicate the direction of differences between observed and expected frequencies based on the χ^2 test. Where comparisons between mature and immature meet assumptions, an asterisk indicates where mature / immature ratio differs from 1:1.

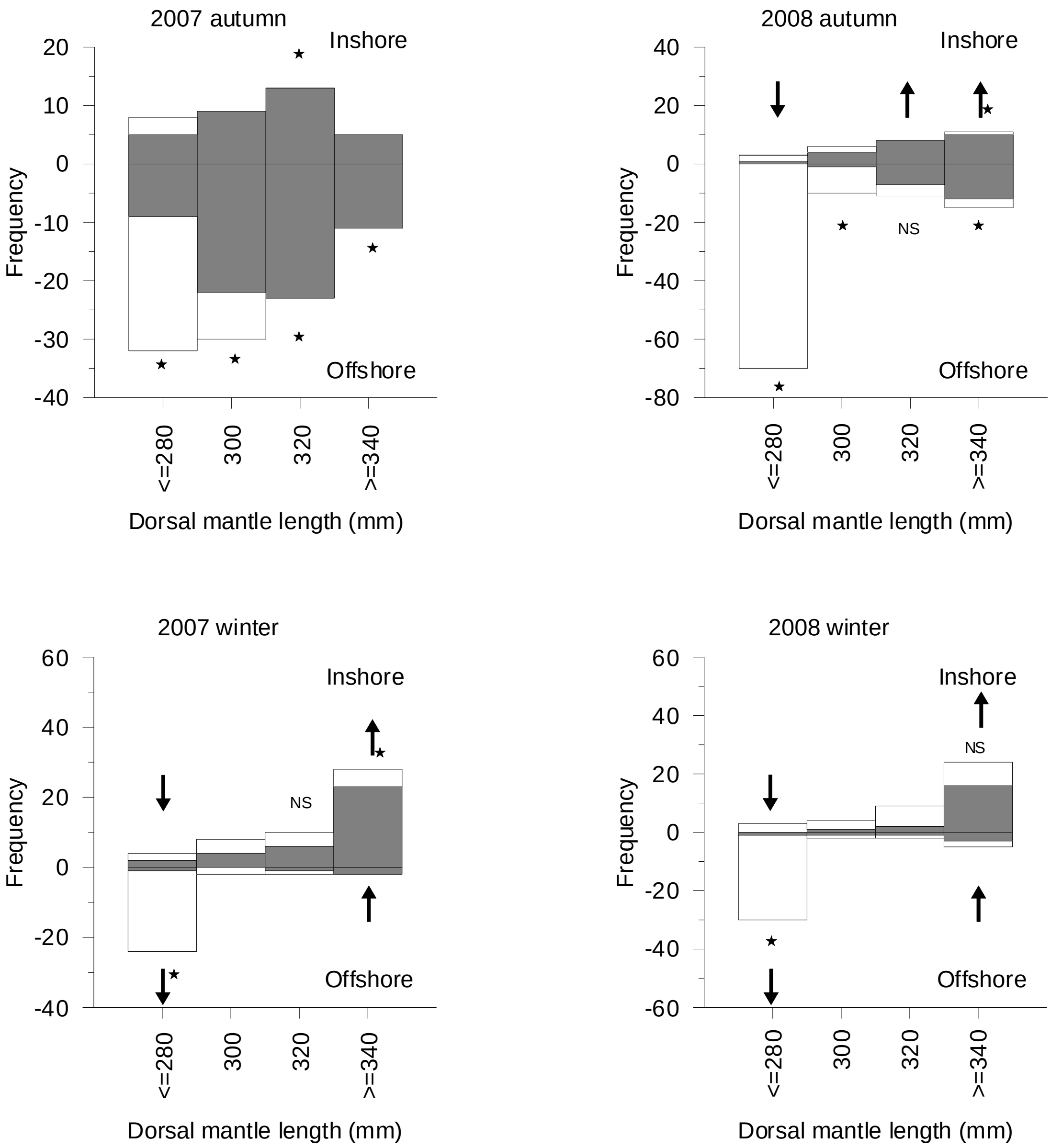

Figure 3-4 Dorsal mantle length frequency distribution of female *N. gouldi* collected from inshore and offshore locations off south eastern Australia by season and year of collection. Positive axis represent inshore collections, negative axis represents offshore collections. Shaded bars represent reproductively mature squid (stage IV–V), open bars represent reproductively immature squid (stages I–III). Arrows indicate the direction of significant differences between observed and expected frequencies based on the χ^2 test. Where comparisons between mature and immature meet assumptions, NS indicates where mature / immature ratio was not different from 1:1; asterisk indicates where mature / immature ratio differs from 1:1.

All male squid collected during 2007 were reproductively mature except for squid caught inshore during autumn where 14% of the 240 mm DML class was reproductively immature (Figure 3-5). In 2008, male squid caught offshore during autumn ≤ 240 mm DML and during winter ≤ 220 mm DML comprised of significantly more mature animals (Table 3-3). For females, a greater number of reproductively mature squid was found inshore (χ^2=26.5, df 1, p<0.001) with a greater number of immature *N. gouldi* caught at offshore locations (χ^2=24.2, df 1, p<0.001) (Figure 3-5). For all years, seasons, and locations, collections of female *N. gouldi* ≤280 mm DML consisted of significantly more immature animals (Table 3-4). During winter female *N. gouldi* collected offshore consisted of significantly greater numbers of smaller immature animals and significantly fewer numbers of larger animals (Figure 3-5).

Table 3-3 Chi-square statistics comparing the mature (M) and immature (I) male *N. gouldi*. Tests are only presented where test assumptions were met.

Season Year	Location	DML class	χ^2	*df*	*p*	*n* M / I
Autumn 2008	Inshore	260	9.94	1	0.002	15/2
Autumn 2008	Offshore	240	5.44	1	0.020	25/11
Winter 2008	Inshore	280	11.26	1	0.001	14/1
Winter 2008	Inshore	300	5.44	1	<0.001	25/3
Winter 2008	Offshore	≤220	6.36	1	0.012	15/4

Table 3-4 Chi-square statistics comparing the mature (M) and immature (I) female *N. gouldi*. Tests are only presented where test assumptions were met.

Season Year	Location	DML class	χ^2	*df*	*p*	*n* M / I
Autumn 2007	Offshore	≤280	6.22	1	0.013	9/23
Autumn 2007	Offshore	300	6.53	1	0.011	22/8
Autumn 2008	Inshore	≥340	7.36	1	0.007	10/1
Autumn 2008	Offshore	300	6.40	1	0.01	1/9
Autumn 2008	Offshore	320	0.81	1	0.366	7/4
Autumn 2008	Offshore	≥340	5.40	1	0.020	12/3
Winter 2007	Inshore	320	0.40	1	0.527	6/4
Winter 2007	Inshore	≥340	11.57	1	0.001	23/5
Winter 2007	Offshore	≤280	20.16	1	<0.001	1/23
Winter 2008	Inshore	≥340	2.66	1	0.101	16/8
Winter 2008	Offshore	≤280	26.13	1	<0.001	1/29

Comparing male and female DML frequency distributions indicated no significant difference for squid caught offshore during winter of 2007 and 2008; however, all remaining season, year and location combinations did show significant difference in frequency distributions between sexes (Table 3-5). Squid caught at both inshore and offshore locations during autumn 2007 and 2008 had a significantly greater number of larger (≥320 mm DML) females compared with males; whereas during winter of

2007 and 2008 this trend was not evident (Figure 3-5). For all season and collection year combinations except winter 2007, a significantly greater number of males compared with females were present inshore locations; however, no significant difference in F:M ratio was found offshore. In winter 2007, the trend was different with significantly more females and males present at inshore and offshore locations respectively.

Table 3-5 Chi-square statistics comparing the dorsal mantle length distribution between sex for inshore and offshore locations for each season and year combination.

Season Year	Location	χ^2	*df*	*p*
Autumn 2007	Inshore	37.08	4	<0.001
Autumn 2007	Offshore	76.96	4	<0.001
Winter 2007	Inshore	11.98	1	<0.001
Winter 2007	Offshore	1.12	2	0.570
Autumn 2008	Inshore	60.56	4	<0.001
Autumn 2008	Offshore	42.68	4	<0.001
Winter 2008	Inshore	14.20	2	<0.001
Winter 2008	Offshore	4.69	2	0.107

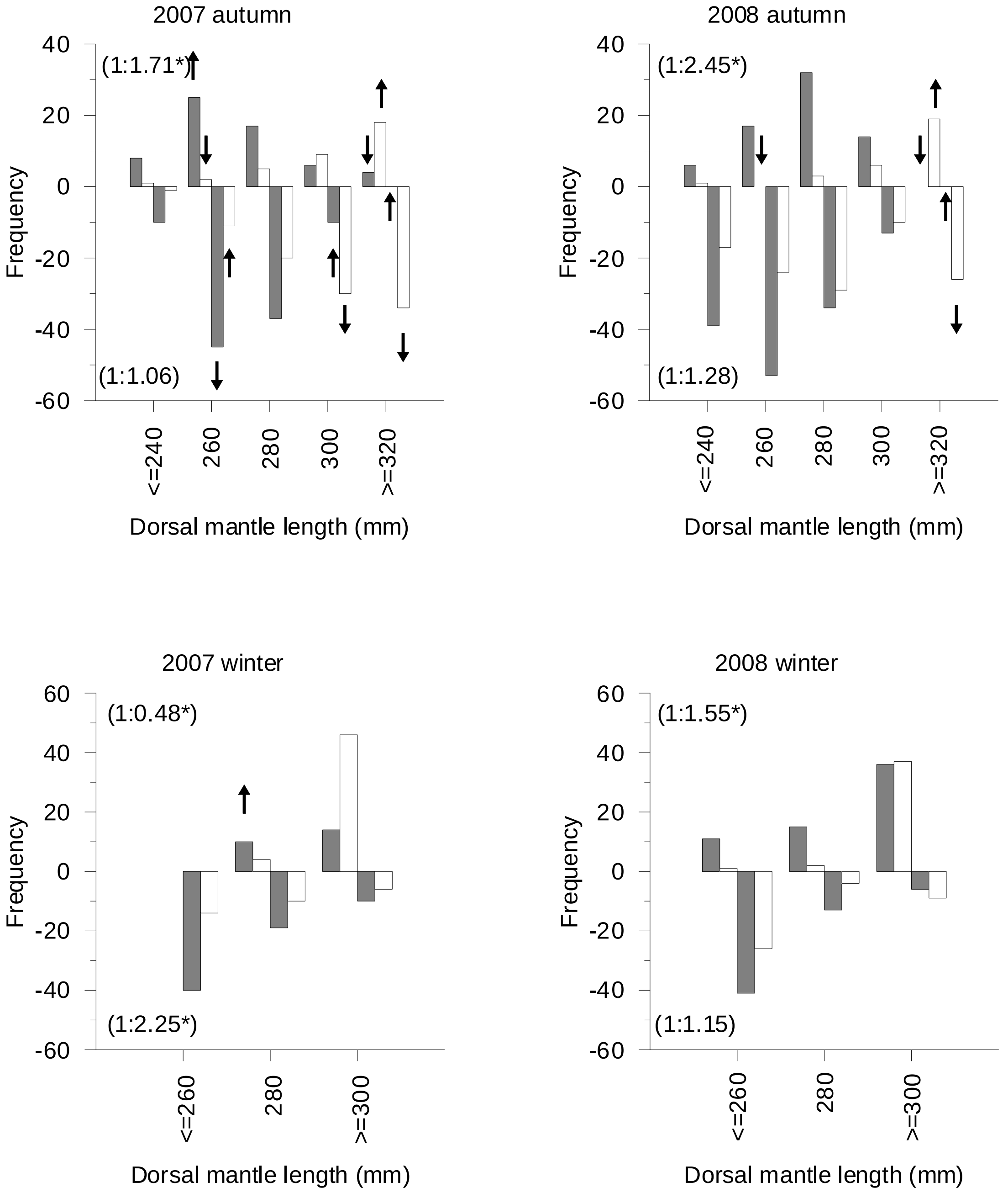

Figure 3-5 Dorsal mantle length frequency distribution of *N. gouldi* by sex. Positive axis represent inshore collections, negative axis represents offshore collections. Shaded bars represent male squid, open bars represent female squid. Arrows indicate the direction of significant differences between observed and expected frequencies based on the χ^2 test. F:M ratio presented in parentheses, Asterisk indicates where F:M ratio differs from 1:1.

Comparing mean residuals of the relationship between testis weight and mantle weight showed significant difference among location, year, and season ($F_{location*year*season}$ = 6.73, df 2,571, p<0.001) with inshore and offshore *N. gouldi* caught during autumn 2007 in better reproductive condition compared all remaining collections (Figure 3-6). For all collection year season combinations no difference in mean residuals was observed between inshore and offshore collections. Reproductive condition was at its poorest during winter in both years of collection.

A three-way ANOVA (location x year x season) was not able to used to compare the mean residual of the relationship between ovary weight and mantle weight due to low number of replicates collected offshore during winter 2007, and inshore and offshore during winter 2008. However, significant differences in mean residuals among remaining combinations of location, year, and season were found ($F_{collection\ combination}$ = 13.13, df 4,168, p<0.001). Greatest reproductive condition was found in *N. gouldi* collected during autumn 2007 compared with all remaining collection combinations (Figure 3-7). No difference in female reproductive condition was found among *N. gouldi* collected inshore and offshore during similar seasons and years of collection.

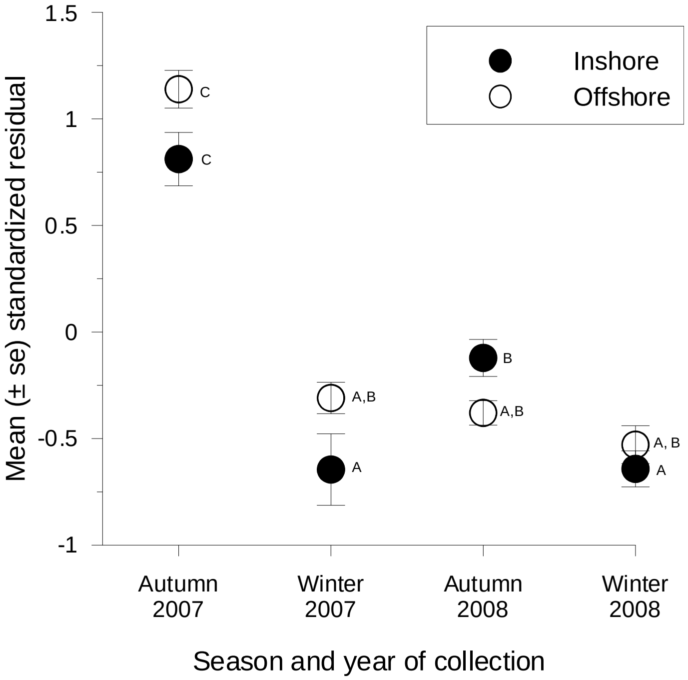

Figure 3-6 Mean standardized residuals (± se) of the relationship between dorsal mantle weight and testis weight by location of capture, season and year of collection. Solid points indicate inshore, open points offshore. Like letters indicate where means are similar using Tukey's post-hoc test.

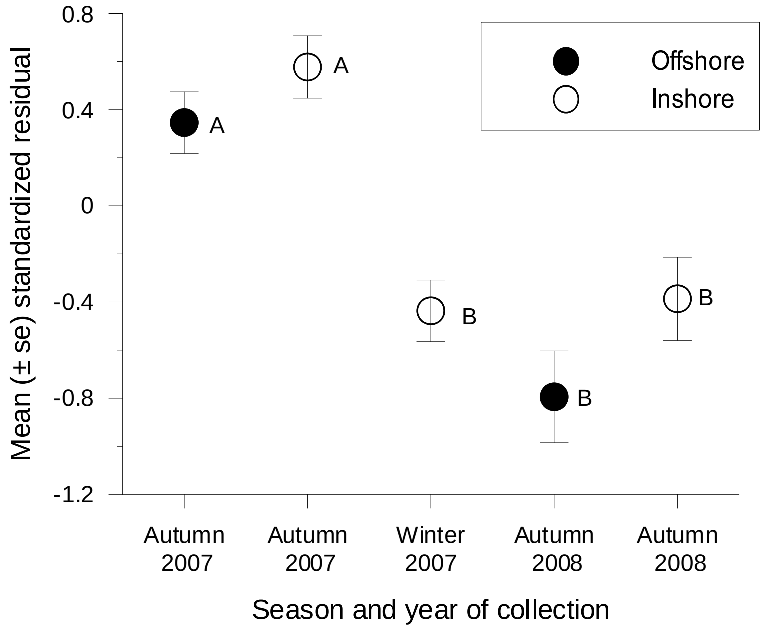

Figure 3-7 Mean standardized residuals (± se) of the relationship between dorsal mantle weight and ovary weight by location of capture, season and year of collection. Solid points indicate inshore, open points offshore. Like letters indicate where means are similar using Tukey's post-hoc test.

Analysing growth showed no significant interaction between sex and location (factors) in the relationships between MWt (response) and estimated age (covariate) ($F_{sex*location*age}$ = 2.13, df 2,1000, p=0.119); however significant interaction was found between sexes and location ($F_{sex*location}$ = 6.37, df 1,1000, p=0.012). Consequently comparing the difference in mean standardized residuals between inshore and offshore collections was conducted on sexes separately. Analysing growth differences by comparing mean standardized residuals of the relationship between Mwt at age showed significant location, year and season interaction for females ($F_{location*year*season}$ = 7.45, df 2,416, p<0.001) and males ($F_{location*year*season}$ = 4.18, df 2,576, p=0.016). Apart from squid caught in autumn 2007, a similar trend in mean residuals was apparent for males and females, with squid caught inshore being larger for their age compared with squid caught offshore for all other seasons and years of collection (Figure 3-8). Both male and female squid collected inshore during winter in 2007 and 2008 were significantly larger for their age compared with all other collections. Independent of season or year, male squid caught offshore were similar in their size-at-age.

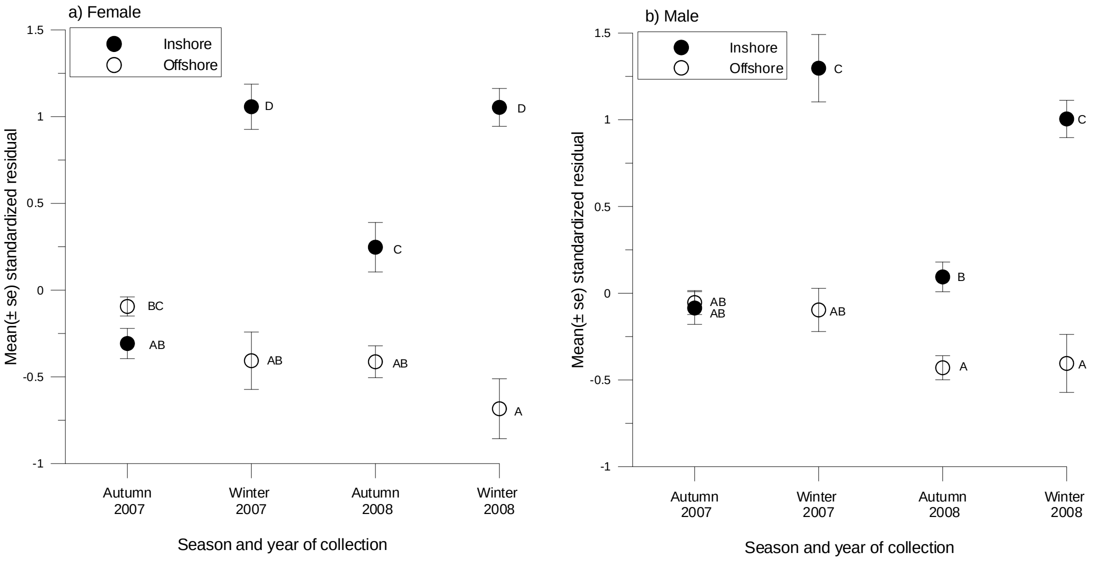

Figure 3-8 Mean standardized residuals (± se) of the relationship between dorsal mantle weight and estimated age by location of capture, season and year of collection for a) females and b) males. Solid points indicate inshore, open points offshore. Like letters indicate where means are similar using Tukey's post-hoc test for each sex.

Comparing age frequencies distributions between inshore and offshore collections for each sex, season and year combination showed significant differences apart from females collected in autumn in 2007 (Table 3-6). For males squid caught during winter 2007 comparisons between inshore and offshore collections could not be made as assumptions (i.e. required numbers) of the chi-square test were not met. The lack of aged squid from this sample was due to difficulties in interpreting incremental structure on statoliths and greater numbers of failed preparations. Age frequency distributions of males caught in 2008 (Figure 3-9) showed that significantly more than expected were found inshore at older age classes (e.g. 240 days and ≥260 d). In contrast significantly younger males were found (≤ 200 d) offshore during similar periods (Figure 3-9). Females showed a similar trend to males with older squid (≥260 d) found inshore and younger squid found offshore.

Table 3-6 Chi-square statistics comparing the estimated age frequency distribution between inshore and offshore collections for each season, year and sex combination. Chi-square statistics not presented for males collected winter 2007.

Season / Year	Location	χ^2	*df*	*p*
Autumn 2007	Male	14.76	3	0.002
Autumn 2008	Male	63.70	3	<0.001
Winter 2008	Male	38.25	3	<0.001
Autumn 2007	Female	6.49	3	0.09
Winter 2007	Female	12.48	2	0.002
Autumn 2008	Female	22.00	3	<0.001
Winter 2008	Female	37.04	3	<0.001

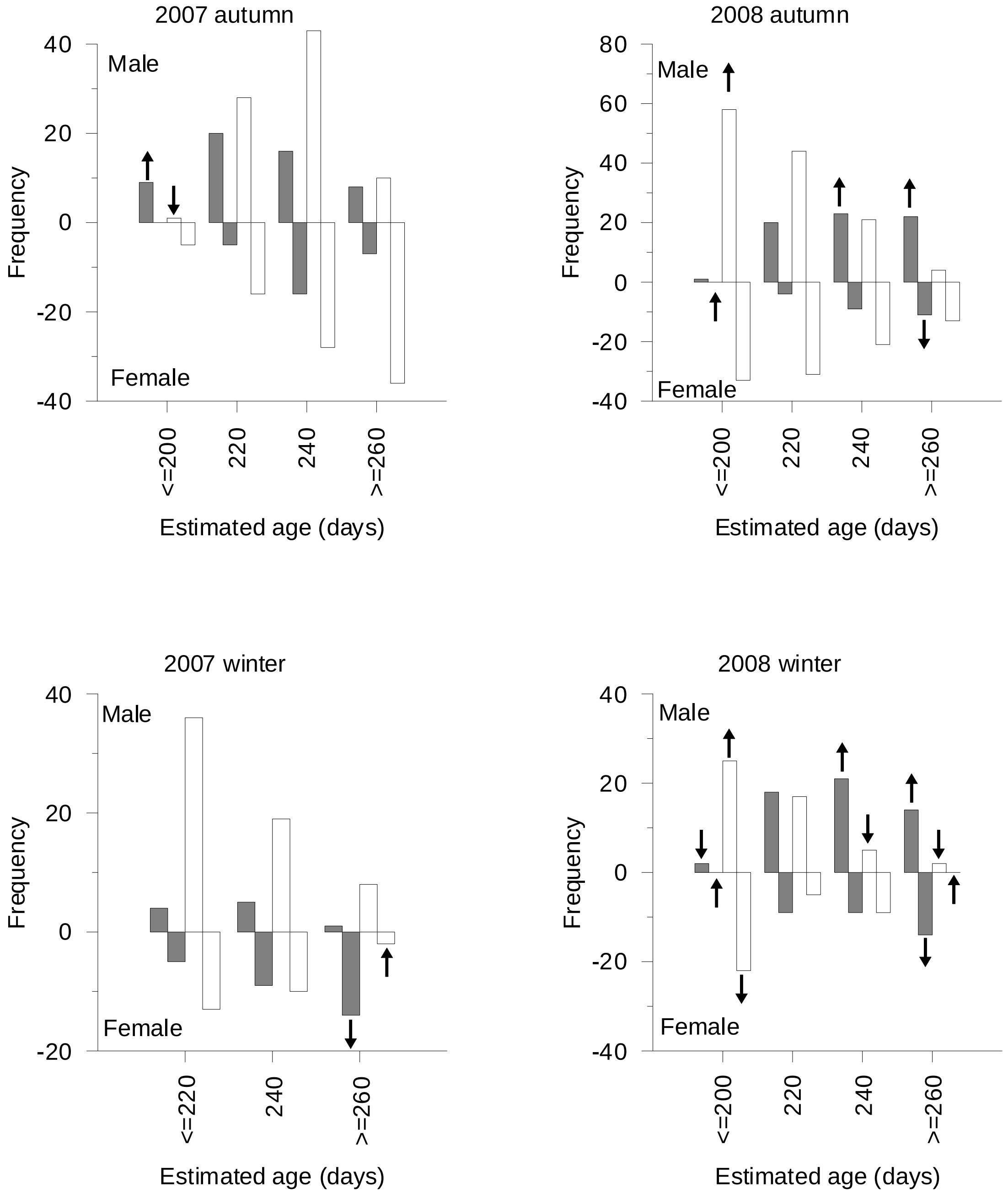

Figure 3-9 Estimated age frequency distribution of *N. gouldi* by sex. Positive axis represent males, negative axis represent females. Shaded bars represent inshore collections, open bars represent offshore collections. Arrows indicate the direction of differences between observed and expected frequencies when comparing inshore and offshore collections based on the χ^2 test for each sex.

Comparing maturity stage frequency distributions between inshore and offshore collections (Figure 3-10) showed significant difference for males collected in autumn (χ^2=27.98, df 2, p<0.001) and winter (χ^2=17.19, df 2, p<0.001). Within the maturity stage frequency distribution a greater proportion of males at stage IV were found inshore compared with offshore. Distribution of maturity stages for females were more complex, compared with males, with significant differences between inshore and offshore during autumn (χ^2=37.6, df 4, p<0.001) and winter (χ^2=41.5, df 4, p<0.001). In both seasons a greater than expected number of stage V females were found inshore, with fewer than expected found offshore (Figure 3-10). Similarly fewer stage I females were found inshore for both autumn and winter. No significant interaction in the mean age at first maturity for males was found between location and season of capture; however, differences among locations ($F_{location}$=4.43, df 1,110, p=0.038) did exist. Mean age at first maturity was 226.0 d (± 9.3se) and 194.6 d (± 9.6se) for males caught inshore and offshore respectively. Mean age at first maturity for females was 255.0 d (± 4.9 se) and 228.0 d (± 38.9 se); however, no significant differences in main effects of interactions of location and season were found.

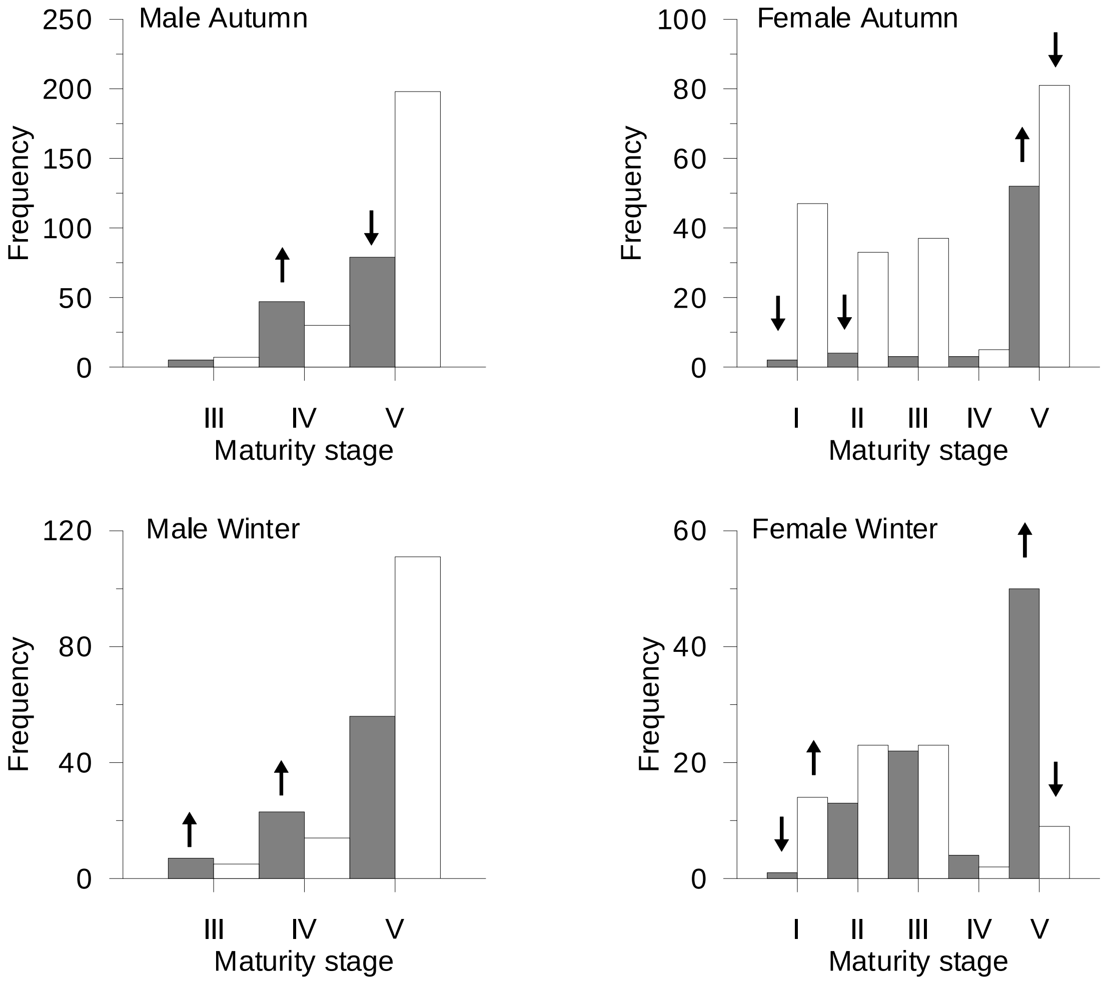

Figure 3-10 Maturity stage frequency distribution for males and females collected in autumn and winter (year of collection combined). Shaded bars indicated inshore collections, open bars indicate offshore collections Arrows indicate the direction of differences between observed and expected frequencies when comparing inshore and offshore collections based on the χ^2 test for each sex and season. For males, stage III consists of stages I–III.

Comparing the concentration of elements in the pre-edge region of statoliths used to analyse inshore / offshore mixing showed no significant difference among location of capture (MANOVA $F_{location}$= 0.270, df 4,19, p=0.89), when incorporating Mg, Mn, Sr and Ba as dependent variables. When comparing the composition of element concentrations from three different ablation regions (core, mid-point, edge), collected from two locations (inshore and offshore) used to analyse ontogenetic stages showed no significant interaction ($F_{location*ablation\ region}$ = 0.657, df 8,158, p=0.73). However, differences were found between ablation regions ($F_{ablation\ region}$ = 5.88, df 8,158, p<0.001). Element concentration of the statolith core was different from both the mid-point and edge concentrations (Figure 3-11). Greater concentrations of Mn compared with all other elements contributed to majority of the difference along the first axis with the core having 1.5 times more Mn compared with the mid-point and twice the concentration of the edge (Table 3-7). Concentrations of magnesium, strontium and barium were highly correlated with one another but there was little variation in these elements among the three regions.

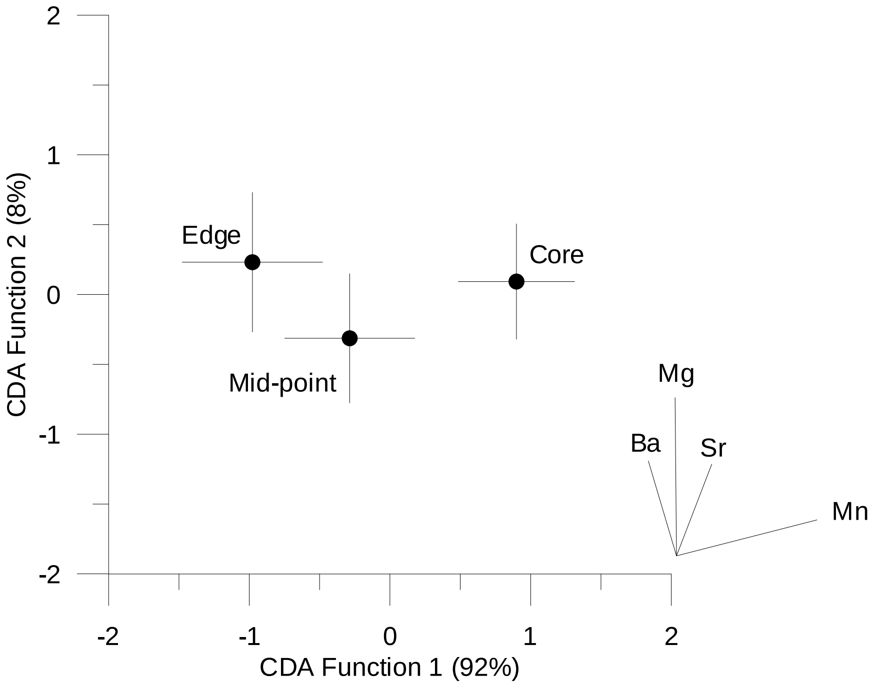

Figure 3-11 Canonical discriminate analysis plot showing the variation in elemental composition of statolith from *N. gouldi* collected at the core, mid-point and edge ablation regions. Error bars represents the 95% confidence intervals around the centroid mean. The length and direction of the vectors for each element indicate the correlation between the elements and the axes Percentages indicated the level of variation explained in each axis.

Table 3-7 Mean (±se) element concentrations (µmol mol^{-1}Ca) for the core, mid-point and edge ablation regions.

	MEAN ABLATION CONCENTRATION (± SE)		
ELEMENT	*Core*	*Mid-point*	*Edge*
Mg	66.82 (6.04)	52.96 (2.99)	71.08 (7.96)
Mn	0.62 (0.05)	0.40 (0.02)	0.31 (0.03)
Sr	7131.78 (131.84)	6853.13 (86.15)	6950.98 (98.69)
Ba	7.14 (0.17)	7.10 (0.11)	7.44 (0.17)

5. DISCUSSION

Nototodarus gouldi caught inshore and offshore using jigs and trawls respectively showed significant differences in biological parameters for size, maturity, age, sex ratio, and growth rate. As jig fishers catch *N. gouldi* at different bathymetries compared to trawlers, it is not possible to determine if differences represent population characteristics or differences in fishing gear performance. The observed differences are likely to be influenced by ontogenetic characteristics, behavioral mechanisms in response to fishing gear, as well as selectivity of fishing methods. Similarities in the composition of elements in the statolith for the 10 days prior to capture between inshore and offshore locations are just as likely to be attributed to similar water chemistry in each location as movement between regions. Statolith ablation regions (core, mid-point, edge) showed significant differences in statolith core element concentrations compared with mid-point and edge ablation regions indicating that ontogenetic changes (e.g. habitat shift or physiological processors) in *N. gouldi* occur.

Male and female *N. gouldi* caught inshore were significantly larger than those caught offshore during similar seasons of capture. This characteristic is similar to other cephalopod fisheries worldwide and directly relate to maturation and subsequent spawning, as well as in response to elevated food requirements (Perez & O'Dor 1998). Although inshore ontogenetic migration of *N. gouldi* cannot be confirmed due to differences in fishing methods at each location, it is likely to be major contributing factor. Ontogenetic migration of adults inshore is known to occur in *Loligo gahi* (Arkhipkin *et al.* 2004b, Arkhipkin *et al.* 2004c), *L. vulgaris reynaudii* (Augustyn 1990) and *Illex argentinus* (Hatanaka 1988). In contrast, female *Moroteuthis ingens* and *Todarodes sagittatus* (from the north-east Atlantic) migrate to deeper water in association with maturity (Jackson 1997) and increasing size respectively (Quetglas *et al.* 1999, Lordan *et al.* 2001). Although little difference in seasonal variation in size composition was evident in *N. gouldi*, seasonal migration differences are observed in *T. pacificus* (Arkhipkin *et al.* 1999). Spatial and temporal growth differences found in *N. gouldi* collections during

2007 and 2008 is a typical characteristic of this species which is similar to previous studies. In 2000 and 2001, variation in *N. gouldi* growth rates using total weight as an indication of size also showed differences presumably due to changes in sea surface temperature, sea surface colour and food availability (Jackson *et al.* 2003). However, since growth is measured over an individual's lifetime, growth variations are difficult to interpret. The mantle weight of males and females were on average heavier at a given age when caught inshore. This may suggest squid inhabiting inshore locations are spending sufficient amount of time to change their lifetime growth rate, although factors contributing to such differences are unknown.

Sex ratio significantly differed with greater number of male *N. gouldi* found inshore. In contrast, all offshore collections showed similar sex ratios (except during winter 2007). In a different study during 2000/01, majority of *N. gouldi* collections made offshore by trawlers also showed similar sex ratios (Jackson *et al.* 2003). Such disparity in sex ratio among inshore and offshore collections suggests sexual dimorphism with respect to ontogenetic migration or alternatively, due to their responsiveness towards fishing gear. For *Loligo vulgaris reynaudii*, females actively feed in between spawning events (Hanlon 1998), which may explain the disparity in F:M ratios caught jigging if *N. gouldi* share similar characteristics. Highly biased numbers of males on spawning grounds is common in other species, for example *Sepia apama* and *Sepioteuthis australis* displayed F:M ratio of 1:11 and 1:6.5 respectively (Hall & Hanlon 2002, Hibberd & Pecl 2007). Ratios naturally skewed toward males increases male competition to mate as well as allowing females to select 'fittest' or most 'attractive' males (Hanlon 1998, Hibberd & Pecl 2007). A greater percentage of mature females were found inshore compared with offshore collections, whereas mature males were found at both regions. Males found inshore were also in lesser reproductive condition compared with offshore collections though reasons are unclear. One theory to explain this could be that males develop reproductively offshore, and then migrate inshore where numbers increase in preparation to spawning. Although males caught during winter 2007 and 2008 shared the lowest reproductive condition, this was not suggested in a different study on *N. gouldi* where a tight correlation between somatic gonad investment occurred over time (McGrath Steer & Jackson 2004). As no measure of spent males was recorded, lesser reproductive condition may be a function of recent spawning activity. Females were in greater reproductive condition during autumn 2007; however, since spent females were not recorded, no indication on spatial variability of spawning females was obtained. Females collected during winter 2007 and autumn in 2008 displayed lower reproductive condition compared other combinations of location, year and season; a result similar to results found by McGrath (2004).

The minimum size class of *N. gouldi* caught was represented in trawl and jig methods indicating that differences attributed to selectivity were the same for small squid. However, squid less than the 220 mm DML size class were not found indicating that juveniles were not represented in the present population or significant selectivity of *N. gouldi* is evident in both fishing methods. This is advantageous in a management perspective as it allows a greater portion of younger squid time to grow mature and later reproduce rather than being exploited. Using similar trawling techniques off the coast of Portland in 2000 also indicated that although squid 90 mm DML were captured, most were >200 mm DML as a direct result of mesh selectivity (Jackson *et al.* 2005). Information on trawling selectivity for teleosts fisheries is quite extensive; however, limited for cephalopods. Differences in selectivity is found between square and diamond mesh for *Loligo vulgaris* (Ordines *et al.* 2006) as well as *Illex coindetii* (Sala *et al.* 2008). For *L. forbesi* Selectivity is a function of size with 75mm diamond mesh codend selecting mantle length (L_{50}) between 139 mm and 149 mm (Hastie 1996). Using a comparable mesh size to trawlers catching *N. gouldi*, 50% of *L. vulgaris* were retained in the codend at 114 mm DML; whereas 80 mm mesh size caught squid 97mm DML (Fonseca *et al.* 2002). Diurnal characteristics may also influence what portions of *N. gouldi* are caught in trawls. As a hypothesis by Nowara (1998), differences in catch rates between daylight and night fishing is likely function of surface feeding migration characteristics. Quantitative assessment of jig caught squid is directly associated with feeding behavior and willingness to attack jigs of both sexes. Although it is possible that larger squid tentacles detach from jigs (Koronkiewicz 1995) selectivity can also be a function of prey size. Previous studies have found that *N. gouldi* prey on pelagic fish, crustaceans and are also cannibalistic (Appendix 1; Machida 1983, O'Sullivan & Cullen 1983b, Uozumi 1998). A significant number of prey items are found in the gut of *N. gouldi* with increasing size indicating larger animals are more likely to feed on larger prey i.e. squid jigs (O'Sullivan & Cullen 1983b). Similarly larger *Todarodes sagittatus* and *L. forbesi* consume larger prey (Collins & Pierce 1996, Quetglas *et al.* 1999). It is also possible that larger *N. gouldi* are more competitive in attacking jigs compared to smaller squid.

Significant differences in biological catch composition suggests similar statolith edge microchemistry concentrations is driven by uniform ocean water chemistries and squid physiological processes, rather than a function of inshore / offshore intermixing. Ocean current systems off the coast of Portland are highly variable, with seasonal fluctuations in environmental variables such as temperature and productivity, influenced by upwelling, topography, canyons, wind and El Niño cycles (Li & Clarke 2004, Middleton *et al.* 2006, Middleton & Bye 2007). Differences between ocean systems have the ability to alter the concentration in otolith chemistry (Campana 1999); however, given the distance between collection locations is relatively short, differences in water chemistry is unlikely. Although no interaction among ablation region and location of capture was evident, a significant difference in element concentration was found in the statolith core compared with the mid-point and edge regions largely due to concentrations of Mn. Manganese is influenced by taxon-specific uptake mechanisms or accuracy variation within the HR-ICP-MS, as well as being more evenly distributed throughout oceans (Arkhipkin *et al.* 2004a) so this result was unexpected. Although no differences between inshore and offshore locations were found, post hatch *N. gouldi* experienced different environmental or physiological conditions probably in response to ontogenetic changes. As no newly hatched individuals have been caught, it can only be hypothesised that ontogenetic changes are likely to affect concentrations based on similar research from other cephalopods.

Research in teleost otoliths and cephalopods statoliths suggest that ontogenetic migration characteristics can be reconstructed using Sr and Ba in particular, though can be species dependent. Positive and negative correlations of Sr concentration with temperature are observed in the statoliths of *Ommastrephes bartrami* as well as *Dosidicus gigas* (Ikeda *et al.* 1996). Greater concentrations of Ba are indicative of increased productivity as well as being correlated with temperature in fish otoliths and statoliths (Elsdon & Gillanders 2003, Arkhipkin 2005). Off the Bonney coastline in Victoria and South Australia, upwelling events occur particularly during November – March (Butler *et al.* 2002a). During upwellings, waters offshore 250 – 300 m encroach inshore waters (<120 m) of the continental shelf (Schahinger 1987). Consequently, similar barium and strontium concentrations could be attributed to inshore and offshore water masses mixing at these bathymetries.

Clear biological differences in the catch composition of *N. gouldi* from Australian waters suggest jig fishers operating inshore catch a larger percentage of the spawning biomass than trawling offshore. Consequently, jig fishers may have greater influence in affecting future recruitment; however, such a scenario is unlikely due to the relatively low numbers caught by jiggers, the extensive distribution and rapid population turnover of *N. gouldi*. Results of depletion analysis based on the catch per unit-effort in the SSJF (2001) estimate an initial biomass of 2,409 t (Triantafillos 2008). The estimate covers the region of the continental shelf west of Cape Otway to approximately 90 km west of the South Australian / Victorian border (Western subzone). Although 99% of squid caught throughout the SSJF were from the Central zone (incorporating Eastern and Western subzones) the western zone represents approximately 50% of the total central zone area. During 2008 and 2009 respectively, only 7% and 13% of the entire SSJF catch was caught based on the initial biomass in the western subzone. Such low catch rates indicate that despite jig fishers catching the spawning portion of the stock, it is unlikely there will be negative ramifications to future recruitment. Although the trawl sector catch a greater tonnage than the jig it is also unlikely trawling will affect spawning aggregation available to jiggers.

As *N. gouldi* are caught inshore using jigs and offshore using trawlers, direct comparison between fishing methods is unknown as ontogenetic characteristics may confound differences. To gain greater understanding on the effects of gear type on *N. gouldi* collections, sampling should be conducted at inshore and offshore locations using both methods; however, this may not feasible. Although trawlers can operate at similar depth as jig fishers; it would be difficult for jigger to operate similar depths as offshore trawlers as gear is likely to become entangled. Using finer mesh size for sampling at inshore and offshore locations is advantageous as the chances of catching *N. gouldi* at most ontogenetic stages increases. Similarly, trawl codends selectively catch squid larger than approximately 220 mm DML; consequently a smaller sized codend cover can be used to determine selectivity of different mesh sizes.

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4. APPENDIX IV

Seasonal variations in diet of Arrow squid (*Nototodarus gouldi*): stomach content and signature fatty acid analysis

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1. ABSTRACT

This study details the feeding ecology of arrow squid, *Nototodarus gouldi*, collected opportunistically from trawlers in waters southeast off Australia in 2007 and 2008. Combined stomach content and fatty acid (FA) signature analyses provided clear evidence of seasonal dietary shifts in prey composition. Teleost fish remains (mainly otoliths) were found in 67% of stomachs with the two mesopelagic planktivorous fish, *Lampanyctodes hectoris* and *Maurolicus muelleri* dominating. Cephalopods and crustaceans were supplementary dietary components, with an increased representation in the diet over winter. Digestive gland lipid content was moderate (16.4 ± 8.4% wet weight) and was rich in triacylglycerol and monounsaturated fatty acids. Multivariate analysis of FA profiles grouped arrow squid with profiles of mesopelagic fish and cephalopods, thus supporting the findings of stomach content analysis. Seasonal differences in total lipid content were likely related to summer upwelling events and local changes in productivity, while intraspecific differences in lipid class and FA composition were related to seasonal differences of prey consumption. FA analyses also demonstrated dietary differences associated with gender, size and female maturation. Such relationships demonstrate that the diet of *N. gouldi* is closely linked to prey size, abundance and availability and possibly also, to key life-history stages.

Keywords: trophic ecology, cephalopoda, signature fatty acids, intraspecific dietary variation

2. INTRODUCTION

In all the world's oceans, squid are integral components of marine ecosystems. However, they remain poorly represented in ecological studies in many geographical regions and there is very limited information on the diet of a large number of species. The Arrow squid, *Nototodarus gouldi*, is an abundant, moderately sized, sexually dimorphic ommastrephid distributed in coastal and off-shore waters in southern Australia and New Zealand (Dunning, 1998). Previous dietary studies of *N. gouldi* show that they consume pelagic fish, crustaceans and other squids (O'Sullivan & Cullen, 1983; Smith, 1983; Uozumi, 1998) and there is evidence to suggest temporal variation in diet composition (O'Sullivan & Cullen 1983). *N. gouldi* aggregate in the lower water column during the day and feed primarily at night relatively close to the surface (Nowara & Walker 1998; O'Sullivan & Cullen, 1983). Arrow squid are a key prey species of many fish (Young *et al.*, 1997; Lansdell & Young, 2007), seabirds (Hedd & Gales, 2001) and marine mammals (Gales *et al.*, 1993). The contribution of *N. gouldi* to the diet of these higher predators has been found to vary spatially, seasonally and inter-annually.

To date, most dietary studies of *N. gouldi* have used stomach contents analysis, which although it can provide important taxonomic prey information, is restrictive and potentially biased (Ibáñez *et al.,* 2008). Signature fatty acid (FA) analysis is increasingly being applied to high-order predators, and has been particularly successful in determining interpopulation, spatial and temporal changes in prey composition (Iverson *et al.,* 1997; Bradshaw *et al.,* 2003). This technique has also been used effectively on the Onychoteuthid squid, *Moroteuthis ingens*, collected at Heard and Macquarie Islands (Phillips *et al.*, 2002, 2003), where the existence of a copepod−myctophid−squid food chain was established and several temporal and seasonal dietary differences were revealed. Typically, analysis of the digestive gland is used for such studies in squid as it is a site of minimal FA modification and indicates a longer time-series of dietary uptake than that obtained from stomach contents (Semmens, 1998).

Commercially, *N. gouldi* are targeted by jig and trawl fishery operations. They are the most commercially exploited cephalopod in Australia (in volume of catch) and, although the scale of the fishery is currently small (fluctuating from 784 tonnes in 2009 to 2334 tonnes in 2005; AFMA 2010), there is considerable potential for expansion if their market value increase (Jackson & McGrath Steer, 1999). To manage fisheries sustainably, it is critical to understand key trophic interactions, including the extent of competition for food resources and the interactions between prey, predators and fisheries. In this context, the aims of this study are to provide complementary stomach content and signature fatty acid analysis of the diet of *N. gouldi* in waters off the southeast Australia. We also assess the effects of season, gender, body size and maturity stage on diet and evaluate predator-prey size relationships. Such information will provide valuable data for the implementation of ecosystem-based management practices.

3. METHODS

A total of 538 *N. gouldi* (including 217 females, 321 males) were collected from coastal waters off eastern South Australia and western Victoria (between 37°56' – 38°43' S and 139°59' – 141°36'E). Samples were taken on an opportunistic basis on seven occasions between 1/03/2007 and 19/02/2008 (Table 4-1) from a commercial trawler, FV *San Tangaroa* collecting mainly blue grenadier *Macruronus novaezelandiae* and silver warehou *Seriolella punctata*. All samples were stored frozen whole until dissection when the following measurements for each specimen were recorded: total weight, dorsal mantle length, and maturity stage (I to VI where I equals immature, taken after Lipinski, 1979).

3.1. Stomach content analyses

Stomachs were thawed, individually cut open and assigned a state of digestion rating (0–6 where 6 is completely digested) and a stomach fullness (0–5, where 0 is empty) according to Jackson *et al.*, (1998). Contents were then sieved, and diagnostic prey items were removed for identification. All contents were initially separated into three broad prey categories: teleost, cephalopod or crustacean. Further taxonomic resolution of cephalopod beaks and crustacean remains was not determined. However, teleost taxonomic resolution was determined to genus and where possible species level using sagittal otoliths. Identification of otoliths was made by comparisons with reference collections (CSIRO Marine Research) and atlases (Furlani *et al.*, 2007; Williams & McEldowney, 1990; Smale *et al.*, 1995). Otolith length (OL, ±0.01mm) of identified fish taxa were measured with vernier callipers. Regression equations from Furlani *et al.* (2007), Smale *et al.* (1995) and Battaglia *et al.* (2010) were then used to estimate the length (mm) of fish prey.

3.2. Lipid and fatty acid analyses

Total lipid was extracted quantitatively by the modified Bligh and Dyer (1959) method. Total lipid content and lipid class composition of samples were determined by an Iatroscan Mark V TH10 thin layer chromatograph (TLC) coupled with a flame ionisation detector (FID). All samples were developed in a polar solvent system (60:17:0.1 v/v/v hexane:diethyl-ether:acetic acid) lined with pre-extracted filter paper for 25 min. A non-polar solvent system (96:4 v/v hexane: ether) was then used to resolve hydrocarbon from wax esters and diacyglyceryl ethers from triacylglycerols. All samples were run in duplicate along with standards. Peaks were quantified using DAPA Scientific Software (Kalamunda, Western Australia).

For FA analysis, an aliquot of the total lipid extract (TLE) was transmethylated to produce FA methyl esters (Nichols *et al.*, 1994). Samples were then silyated by the addition of N-O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) (Nichols *et al.*, 1994) and heated at 60ºC overnight before excess reagent was removed under a stream of nitrogen gas and an internal injection standard (C_{19} FAME) was added. Gas chromatographic (GC) analyses were performed with an Agilent Technologies 6890N GC (Palo Alto, California, USA) equipped with an HP5 cross-linked methyl silicone fused silica capillary column (50 x 0.32 mm i.d.), an FID, a splitless injector and an Agilent Technologies 7683 Series auto sampler. Selected FA samples were analysed further using gas chromatography-mass spectrometry (GC-MS) to verify component identifications.

3.3. Profile comparisons of prey and squid species

FA profiles of squid digestive glands were compared to published data on potential prey genera and species (Pethybridge *et al.*, 2010). Specifically, the mean FA profile of males and females collected over the 7 sampling periods were individually tested against 13 species of myctophids, 13 other teleosts, 8 cephalopods, 1 octopod and 2 crustaceans collected from south-east Australia. This included FA profiles of 8 teleost species found in the stomach content analysis as indicated in Table 1. Data used in comparisons were in percent composition (of total fatty acids) form. FA used were those that made up >0.5% of the total FA and included: 16:0, 18:0, 16:1ω7, 18:1ω7, 18:1ω9, 20:1ω9, 20:1ω11, 22:1ω9, 22:1ω11, 24:1ω9, 20:4ω6, 20:5ω3 (EPA), 22:6ω3 (DHA).

3.4. Statistical analyses

For stomach content analyses, prey groups and prey species were analysed using number of individual prey (N) and percent frequency of occurrence (%O). All results are expressed as mean ± standard deviation. Statistical treatment of all data entailed the application of univariate or multivariate analysis of variance (ANOVA or MANOVA). This included assessing the main effects of season, gender, body size and female maturity stage on diet composition (as determined by the proportion of a major food type found in stomach contents), total lipid content, major lipid classes and various FA constituents. The interaction among the independent variables and the association between dietary base variables was also tested. Such analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL). Non-metric multi-dimensional scaling (MDS) ordinations based on Bray-Curtis matrices were used to assess groupings within the dataset and analysis of similarity (ANOSIM) was used to determine which levels differed within a significant fixed effect ($p<0.05$). The ANOSIM-R value indicated the extent to which the groups differed ($R> 0.75$: well-separated groups; $R = 0.50 – 0.75$: separated but overlapping groups;

0.25 – 0.50: separated but strongly overlapping groups; R <0.25: barely separated groups). MDS and ANOSIM analysis was performed with PRIMER6 software (PRIMER-E, Plymouth, UK).

4. RESULTS

4.1. Stomach contents

Of all stomachs analysed, 22.9% were empty (mean stomach fullness 1.3), and most of stomach contents were more than 90% digested (mean digestive stage = 4.9). Squid caught during 6/8/2007 and 15/10/2007 had a greater proportion of samples with empty stomachs and had a lower number of prey items per stomach in comparison to squid collected on other dates (Table 4-1). Overall, the mean number of prey items per stomach was 2.5 while up to 40 individual prey items were observed in a single stomach.

A total of 1365 prey items was identified, with fish the most dominant prey group (N = 1088; 66.6%O, Table 1). For the teleosts, 15 taxa were identified with the myctophid, *Lampanyctodes hectoris*, showing the greatest contribution of any fish species (N = 461, 17.1%O). The next most important fish included the sternoptychid *Maurolicus muelleri* (N = 227, 11.5%O). The estimated mean lengths of fish prey were within their known size ranges. The smallest fish species identified and measured was the lanternfish *H. hanseni* (21.7mm SL). The largest estimated fishes consumed were the lightfish, *P. argenteus* (as large as 391.4mm FL), the toothed whiptail, *L. denticulatus* (as large as 342.1mm SL) and the New Zealand dory, *C. novaezelandiae* (as large as 246.5mm TL). Comparing the mantle length of individual squid with fish length estimates, it is evident that arrow squid can consume prey items almost as large (up to 92%) as its own mantle length. The predator size-prey size relationship however was not significant (ANOVA, $p>0.05$).

Cephalopods were the second most important prey group (N = 205; 33.3%O), followed by the crustaceans (N=72; 7.6%O). Of those cephalopods, most were identified as squid with very few octopus beaks recorded. Cephalopod tentacle remains consisted of suckers only as opposed to sharp hooks, eliminating squid species from the families Onychoteuthidae or Octopoteuthidae. Crustacean material was primarily composed of decapods and amphipods.

Over the yearly sampling period some seasonal trends in biology and diet are observed for *N. gouldi*. We found that smaller specimens (total body weight and digestive weight) were collected during June and October 2007, and February 2008, whereas size peaked during August 2007 (Table 4-1). There was a greater occurrence of empty stomachs in September and October 2007 and a higher number of total prey items per stomach during the summer/autumn sampling periods (Table 4-1).

Table 4-1 Diet of arrow squid *Nototodarus gouldi*, collected from 38S, 139–141E between March 2007 to February 2008. M-male; F-female; SD – standard deviation; DG- digestive gland; N – number of occurrence; %NA – percent numerical abundance of total prey items or of total fish prey; UnID – unidentified otoliths. * indicate fish species of which fatty acid profiles where available

Season	Autumn				Winter				Spring				Summer	
Collection Date	1/3/2007		27/3/2007		7/6/2007		6/8/2007		19/9/2007		15/10/2007		19/2/2008	
number	72		79		75		79		73		81		79	
Sex ratio (M:F)	1.4:1		0.8:1		2.9:1		2.8:1		1.7:1		2.2:1		0.6:1	
Total wt(g) ±SD	898.8 .	±251.4	776.7	±229.3	664.3	±238.7	898.8	±251.4	901.7	±298.4	555.3	±218.1	410.5	±95.9
Total DG wt(g) ±SD	45.5	±30.6	51.1	±20.0	31.1	±15.8	45.5	±30.6	42.1	±21.4	30.2	±16.3	27.9	±10.9
% stomachs empty	12.5		6.3		6.7		27.8		47.9		48.1		10.1	
Stomach Fullness ±SD	1.1	±1.0	2.0	±1.2	1.5	±0.7	1.1	±1.0	0.8	±0.9	0.8	±0.9	1.9	±1.1
State of digestion ±SD	4.9	±0.9	4.5	±0.8	4.5	±1.0	4.9	±0.9	5.4	±0.7	5.4	±0.7	4.8	±0.8
Mean No. prey ±SD	2.1	±2.5	6.0	±7.1	2.8	±2.2	1.2	±1.6	0.9	±1.7	0.6	±0.7	4.1	±5.9
Range of prey items per stomach	0	–17	0	–39	0	–11	0	–13	0	–12	0	–3	0	–40
	N	%O	N	%O	N	%O	N	%O	N	%O	N	%O	N	%O
TOTAL CEPHALOPODS	8	11.1	24	30.4	56	70.8	41	40.5	24	17.8	11	12.3	41	48.1
TOTAL CRUSTACEANS	4	5.5	1	1.3	56	37.3	2	2.5	1	1.3	1	1.2	7	7.6
TOTAL TELEOST	139	83.3	452	86.1	102	76.0	49	50.6	38	41.1	39	43.2	269	77.2
Myctophidae (lanternfish)	67	45.1	284	51.9	6	6.7	2	2.7	8	5.5	8	3.7	121	36.7
Diaphus ostenfeldi	6		0		0		0		0		0		0	
Diaphus danae *	0		0		0		1		0		0		2	
Diaphus sp.	11		1		1		0		0		0		0	
Electrona rissoi *	0		1		0		0		0		0		0	
Hygophum hanseni *	0		0		1		0		0		0		0	
Lampanyctodes hectoris *	48		282		2		1		8		5		115	
Lampanyctus cf. *australis* *	0		0		1		0		0		3		0	
Macrouridae (whiptails)	9	7.0	26	20.3	2	2.7	1	1.3	0	0	2	2.5	1	1.3
Caelorinchus sp. *	1		9		1		1		0		1		1	
Lepidorhynchus denticulatus *	6		11		1		0		0		1		0	
Percichithyidae (temperate basses)	6	7.0	2	2.5	4	6.7	2	2.7	1	1.4	0	0	18	
Sternoptychidae (hatchetfish)	15	2.8	79	25.3	66	37.3	2	2.7	0		3		62	12.7
Maurolicus muelleri *	15		79		66		2		0		3		62	
Cyttidae (dories)	1	1.4	3	3.8	0	0	0	0	1	1.4	3	3.7	1	2.5
Cyttus novaezelandiae	1		3		0		0		0		2		0	
Cyttus australis	0		0		0		0		1		1		1	
Clupeidae (herring)	0	0	0	0	0	0	0	0	2	2.7	0	0	0	0
Sardinops neopilchardus	0		0		0		0		2		0		0	
Phosichthyidae (lightfish)	11	7.0	1	1.3	0	0	0	0	0	0	2	2.5	12	6.3
Phosichthys argenteus	11		1		0		0		0		2		12	
Unidentified	30	28.2	57	19.0	24	40.0	42	61.3	26	41.1	21	34.6	54	46.8
No. Nematodes	49		60		184		344		431		96		24	

The contribution of fish to the diet of *N. gouldi* significantly different among seasons (ANOVA F_3=158, p<0.001), but not among size-classes or gender (p=0.15 and p=0.35 respectively). For both genders, fish strongly dominated (>90% occurrence) the diet in March 2007 and numbers of cephalopods and crustaceans increased during winter months. Seasonal dietary shifts in the dominant prey species were observed with *L. hectoris* having greater representation in the diet during the summer months (particularly collection date March 2007), compared other sampling months (Table 4-1). Furthermore, an increased occurrence of *M. muelleri* was observed during July, and there was an increase of unidentified otoliths during the winter months in 2007 (6/8, 19/9, and 15/10). Despite the seasonal differences in the quantity consumed, both *L. hectoris* and *M. muelleri* were identified in the diet of squid collected over the entire sampling period. Teleosts that occurred exclusively in squid collected on only one sampling date included: *Electrona rossi*, *Hygophum hanseni*, and *Sardinops neopolchardus* (Table 4-1). The occurrence of parasitic nematodes (including *Anisakis* species) on the stomach walls increased in August and September.

4.2. Lipid content and composition

Total lipid content of digestive gland averaged 16.4 ± 8.4% ww (Table 4-2). The lipid composition was mostly dominated by triacylglycerols (TAG, 46.1 ± 17.6%). Polar lipids (PL), wax esters (WE), free fatty acids (FFA), and sterols (ST) were also present in decreasing importance. Free fatty acids accounted for relatively high proportion of the total lipid (13.2 ± 6.3%), indicating that samples may have undergone some deterioration since their collection. FFA levels were lowest in those specimens collected during March 2007 and highest on 15/10/2007 (Table 4-2).

Differences in lipid class composition were significantly affected by season (P<0.001, Table 4-3), with mean TAG and total lipid content higher during the summer collection months (1/3/2007, 27/3/2007 and 19/2/2008) compared to all other sampling dates (Table 4-2). Multiple comparison (Tukey HSD) tests demonstrated significant differences between the total lipid content of squid collected in autumn and other seasons ($p \leq 0.004$), while no significant differences were observed between the other months. Relative proportions of TAG and ST significantly differed across all seasons (p<0.001), with the exceptions of those between autumn and summer and between winter and spring. Proportions of PL significantly differed between squid collected in summer and both winter and spring while FFA in squid collected in autumn significantly differed to those collected in other seasons (p<0.001). WE only significantly differed between those squid collected in autumn and spring (p=0.012). As individual factors, size-class and gender had limited influence on lipid composition, with the exception of that on FFA when season was tested as a co-factor (Table 4-3). Maturity stage had no significant influence on lipid profiles (ANOVA, p=0.60).

Table 4-2 Percent lipid class composition (of total lipid) and total lipid content (as % wet weight) of the digestive gland of arrow squid, *Nototodarus gouldi*. Values are means ± SD. Gender, maturity, size and collection period comparisons are shown also.

	Percent composition							% Total lipid
	n	total wt (g)	WE	TAG	FFA	ST	PL	
Digestive gland (All specimens)	150	249–1444	13.1 ±5.1	46.1 ±17.6	13.2 ±6.3	9.0 ±5.1	18.6 ±5.4	16.4 ±8.4
Comparisons - collection period								
1/3/2007	34	256–953	8.3 ±10.2	63.2 ±19.1	8.2 ±4.9	3.8 ±5.9	16.6 ±6.8	24.1 ±10.3
27/3/2007	10	380–673	5.4 ±9.9	71.0 ±15.1	4.9 ±0.9	3.3 ±5.1	15.4 ±7.2	30.0 ±10.4
7/6/2007	19	360–1159	11.5 ±8.1	42.2 ±30.8	9.6 ±6.3	13.1 ±12.7	23.6 ±13.9	11.4 ±6.8
6/8/2007	20	385–876	19.1 ±11.1	37.1 ±22.7	13.0 ±6.8	10.9 ±10.1	19.8 ±9.1	12.9 ±5.5
19/9/2007	20	353–1113	16.7 ±9.3	26.9 ±19.2	15.7 ±4.7	14.9 ±10.4	25.8 ±9.2	8.7 ±3.3
15/10/2007	23	622–1444	17.9 ±8.1	26.5 ±13.6	23.4 ±7.3	12.9 ±6.7	19.4 ±11.7	7.9 ±2.9
19/2/2008	23	249–1274	13.1 ±9.2	55.8 ±20.6	17.7 ±6.8	3.9 ±3.0	9.6 ±6.5	19.6 ±7.3

Abbreviations: WE, wax ester; TAG, triacylglycerol; FFA, free fatty acid; ST, sterol; PL, polar lipid (mostly phospholipid).
Note: samples may contain small amounts of diacylglyceryl ether or hydrocarbon as they coelute with TAG and WE

Table 4-3 Summary of MANOVA on total lipid, lipid class composition and fatty acid composition of *Nototodarus gouldi* depending on collection period (seasons), size classes (< and > 300 mm ML) and gender.

		Total lipid	Lipid class composition					Fatty acid composition					
			WE	TAG	FFA	ST	PL	ΣSAT	ΣMUFA		ΣPUFA		
										18:1w9c		22:6w3	20:5w3
Season	F-value	8.62	5.70	27.22	16.07	15.04	8.80	6.20	3.05	3.00	5.55	4.44	4.14
	p	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.031	0.033	0.001	0.005	0.008
Size	F-value	0.01	0.47	0.11	0.63	0.40	0.01	0.29	2.41	7.20	5.15	6.63	4.67
	p	0.946	0.496	0.739	0.431	0.527	0.912	0.589	0.123	0.008	0.025	0.011	0.033
Gender	F-value	2.45	0.11	0.17	3.40	0.11	0.46	0.22	1.56	3.01	0.81	3.99	0.16
	p	0.120	0.739	0.685	0.068	0.737	0.499	0.639	0.213	0.085	0.371	0.048	0.693
season x size	F-value	0.53	1.69	0.59	3.34	0.76	2.79	0.35	2.10	0.74	2.60	4.22	1.50
	p	0.592	0.188	0.558	0.039	0.471	0.066	0.787	0.103	0.531	0.055	0.007	0.218
season x gender	F-value	2.46	1.16	0.91	8.53	0.13	1.06	7.38	10.74	1.59	10.98	10.96	7.87
	p	0.090	0.316	0.407	<0.001	0.881	0.349	<0.001	<0.001	0.196	<0.001	<0.001	<0.001

Significant probabilities are in bold (P<0.05). Note: no significant interactions were observed between size x gender and any variable with the exception of 22:6w3 (F_1=5.55, p=0.020).

4.3. Fatty acid profiles

Twenty nine different FA were found in greater than trace levels (>0.5%) in all digestive gland samples (Table 4-4). The major FA that dominated in the digestive gland consistently were: 16:0, 22:6ω3, 20:1ω9, 18:1ω9, 18:0, 22:1ω11, 20:5ω3, 24:1ω9, 22:1ω9 and 18:1ω7, in decreasing order of relative abundance. For all specimens combined, SAT accounted for 30.5 ± 7.9%, MUFA 40.8 ± 10.7% and PUFA 24.6 ± 10.1%, although variations between the sampling periods were observed (Figure 4-1). The iso-SAT and branched fatty acids accounted for 0.6% and 2.1% of the total FA, respectively.

A three-way MANOVA revealed that season significantly affected all the FA constituents tested, while only selected FA constituents were affected by size and gender (Table 4-3). Using Tukey HDS post-hoc multi comparison test, SAT levels significantly differed in autumn compared to other seasons ($p \leq 0.008$). Significant differences in the proportions of MUFA were evident between autumn and spring (mean difference, I-J=6.24, $p=0.021$), while PUFA differed between autumn and winter (I-J=5.94, $p=0.013$). Significant differences between the two size classes for PUFA (including 22:6ω3 and 20:5ω3) and 18:1ω9 were observed (Table 4-3). Higher levels of dietary derived PUFA was observed in smaller (< 300mm ML) specimens and higher proportions of 18:1ω9 was found in larger (>300mm ML) specimens. Gender only significantly influenced proportions of 18:1ω9 and 22:6ω3 (Table 4-3), with higher levels of 18:1ω9 and lower levels of 22:6ω3 observed in males than females. The interaction between gender and season also showed a significant effect on a number of lipid class and FA constituents (Table 4-3). Significantly higher levels of 18:0 and 22:1ω9, and lower levels of 16:0 and 22:1ω9 were found in females of maturation stage 5, while highest levels of the long-chain PUFA - 22:6ω3 and 20:5ω3 - were observed in females of a maturity stage of 4 ($p<0.01$). Male fatty acid profiles were most similar to that of females with a maturity stage of 3 as opposed to those that had a maturity stage of 4 and 5.

Using non-parametric multi-dimensional scaling (MDS, Figure 4-2) ordinations and ANOSIM, we tested group associations of squid fatty acids profiles. In general males and females demonstrated limited dissimilarity in their fatty acid profiles (ANOSIM $R=0.11$) while collection period differences were evident between males and females. The most dissimilar ($R>0.75$) male and female profiles occurred between those caught in February 2008 and those caught in March and October 2007. Male and female squid collected in October showed dissimilarity from those caught in August and September, and from squid collected in June and August. Between other collection months, more similar profiles between males and females were displayed ($R \leq 0.5$, Figure 4-2).

Table 4-4 Average fatty acid composition of the digestive gland of *Nototodarus gouldi* collected off south-east Australia

	Digestive gland profile
	All specimens
n	144
total wt (g)	693.8±244.4
DG wt (g)	39.3±17.9
Fatty acids	
14:0	1.9±1.0
15:0	0.6±0.2
16:0	17.5±4.6
17:0	1.0±0.5
18:0	7.8±3.1
19:0	0.5±0.2
20:0	0.5±0.1
22:0	0.5±0.2
∑SAT	30.5±7.9
16:1ω7	1.9±0.9
17:1ω8+16:1ω 9	0.6±0.2
18:1ω9	10.7±4.0
18:1ω7	2.1±0.7
18:1ω5	0.3±0.1
20:1ω9	13.1±5.7
20:1ω7	0.7±0.2
22:1ω11	5.7±4.9
22:1ω9	2.3±4.1
22:1ω7	0.4±0.3
24:1ω9 (+11)	2.5±0.7
∑MUFA	40.8±10.7
18:2ω6	0.7±0.3
20:2ω6	0.5±0.1
20:4ω6 (AA)	1.4±1.0
18:4ω3	0.5±0.3
20:4ω3	1.1±0.6
20:5ω3 (EPA)	4.4±2.0
22:4ω3	0.5±0.3
22:5ω3 (DPA)	1.5±0.6
22:6ω3 (DHA)	14.2±7.1
∑PUFA	24.6±10.1
Iso-SAT	0.6±0.2
br17:1+7Me17:1	2.1±0.6
∑others*	2.1

N: number, DG: digestive glands, SAT: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids. Others FA include those less than 0.2%: 21:0, 24:0, 16:1ω13t, 16:1ω5c, 24:1ω7, 18:3ω6, 21:5ω3, 22:2ω6, 22:3ω6. Iso-FA include: i15:0, i16:1, i17:1, i16:0, i17:0, i18:0.

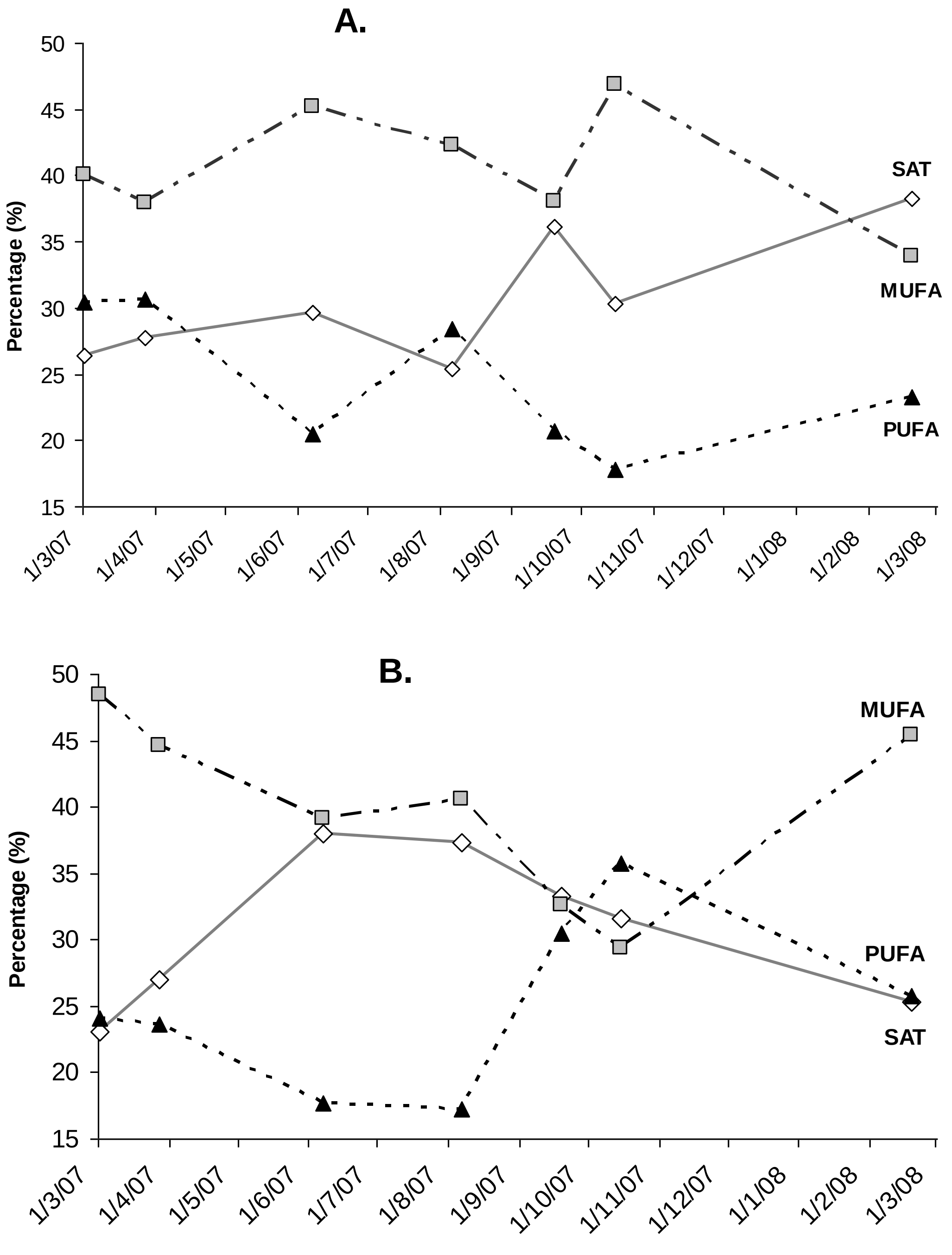

Figure 4-1 Collection date comparison of the major FA groups, saturated (SAT), monounsaturated (MUFA), and polyunsaturated (PUFA), for A) males only and B) females only.

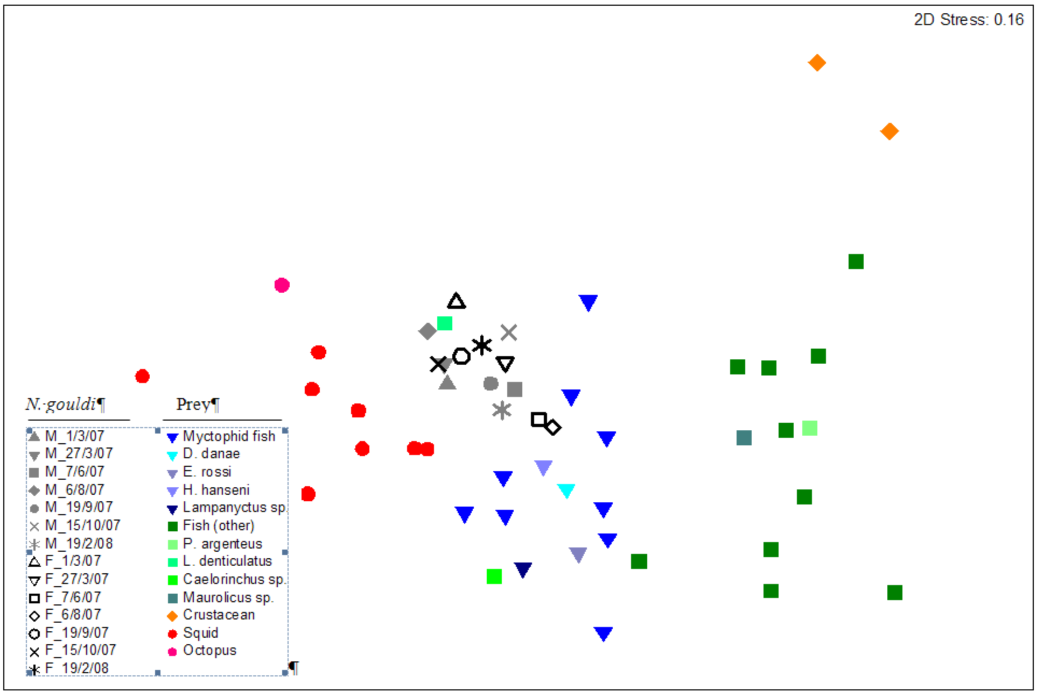

Figure 4-2 Scatterplot of multidimensional scaling (MDS) based upon the mean fatty acid composition of various potential prey species and the digestive gland of male (M) and female (F) N. gouldi collected over various dates. Axis scales are arbitrary in non-metric MDS and are therefore omitted.

4.4. Prey profile comparisons

Mean FA profiles of potential prey species were compared to those of digestive gland of male and female *N. gouldi* collected over the 7 sampling dates using an MDS scatter plot (Figure 4-2). All squid were closely grouped together regardless of gender or collection season, although were most closely related to the FA profiles of various myctophid fish, other fish species and cephalopods. Myctophid fish demonstrated the highest association with arrow squid digestive gland (ANOSIM R=0.037 – 0.6) with greatest similarity between male squid collected in 1/3/07, 7/6/07 and 19/02/08 (R=0.025, 0.037 and 0.037, respectively) and in female squid collected in June (R= 0.062). Fish species that showed greatest similarity included *H. hanseni* and *D. danae* (R=0.02 and 0.05, respectively). As a prey group, other fish demonstrated less association with arrow squid profiles (R=0.57 – 0.92) with the exception of *L. denticulatus*,which was very similar in profile (R=0. 01, Fig 2). For male and female arrow squid there was limited association with other squid prey profiles (R=0.51 – 0.79) and no association (R=1) was displayed with the two crustaceans (shrimps *Acanthephyra* sp. and *Sergia potens*).

5. DISCUSSION

The diet of *Nototodarus gouldi* as indicated by stomach content and digestive gland lipid and FA profiles varied markedly over the one year sampling period. Stomach contents provide clear evidence for dietary shift in prey groups consumed by *N. gouldi*. Multivariate comparisons of lipid and FA profiles also indicated seasonal differences in the diet of *N. gouldi*. Evidence of diet variation between gender, two size-classes (< or > 300mm ML) and life-history (maturation) stages of females was also revealed with lipid and FA profile comparisons. Stomach contents and FA results are discussed in detail below.

5.1. Stomach contents

The diet of *N. gouldi*, as determined by stomach content analysis, is typical of adult ommastrephid squid that feed on a diverse range of fish, cephalopods and crustaceans (Dawe *et al.,* 1997; Rodhouse & Nigmatullin, 1996; Philips *et al.*, 2001, 2002). During the one year sampling period, *N. gouldi* fed largely on fish, consisting of two dominant taxa, and supplemented its diet with a range of other prey. During the summer months, the myctophid, *L. hectoris,* dominated while during the winter period cephalopods (as a prey group) and the pearlside (*M. muelleri*) dominated. *L. hectoris* is one of the few species of lanternfish to inhabit shallow waters, and in those waters it is one of the most abundant species of fish, and central to the food chain of the upper continental slope (Young, 1998). In contrast, pearlsides (*M. muelleri*) are small shoaling fish which occur along the continental shelf break, mainly confined to waters between 300 to 400 m (Clark, 1982). Both species experience large seasonal fluctuations (Rosland & Ciske, 1997; Williams & Koslow, 1997) which is reflected in the diet of *N. gouldi*. Similar to the trend found here, Williams & Koslow (1997) reported that populations of *M. muelleri* occurring in Tasmanian waters were much more abundant in autumn. In contrast, Williams & Koslow (1997) reported that *L. hectoris* were only present in spring, whereas in our study the occurrence of *L. hectoris* was higher in summer months. In other parts of the world, mesopelagic fish (especially *L. hectoris*) are known to move offshore in winter because of their spawning migration into deeper water (Hulley & Lutjeharms, 1989), which may account for the reduction of lanternfish in the diet of *N. gouldi* during that period.

In the pelagic ecosystem off the south coast of South Australia and western Victoria, a diverse suite of small pelagic fishes occurs, largely dominated by members of family Clupeidae (herring-like fishes) (Ward *et al.,* 2008). Key species include sardine (pilchard, *Sardinops pilchardus*), Australian anchovy (*Engraulis australis*), sandy sprat (*Hyperlophus vittatus*), blue sprat (*Spratelloides* spp.), blue mackerel (*Scomber australasicus*), redbait (*Emmelichthys nitidus*) and saury (*Scomberesox saurus*) (Ward *et al.*, 2008). However, in this study only 2 occurrences of sardines (*Sardinops neopilchardus*) were observed in the month of September. The absence of these prominent fish species in this study, particularly for those specimens collected over the winter and spring sampling months (Table 1), is likely to be related to the different distributions of these particular fish species in the waters off south-eastern Australia and emphasizes the generalist foraging behavior of *N. gouldi*. For example, in arrow squid collected in Bass Strait, pilchards (*Sardinops pilchardus*) and juvenile barracouta (*Leionura* *atun*) were reported as the most common fish prey species identified (O'Sullivan & Cullen, 1983), while in squid collected in the eastern Great Australian Bight, redbait (*Emmelichthys nitidus*) and jack mackerel (*Trachurus declivis*) have greater representation in the diet (Braley *et al.*, 2010). Alternatively, as these species can be caught as bycatch in coastal trawl operations, these finding may suggest some bias associated with fishing gear as has been described by Ibáñez *et al.* (2008).

Arrow squid was found to consume a large range of prey sizes with some individuals consuming large fish relative to their mantle length. However, most fish consumed were small (< 20 cm TL), including the two dominant prey species *L. hectoris* (max 11 cm SL) and *M. muelleri* (max 7 cm SL). These results suggest that *N. gouldi* feed predominantly on small mesopelagic prey, but are capable of exploiting an array of small to medium sized prey. Such patterns in the size-selectivity of squid diet have been observed in many species (reviewed by Rodhouse & Nigmatullin, 1996) and can be influenced by seasonal changes in size of prey (Collins & Pierce, 1996).

The high occurrence of parasitic loadings of the larval nematode *Anisakis* spp. suggests that *N. gouldi* is an important intermediate host species to higher-order predators (Gonzalez *et al.,* 2003). Such occurrences of not only nematodes but other parasitic fauna such as larval cestodes are very common in a number of ommastrephid squid globally (Abollo *et al.*, 1998; Pardo-Gandarillas *et al.*, 2009). The increased number of infestations during August and September compared to other sampling months indicate that *N. gouldi* may predate in different trophic pathways during these times. This seems to be governed by the increased consumption of cephalopods during these months. Alternatively, it may be governed by the distribution of marine mammals which are the final hosts required by nematodes to complete their life cycle (Haimovici *et al.*, 1998).

5.2. Lipid content

The lipid content of *N. gouldi* is lower than that reported in other ommastrephid squid (e.g. *Nototodarus sloanii,* Vlieg, 1984; *Todarodes pacificus*, O'Dor & Weber, 1986; *Todarodes filippova*, Pethybridge, 2004). In contrast, *N. gouldi* has higher lipid content than that reported in most coastal species (*Sepioteuthis australis*, Phillips *et al.*, 2002; *Sepia offinalis*; Blanchier & Boucaud-Camou, 1984). Interspecific differences may be an indicator of the food availability and system bioenergetics in different regions as proposed by Abolmasova *et al.*, (1990). For example, in the southern ocean onychoteuthid squid, *Moroteuthis ingens*, collected from three geographically isolated areas, Phillips *et al.*, (2003) correlated different lipid contents to differences in regional productivity. In the present study, mean lipid content of *N. gouldi* was higher (mean > 19%) during summer collection months and was consistently low (mean < 13 %) during other months (Table 2). This is likely to reflect known seasonal differences in primary productivity off South Australia, in which upwelling events occur during summer and autumn (Middleton 2000). It may also reflect the decrease in consumption of myctophid and other oily (high calorific energy) fish species (Pethybridge *et al.*, 2010) which are less available in coastal waters during winter (Hulley & Lutjeharms, 1989). Castellanos (1960) estimated that lipid stored in the digestive gland of *Illex argintinus* was sufficient to fuel a spawning migration of up to 21 days. However, given that cephalopods have a protein-based metabolism (O'Dor & Webber, 1986), it may be that squid are unable to handle the high levels of lipids from predation on oily fish and the lipids are getting dumped into the digestive gland for excretion, as has been shown in loliginid squid (Semmens, 1998).

5.3. Lipid class and fatty acid composition

Lipid class and FA profiles of the digestive gland of *N. gouldi* demonstrated dietary variability which is explained by seasonal, gender and size-related differences. Similarly to stomach contents, multidimensional scaling analysis of FA profiles highlighted that myctophids, squid and certain non-myctophid fish are strongly represented in the diet of *N. gouldi*, and that crustaceans are unlikely to be more than supplementary components. Although prey profile comparisons were undertaken, FA data was not used to determine which prey species may be responsible for intraspecific variation, as temporal, size and spatial variation in FA content has been recorded in several mesopelagic fish, cephalopods and crustaceans (Lea *et al.*, 2001; Iverson *et al.*, 2002; Darlsgaard *et al.*, 2003). Instead, we used signature lipid and FA to investigate which biological and temporal variables are most responsible for intraspecific variation as it relates to diet.

5.4. Intraspecific variation

There are apparent trends in the FA and lipid class profiles that indicate dietary differences among sampling dates, consistent with findings in the stomach contents. Digestive glands from *N. gouldi* collected during summer months have higher relative levels of TAG, but lower PL and ST in comparison to collection months between 7/6/07 and 15/10/07 (Table 2). These seasonal differences are likely to be attributed to diet, with high levels (>70%) of TAG associated with some species of myctophid fish (e.g. *L. australis* and *H. hanseni*; Pethybridge *et al.*, 2010). Therefore, high TAG during summer months is likely to be due to an oil-rich myctophid diet, as shown in the stomach contents. Most other fish species (including *Maurolicus australis*), along with cephalopods, have lower relative levels of TAG and increased PL (Pethybridge *et al.*, 2010). A smaller number of fish contain WE as their major storage lipid, which is reflected in the lipid class profiles of arrow squid.

FA profiles of digestive glands collected in autumn were most dissimilar to other sampling dates and particularly to winter FA profiles, being significantly lower in 22:6ω3 and significantly higher in 16:0 and 18:1ω9. As found in stomach contents, FA results agree with the high consumption of mesopelagic fish in autumn followed by a dietary shift to greater proportions of crustaceans and cephalopods during the winter period. However, some conflicting results were observed between FA profiles and stomach content analyses. For example, digestive glands collected in September demonstrated several significant differences to other sampling dates in the relative proportion of individual FA, containing higher 20:4ω6 and 24:1ω9 and lower 20:1ω9, 18:1ω9 and 24:1ω11. Such a profile suggests potential increased consumption of crustaceans, octopus and myctophids (Pethybridge *et al.*, 2001) which was not demonstrated by analysis of the stomach contents. Such a divergence between the stomach contents and FA data could be expected as in a controlled study, Stowasser *et al.*, (2006) identified that FA of the squid, *Lolliguncula brevis* start to reflect their diet after 10 days.

Ontogenetic variation was displayed by FA profile comparisons, with squid smaller than 300mm ML having higher PUFA (22:6ω3 and 20:5ω3) and lower levels of 18:1ω9 than larger specimens (Table 3). Higher levels of these PUFA are typical features of various crustaceans, mesopelagic squid and octopus (Pethybridge *et al.*, 2010). Such a trend is aligned with other dietary studies of *N. gouldi* that demonstrated an increase in the occurrence of cephalopod prey with size (O'Sullivan & Cullen, 1983; Uozumi, 1998). In contrast, high levels of MUFA are a typical feature of teleost (Pethybridge *et al.*, 2010) and higher relative levels of 20:1ω9, 22:1ω11 and 22:1ω9 in smaller squid, are possibly indicative of a diet rich in copepods (Dalsgaard *et al.*, 2003) or more likely a diet rich in dominant predators of copepods such as myctophid fish. A similar finding is observed for large squid where significantly higher levels of 18:1ω9 and slightly higher levels of 16:0 are indicative of a diet rich in mesopelagic fish (Pethybridge *et al.*, 2010) including myctophid fish (Saito & Murata, 1996). In agreement with our findings, O'Sullivan & Cullen (1983) also observed that the consumption of fish remains relatively constant over all sizes of *N. gouldi*. Such findings may be related to the age and growth of *N. gouldi* as it has been shown to be highly variable over time and space (Jackson *et al.*, 2003). The relatively small sample sizes used in our study may also have limited our conclusions.

In general, there were few differences between the FA profiles of male and female squid (Table 3). The most significant difference was related to the PUFA, 22:6ω3, which was significantly higher in males than females. Higher levels of dietary derived long-chain PUFA and particularly 22:6ω3 may indicate an increased consumption of crustaceans and mesopelagic squid (Pethybridge *et al.*, 2010). However, the interaction between season and size-class also contributed to significant gender-related differences in the proportions of 22:6ω3. Thus, males and females may be feeding on different prey or prey-sizes throughout parts of the year. Arrow squid display sexual dimorphism; therefore this result is not surprising as smaller males would be more likely to consume smaller prey species due to morphological restrictions imposed. However, while size-class only correlated with gender for 22:6ω3, many more FA constituents were affected by the interaction among season and gender. Sexual segregations in timing and pathways of migrations have been documented for other ommastrephid squid (Arkhipkin & Middleton, 2002) and thus are not unlikely in this species. These segregations in time and space are likely to be reflected in the diet as each gender encounters different available prey groups.

In addition to gender-seasonal differences there is evidence of female life-stage (maturity stages 3–5) differences in FA profiles of the digestive gland. For example, high levels of 22:1ω11, 14:0 and 16:0 were characteristic of low maturity (stage 3) females. This finding is likely to be related to similarities in sizes as mature females are larger. However, to some extent, it may indicate different stages of the maternal provisioning of essential nutrition to the developing embryos as changes in life stages are often associated with changes in diet. Although evidence suggests that the digestive gland is not used as a storage organ and is unlikely to be used during reproductive development (Semmens, 1998), future research would benefit from discerning the role of these (and other) potential factors in influencing the FA profiles of arrow squid.

Overall, this study provides new data on the diet of *N. gouldi*, an abundant squid in pelagic ecosystems in south Australian waters. The combined use of stomach contents and signature FA analysis confirmed that diet varies seasonally. These seasonal differences are likely associated with changes in local productivity and thus, species composition and availability, as indicated by seasonal changes in the total lipid content of digestive gland. Whereas stomach content analysis identified major changes between certain prey taxa, FA profiles established that diet differs between males and females and between different size classes. Such results emphasize the power of using multiple methods for exploring not only diet composition but also longer-time integrated dietary trends as they relate to multiple biological and environmental factors. Ecosystem-based management practices should look to incorporating the results from both dietary techniques in dynamic mixing or statistical models so that the impact of small-scale (e.g. monthly) perturbations on local marine food resources can be projected.

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5. APPENDIX V

An analysis of spatial and temporal population structure of arrow squid Nototodarus gouldi from southern Australia using statolith elemental composition and shape

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1. ABSTRACT

Arrow squid, *Nototodarus gouldi,* are managed as a single population in Australia. It is however, unknown whether *N. gouldi* caught in Victoria and the Great Australian Bight are from a single population or are separate stocks. This study examines whether *N. gouldi* caught in these two spatially separated locations of southern Australia are different stocks. Fourier shape analysis of statoliths was used for the first time to determine whether discrete phenotypic stocks exist; whereas statolith element concentrations were used to determine where individuals from both locations are likely to have hatched. Comparing statolith shape from squid collected in Victoria to squid collected in the Great Australian Bight suggested significant phenotypic heterogeneity in stocks; whereas elemental composition analysis suggests that *N. gouldi* caught at either location hatched throughout their distribution. Although *N. gouldi* caught in the Great Australia Bight and Victoria appear as separate stocks, squid from both regions are important for recruitment.

2. INTRODUCTION

Stock identification and discrimination are fundamental requirements of fisheries assessments; though for some cephalopod fisheries, managers assume a single population with spatial integrity. Given their short life history coupled with high variability in catch, growth, recruitment, age and size at maturity, the assumption of a panmictic population is justified but not always the case (Anderson & Rodhouse 2001b, Rodhouse 2001a, Agnew *et al.* 2002, Jackson *et al.* 2003, Boyle & Rodhouse 2005). For example, two stocks of *Todarodes pacificus* have been identified and managed separately in the

Japanese and Pacific Seas (Kidokoro & Mori 2004). Although objectives of cephalopod management are fishery specific, catch controls are mainly by effort restrictions (Boyle & Rodhouse 2005). Areas of government jurisdiction, species spatial distribution, or location of artisanal fishing grounds typically define squid fisheries; however, without identifying the possible existence of multiple stocks, management may be rendered ineffective.

Arrow squid, *Nototodarus gouldi* (McCoy 1888), are the primary oceanic squid distributed in waters south of latitude 27° off the Australian coast, as well as New Zealand (Dunning & Forch 1998). Based on collections from six locations around southern Australia (700 – 4300 km separation) allozyme electrophoresis found that *N. gouldi* is a single species with little support that the metapopulation is panmictic (Triantafillos *et al.* 2004). Like other ommastrephids, *N. gouldi* is a fast growing species capable of spawning multiple times a year, exhibits spatial and temporal variation in growth rates, reproduction, maturity and movement (McGrath & Jackson 2002, Jackson *et al.* 2003, McGrath Steer & Jackson 2004, Triantafillos *et al.* 2004, Jackson *et al.* 2005, Stark *et al.* 2005). Variability in catch rates and biological parameters in *N. gouldi* is attributed to environmental and oceanographic variables, as for *Loligo gahi*, *D. gigas*, *Illex illecebrosus* and *I. argentinus* (Dawe *et al.* 1998, Anderson & Rodhouse 2001b, Middleton & Arkhipkin 2001, Agnew *et al.* 2002, Jackson & McGrath-Steer 2004b). *Nototodarus gouldi* is an important resource for domestic and international markets (Lynch 2005) targeted by jig fishermen from the southern squid jig fishery (SSJF) between January and July primarily off Victoria and Tasmania, and caught as a trawling bycatch year round in the Commonwealth trawl sector (CTS) and Great Australian Bight trawl sector (GABTS) . Managed as a single population within the southern and eastern scalefish and shark fishery (SESSF), *N. gouldi* caught within the boundaries of the SSJF, CTS and GABTS contribute to allowable catch limits; however, it is unknown whether *N. gouldi* caught are from a single population with spatial integrity or consists of multiple stocks. With stock identification a prerequisite for stock assessment (Cadrin *et al.* 2005), knowledge of whether a discrete stock of randomly mating individuals exist in southern Australia will be valuable information for efficient management of the *N. gouldi* fishery.

For cephalopods, various techniques are used to answer stock identification and migration questions, including genetic (Martinez *et al.* 2005, Buresch *et al.* 2006), meristic (Martinez *et al.* 2002, Vega *et al.* 2002), tagging (Stark *et al.* 2005, Replinger & Wood 2007), and morphometric comparisons of body shape (Baron & Re 2002, Martinez *et al.* 2002). In teleost research, both otolith shape and element composition analyses have also been useful, with interest in element composition growing in cephalopod population biology (Semmens *et al.* 2007). Several techniques are available to describe otolith shape including the use of wavelets (Lombarte *et al.* 2006) and morphological characterisation (Begg *et al.* 2001, Bergenius *et al.* 2006); however, many have been successful using Fourier analysis (Campana & Casselman 1993). Fourier analysis of otolith shape has been used in elucidating stock structure of haddock *Melanogrammus aeglefinus* (Begg *et al.* 2001), orange roughy *Hoplostethus* *atlanticus* (Smith *et al.* 2002), and Atlantic mackerel *Scomber scombrus* (Castonguay *et al.* 1991). The effectiveness of otolith shape analysis is sometimes questioned because within stocks there are differences between males and females as well as among year classes (Castonguay *et al.* 1991, Begg & Brown 2000); however, the technique has still provided useful information for fisheries models and assessments. Otolith element composition has also been effective in discriminating stocks of cod *Gadus morhua* (Campana *et al.* 2000), orange roughy *Hoplostethus atlanticus* (Edmonds *et al.* 1991), and Spanish mackerel *Scomberomorus sierra* (Ramírez-Pérez *et al.* 2010) with only a few papers using both methods complementarily (Bergenius *et al.* 2006, Turan 2006).

Squid statoliths are paired calcareous concretions found within the statocysts of the cephalopod cranium and are composed calcium carbonate ($CaCO_3$) in the form of aragonite crystals (Clarke 1978, Radtke 1983, Morris 1991). Similar in form and function to fish otoliths, they grow continuously throughout their life and are thought to be capable of recording life history events (Jackson 1994, Arkhipkin 2005) useful for stock assessment. There is interest and potential to apply statolith shape analysis and element composition techniques to obtain additional biological and historical lifecycle data of squid. The limited use of these techniques may be due to the small size of statoliths (≈1 mm total length) which can pose challenges when preparing and examining these balance organs. However, recent technological advancements (e.g. microscopy, image analysis, laser ablation inductively coupled plasma mass spectrophotometer) have illustrated that both techniques have potential to increase existing knowledge of squid fisheries for modelling and assessment purposes (Gonzalez & Guerra 1997, Semmens *et al.* 2007). Biomineralisation studies found that statoliths comprise of minor and trace elements including Mg, Mn, Sr, and Ba (Hurley *et al.* 1985, Durholtz *et al.* 1997, Lipinski *et al.* 1997, Yatsu *et al.* 1998, Arkhipkin *et al.* 2004a). Although the process of elemental uptake from surrounding waters in statoliths is unknown in cephalopods (Zumholz *et al.* 2007b), element concentration is related to both spatial and environmental influences (Ikeda *et al.* 1998, Arkhipkin *et al.* 2004a, Arkhipkin 2005), and physiological processors (Hanlon *et al.* 1989, Bettencourt & Guerra 2000). For example, strontium concentration is used to help define ontogenetic migrations (Yatsu *et al.* 1998, Ikeda *et al.* 2003, Zumholz *et al.* 2007b), whereas barium is related positively to water temperature and productivity (Zumholz *et al.* 2007a, Zumholz *et al.* 2007b). As well as incorporating elements, the rate of statolith growth may influence their shape over time. Statolith shape differences particularly in the dorsal dome occurs during all ontogeny stages from juveniles to adults (Lipinski 1993, Gonzalez & Guerra 1997, Thomas & Moltschaniwskyj 1999) and shape has been used to discriminate species within the genus *Loligo* (Pineda *et al.* 1998). Morphological features of statoliths from squid of early ontogenetic stages are relatively complex compared to otoliths (Arkhipkin & Bizikov 1997) indicating that statolith shape has significant phylogenetic value (Clarke 1978). Although genetics may dictate the initial shape of otoliths, environmental variability among geographically separated regions may influence their shape characteristics as they grow (Neilson *et al.* 1985, Campana & Casselman 1993).

Over broad spatial scales genetic homogeneity can be maintained where reproductive exchange is limited (Waples 1998). In such situations, genetically homogenous populations can comprise of distinct phenotypic stocks that are crucial for understanding structure for management purposes (Begg & Waldman 1999). As *N. gouldi* are distributed over a vast geographical area they may be subjected to little genetic variation as a result of large genetic drift (Ward *et al.* 1994) so phenotypic variation in both shape and elemental concentrations can be advantageous when investigating putative stock identities (Campana 1999, Campana *et al.* 2000, Jonsdottir *et al.* 2006). The aim of this study was to determine whether *N. gouldi* caught in the Great Australian Bight (GAB) are a different stock compared with squid caught in Victoria using statolith shape and element composition analyses. Statolith shape will be used to determine whether discrete phenotypic stocks of *N. gouldi* exist in the GAB and Victoria; whereas element composition near the statolith core will be used to ascertain where individuals are likely to have hatched.

3. METHODS

3.1. Samples

Nototodarus gouldi were collected from commercial trawling vessels operating in the GAB (33°15S, 130°00E) from June 2008 – June 2009; and off the coast of Portland, Victoria, Australia (38°45S, 141°30E) from March 2007 – April 2009 (Figure 5-1). Animals were collected approximately bimonthly from the GAB; whereas samples from Victoria were collected approximately monthly (Table 5-1). Frozen squid were shipped to the University of Tasmania or the Marine and Freshwater Fisheries Research Institute in Victoria for dissection, sample preparation, and statolith analysis. Data collected from each individual included total weight (g), dorsal mantle length (DML, mm), sex, and age (d) estimated using statoliths. Statolith pairs were removed, cleaned with distilled water, and stored dry at room temperature in cavity trays. For age estimation, statoliths were ground and aged by counting incremental structure from the hatch mark to the edge of the dorsal dome (as per Jackson, 2005).

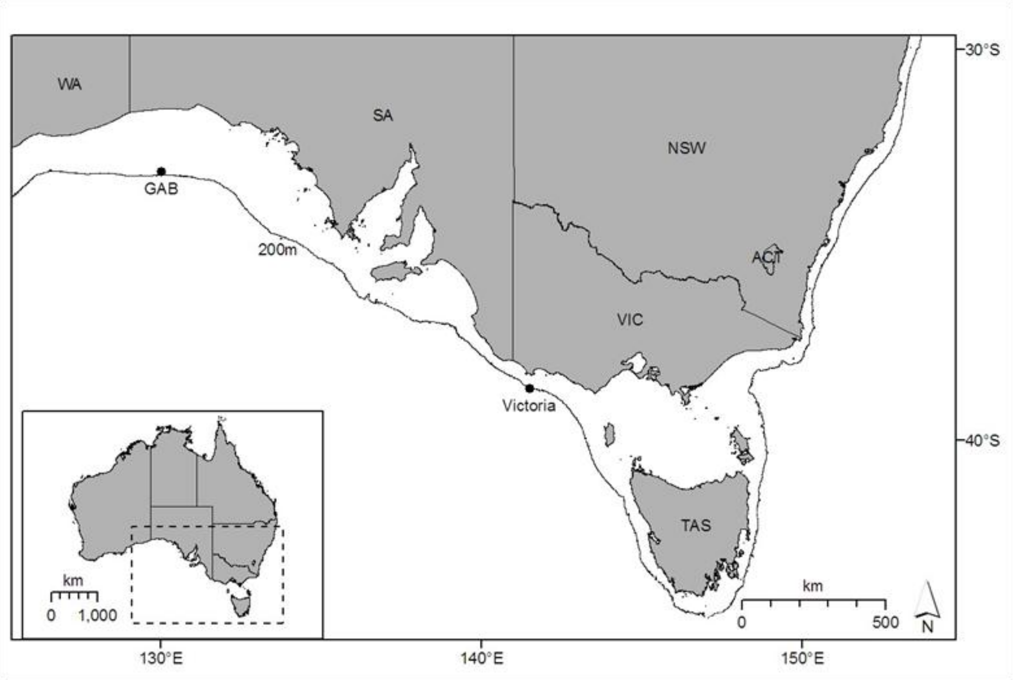

Figure 5-1 Map of south eastern Australia illustrating the two collection regions, Great Australian Bight (GAB) and Victoria.

Table 5-1 Number of *Nototodarus gouldi* from which statoliths were used for shape analysis and/or statolith element analysis from each site (GAB and Victoria). Blank cells indicate no samples collected or used in that analysis.

			SHAPE ANALYSIS		ELEMENT ANALYSIS	
YEAR	MONTH	SEASON	*GAB*	*Victoria*	*GAB*	*Victoria*
2007	Mar	Autumn 07		149		
	Jun	Winter 07		91		
	Aug	Winter 07		88		
	Sep	Spring 07		82		
	Oct	Spring 07		86		
Total				*496*		
2008	Feb	Summer 07/08		85		
	Mar	Autumn 08		84		
	May	Autumn 08		171		
	Jun	Winter 08	99	85	17	16
	Jul	Winter 08		88		
	Aug	Winter 08	248	195	17	16
	Sep	Spring 08		105		20
	Nov	Spring 08	102	108	17	
	Dec	Summer 08/09	64	110	17	15
Total			513	1031	68	67
2009	Jan	Summer 08/09		89		
	Feb	Summer 08/09	126	121	18	18
	Mar	Autumn 09		86		18
	Apr	Autumn 09	76	66	14	
	Jun	Winter 09	114	63		
Total			316	425	32	36
Total over all years			**829**	**1952**	**100**	**103**

3.2. Fourier shape analysis

In teleost research, the Fourier transformation is considered the most objective and powerful of the shape analysis techniques available (Campana & Casselman 1993) and consequently used as a technique trialled to discriminate putative *N. gouldi* stocks. Both statoliths from all animals were used in shape analysis prior to further preparation used to obtain age and elemental concentrations (Table 5-1). Statoliths were attached to glass microscope slides using the thermal plastic glue Crystalbond™ and arranged with the convex (posterior) side facing up. Using a compound microscope (Leica DM3000) and reflected light, images were digitally captured using a 25x objective coupled with a 0.70x ‘C-type’ camera mount. Image analysis software Optimas™ was used to capture and save statoliths images in tiff (Tagged Image File Format, 768x576, 24-bit True Colour RGB) format and later converted to a jpeg (Joint Photographic Experts Group, 384x288, 8bit, Greyscale) image. Broken statoliths were not used for analysis. Statoliths are attached to the sensory *macula statica princeps* within the statocyst of the squid cranium (Arkhipkin 2003); however, when dried this non-calcareous attachment point dehydrates, becomes brittle, and often fractures. Statolith shape was therefore described using only the calcareous parts. The perimeter of each statolith was automatically (and sometimes manually) traced from the rostrum tip, along the lateral dome and completed at the margin between the dorsal dome and the statolith attachment point (Figure 5-2). The number of coordinates used as inputs to the Fast Fourier Transform is bound by 2^n complex numbers and was identical to those used to describe the otolith shape of the deepwater teleost orange roughy *Hoplostethus atlanticus* (Smith *et al.* 2002). For orange roughy, 128 x–y pixel coordinates are extracted at equidistance intervals around the perimeter of individual otoliths. As statoliths are less complex than orange roughy otoliths, 128 coordinates was considered to be sufficient to describe statolith shape. A relative statolith area was calculated representing the area between the first (x_0–y_0) and last (x_{127}–y_{127}) pixel coordinates bounded by the perimeter of the calcareous region (Figure 5-2).

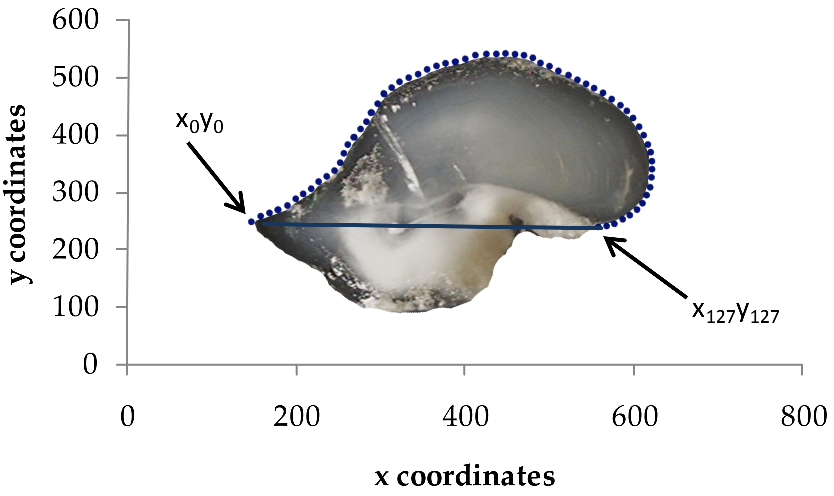

Figure 5-2 Posterior side of a *N. gouldi* statolith displaying the trace of the calcareous region from the rostrum tip (x_0y_0) to the margin between the dorsal dome and the statolith attachment point ($x_{127}y_{127}$). A relative statolith area was calculated as the number of pixels represented between the first and last x–y pixel coordinates (solid line) bounded by the perimeter of the calcareous region (dashed line).

Using x–y coordinates as the complex number input, a Fast Fourier Transform (FFT) was performed using the Microsoft Excel™ FFT function. The resulting array of 128 Fourier descriptors (also represented as a complex number) was calculated for all statolith trace coordinates using a Visual Basic™ macro. To normalize the location of the start of the trace, the 0th descriptor was set to $0+0i$. To normalise for statolith size, the 1st descriptor was divided by itself and the remaining 126 descriptors. All descriptors now represent the shape of a statolith and corrected for trace location and statolith size. Although the initial descriptors describe the majority of shape, all Fourier descriptors were used in the analysis. The Harmonic value was calculated for each descriptor within the array using the formula Eq 5.1.

$$(\mathrm{H}) = |z| = \sqrt{a^2 + bi^2} \qquad \text{Eq 5.1}$$

where *a* and *bi* are the real and imaginary components of each Fourier descriptor (Lestrel 1997). To determine the difference in statolith shape between two specified groups of squid, the harmonic difference between each group was calculated using Eq 5.2,

$$DH_{jk} = \sqrt{\sum_{i=2}^{127} (\overline{H}_{ij} - \overline{H}_{ik})^2} \qquad \text{Eq 5.2}$$

where *DHjk* is the Observed Harmonic Difference (OHD) between group *j* and group *k*. *Hij* is the *i*th mean harmonic value from group *j*. *Hik* is the *i*th mean harmonic value from group *k* (Lestrel 1997). Only equal numbers of samples from each group were used in analyses.

A pairwise randomisation technique was used to explore whether the global shape of statoliths from one group was similar to the global shape of another group. A Randomised Harmonic Difference (RHD) was calculated by creating a homogenous group of statolith shapes by randomly sorting the harmonic values from two groups of statoliths (e.g. Group A and B mixed) and allocating in a 50:50 ratio to two new groups (e.g. Group 1 and 2). The RHD was calculated between Group 1 and 2 using the same formula used to calculate the OHD. The randomisation was repeated 5000 times to obtain a distribution of RHD values (Galley *et al.* 2006). The probability that two groups expressed similar shapes was estimated as the proportion of randomisations for which the OHD was greater than the RHD divided by the number of randomisations used. A Bonferroni correction was applied to the significance level ($\alpha = 0.05$) to reduce the likelihood of a type I error when multiple significant tests are used. The adjusted p-value of $\alpha = 0.0125$ based on four different groups (two locations for each of the two sexes) was compared with randomisation test results.

Left and right statoliths from each animal were compared using Fourier analysis to determine if they were similar in shape. If similar, only one statolith (left or right) would be required for comparison among putative stocks. A pairwise randomisation test showed that there was no significant difference between left and right statoliths for either sex (females p=0.91, n=110; males p=0.57, n=160). As a result, either the left or the right statolith was randomly selected from collections for subsequent analysis. The relationship between statolith and somatic growth is variable in many squid species (Arkhipkin *et al.* 1999, Thomas & Moltschaniwskyj 1999); therefore shape may be a function of statolith area, animal size, or a combination of these and other factors. Comparing statolith shape between two groups requires that comparable groups of animals be selected ‘a priori’. To determine whether to select samples based on statolith area or mantle length, an ANCOVA was used to analyse the relationship between statolith area and DML of squid collected in the GAB and Victoria, using DML as the covariate. Although no significant interaction was observed between location, sex and the covariate DML ($F_{\text{location*sex*DML}} = 2.81$, df 1,2745, $p<0.094$), differences in the relationship between statolith area and dorsal mantle length (Figure 5-3) was evident between sexes ($F_{sex*DML} = 33.42$, df 1,2745, $p<0.001$). A significant positive linear relationship existed for males and females with statolith area increasing a greater rate for males by 1.92 $\times 10^{-4}$ $mm^2\,mm^{-1}$ DML (Table 5-2). Analysing the relationship between DML and statolith area between locations showed no significant difference ($F_{location*DML} = 3.84$, df 1,2745, $p=0.05$). Estimated marginal means however, did indicate that a difference was observed between sexes ($F_{sex} = 267.3$, df 1,2750, $p<0.001$) with males displaying larger statoliths on average than females by $0.042mm^2$ (Figure 5-4). As sexual dimorphism in statolith area with respect to mantle length was observed, selecting samples of equal DML will yield a range of different sized statoliths for each sex. Therefore statolith area was used as a standardising factor when selecting samples for Fourier analysis with comparisons between sexes made separately.

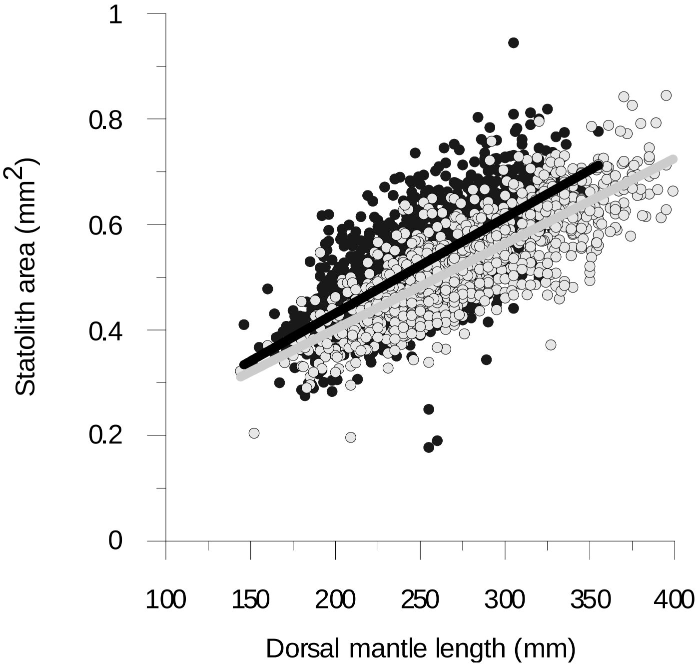

Figure 5-3 The relationship between statolith area and dorsal mantle length by sex (locations combined), black symbols male, grey symbols female. Linear regression displayed for male (black line) and female (grey line).

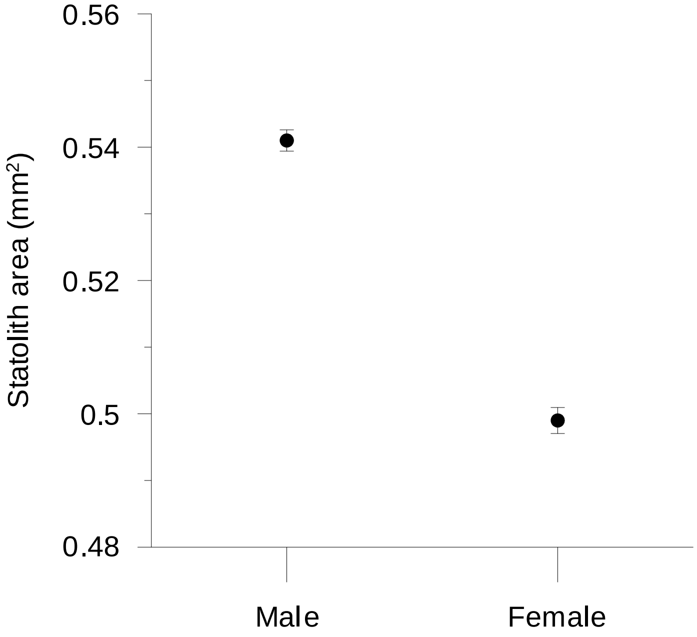

Figure 5-4 Estimated marginal means (±se) of statolith area for male and female *N. gouldi* (combined locations).

Table 5-2 The linear relationship between statolith area and dorsal mantle length (DML) for male and female *N. gouldi* (combined locations). H_o for t test: $b = 0$. Standard errors are represented in parentheses.

Sex	n	Slope ±se	Intercept (±se)	r^2	F	P
Female	1107	$1.62\text{x}10^{-3}(3.69\text{x}10^{-5})$	$7.83\text{x}10^{-2}(1.03\text{x}10^{-2})$	0.64	1924.2	<0.001
Male	1646	$1.81\text{x}10^{-3}(4.59\text{x}10^{-5})$	$7.00\text{x}10^{-2}(1.16\text{x}10^{-2})$	0.49	1554.3	<0.001

The relationship between statolith size and shape was also analysed to determine if statoliths change shape, as they get larger. If shape is consistent throughout the life of a squid, statoliths of different sizes could be pooled for analysis. The shape of small statoliths was compared with the shape of large statoliths for each location and sex. A frequency distribution of statolith area was used to develop criteria that defined small (<25th percentile of statolith area) and large (>75th percentile of statolith area). For Victoria caught squid (combined collections), statoliths < 0.457 mm^2 and > 0.595 mm^2 were denoted as being small and large statoliths respectively; whereas GAB caught squid statoliths <0.457 mm^2 and >0.549 mm^2 were classified as small and large respectively. Results of the randomisation test indicate that small statoliths are different in shape compared with large statoliths for male and female squid caught in locations GAB and Victoria (Table 5-3).

Table 5-3 Shape analysis test between pairwise comparison groups. Equal statolith areas are represented in each group for comparisons between sex and between locations. Due to multiple tests the critical P values has been adjusted using a Bonferroni adjustment to 0.0125.

Pairwise comparison	Location	N	Year(s)	Observed Harmonic Difference (x10^{-2})	Average (± se) Randomised Harmonic Difference (x10^{-2})	Significance (p)
Small vs large male	Victoria	556	2007–09	9.18	1.86 (0.01)	<0.001
Small vs large female	Victoria	352	2007–09	7.16	2.26 (0.02)	0.003
Small vs large male	GAB	250	2008–09	9.36	2.79 (0.02)	0.002
Small vs large female	GAB	166	2008–09	14.06	3.82 (0.03)	<0.001
Female vs male	Victoria	1478	2007–09	8.75	1.07 (0.01)	<0.001
Female vs male	GAB	682	2008–09	8.67	1.76 (0.02)	<0.001
Victoria v GAB female		730	2007–09	5.81	1.64 (0.01)	<0.001
Victoria v GAB male		920	2007–09	5.36	1.42 (0.01)	<0.001

Both sexual dimorphism in the relationship between DML and statolith area coupled with changes in statolith shape with respect to statolith size indicates that statolith shape discrimination between squid caught in the GAB and Victoria needs to be conducted on sexes separately as well as being based on statolith size. If the frequency distribution of statolith area from each location of capture were different, then differences in shape between the two locations may be attributed to the size of the statolith as well as the phenotypic characteristic of squid in each area. Consequently, the influence of statolith size on statolith shape was removed by randomly selecting data from each group so that an equal distribution of statolith area was represented in each test group. To maximise the sample size for shape analyses, individuals collected across the years were combined, on the assumption that the shape is temporally consistent over the collection period.

3.3. Statolith element composition analysis

Squid collected from the GAB and Victoria in June 2008 – April 2009 were organised into approximately six bimonthly groups (Table 5-1). Up to twenty animals from each location and bimonthly group were randomly selected for element composition analysis (n=203). Either the left or the right statolith was randomly selected and ground in the posterior-anterior plane to achieve a section approximately 140 µm thick. A base layer of Aka Resin™ (and hardener) was poured on to a silicone rubber mold (1 cm^2) and allowed to cure partially. To ensure statoliths were ground in the correct plane, each was positioned on the resin base with the anterior tilted at approximately five degrees so that the core and statolith edge was horizontally aligned. The resin block was labelled and further resin was used to encompass the statolith and allowed to cure overnight at 30°C. An Isomet™ saw was used to trim excess resin from the statolith surrounding until a small block 4mm x 4mm x 2mm was achieved. Using a hotplate, the resin block was attached to a microscope slide using the thermoplastic glue Crystalbond™. Lapping film (3M®) of grades 30 µm and 10 µm were used to grind statoliths so that the region from the core of the statolith to the edge of the statolith was clearly seen, but not exposed; final polishing of the statoliths was done using 5 µm lapping. Preparations were regularly viewed during the grinding process using a Leica compound microscope at up to x400 magnification until the core and edge were clearly visible.

Statolith element composition was determined at the Marine and Freshwater Fisheries Research Institute, Queenscliff, Australia using New Wave Research UP-213 Nd:YAG ultraviolet laser microprobe (Q-switched) in combination with a Thermo Finnigan Element2 High Resolution Inductively Coupled Plasma Mass Spectrophotometer (HR-ICP-MS). To remove superficial contaminants from the ground surface, mounted statoliths were sonicated in Milli-Q water for three minutes, triple rinsed, and allowed to air dry in a laminar flow cabinet overnight. The accuracy of acquired concentrations is affected by room temperature and internal contamination; therefore accuracy will change during consecutive analyses. To compensate for this, the sequence that statolith preparations were analysed was randomised with respect to location and bimonthly collection group. Prior to ablating each statolith, an average background count was determined from the first 50 scans and subtracted from the sample counts for each transect ablation. In the presence of helium, statoliths were ablated along a transect from the core to the edge of the dorsal dome, near to the apex of increment formation (Figure 5-5). To further remove surface contaminants each preparation was pre-ablated along the transect (80 µm, pulse rate 6 Hz, ≈9.5 J/cm^2, 70 µm sec^{-1}). A 30µm spot size ablation (pulse rate 10Hz, ≈9.5 J/cm^2) followed that continuously acquired sample from the core to the edge at 2.0 µm sec^{-1}. The helium/sample combination was mixed with argon prior to analysis in the HR-ICP-MS. To ensure consistency between ablations, the National Institute of Standards (NIST) SRM 612 glass wafer was used after every six transect ablations (Lahaye *et al.* 1997, Hamer *et al.* 2003). Elemental concentration was collected for Mg^{25}, Mn^{55}, Cu^{63}, Zn^{66}, Sr^{88}, Rb^{26}, Ba^{138}, and Pb^{208} with Ca^{44} used as an internal standard to control for variation in the ablation yield. The concentration of calcium in statoliths was assumed similar to fish otoliths and set a constant 388,000 µg g^{-1}. Detection limits were used to reject data that may be the result of unwanted instrument noise and calculated for each element based on three standard deviations of the blank gas and adjusted for ablation yield. Average detection limits (µmol mol^{-1} Ca) were Mg=39.6, Mn=1.67, Sr=7.4, Ba=0.19. Elements Cu, Zn, Rb, and Pb were below detection limits and not used in analysis. Concentrations of Mn were sometimes below the Limits of Detection (LOD), and although the quantitative results may be subjective, data may still be useful for indicating variation between groups or analysing trends (Ben-Tzvi *et al.* 2007). Noise was removed from the transect data using an 11 point running median followed by an 11 point running average (Sinclair *et al.* 1998). Element concentration was calculated and presented as a ratio to Ca in µmol mol^{-1} Ca. Accuracy and precision were calculated on a daily basis using NIST SRM 612. Precision was calculated for individual elements as the average relative standard deviation (RSD) for all NIST samples (Mg=6.3 %, Mn=4.0 %, Sr=4.9 %, Ba=5.2 %). Accuracy was calculated for individual elements as the average percentage recovery of NIST SRM 612 (Mg=101.5 %, Mn=101.8 %, Sr=100.9 %, Ba=100.2 %).

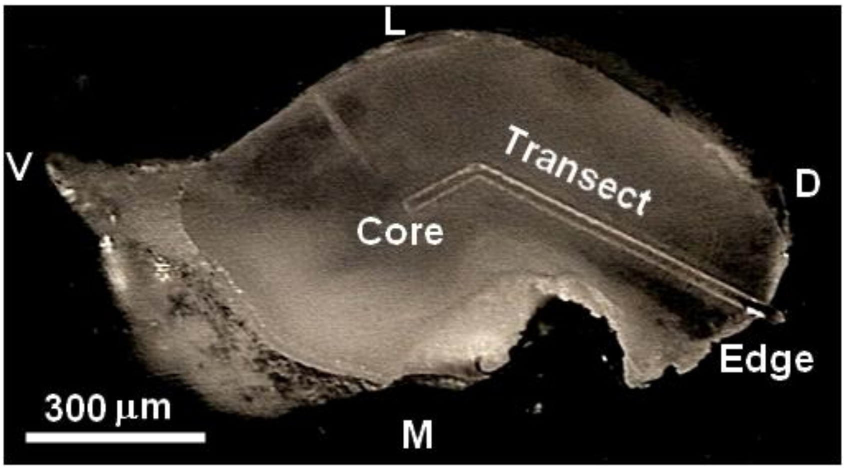

Figure 5-5 Posterior side of a ground *N. gouldi* statolith illustrating the location of the ablation transect from the core to the edge of the dorsal dome. D = Dorsal, V = Ventral, L = Ventral, M = Medial.

The first step to determine the natal origin of individuals from specific times and locations using elemental concentration from statoliths is to ascertain if there are significant spatial and temporal differences in the elemental composition of the hard structure. If this is present, then it will allow discrimination of individuals at those spatial and temporal scales. Each collection from a particular month was converted to a season of capture (summer, autumn, winter, spring) to reduce the complexity of interpretation and increase statistical confidence. Spatial and temporal variability was assessed using the pre-edge composition of statoliths in a MANOVA. Pillai's test was used as the test statistic ($p<0.05$) as it is robust to deviations from multivariate normality (Quinn & Keough 2008). Average element concentration in the 'pre-edge' was calculated approximately over the statolith 30 days pre-capture (i.e. 30 daily growth increments from the statolith edge). Elements Mg, Mn, Sr and Ba were used as dependant variables in the MANOVA from adults caught between winter 2008 and spring 2009. Canonical discriminant analysis was used to ascertain where spatial and temporal differences existed and what elements were influencing differences (Henry *et al.*). ANOVAs were used to illustrate differences between location and season of capture combinations.

Given spatial and temporal difference in statolith elemental composition, a common approach to assign natal origin to animals is to collect newly hatched juveniles from specific locations and times to generate allocation rules based on the suite of elemental concentrations in the core of the statolith. The analysis of statolith elemental composition of adults from this cohort allows the statolith core elemental composition to be compared with the core elemental composition determined from juveniles at particular locations. Using techniques like Linear Discriminant Analysis (LDA) the likelihood of an animal originally coming from a particular location is calculated.

However in this study, juvenile squid could not be collected to generate elemental allocation rules, therefore an alternative approach was adopted. Adult squid were collected from the GAB and Victoria at specific times, at the same time that juveniles were hatching. The recently deposited pre-edge region of statoliths from adult squid were analysed to obtain the elemental concentration at that specific location and time and subsequently used to generate the LDA allocation rules. The elemental concentration in the post-core (representing an average concentration of elements in the first 30 days post hatch) region of squid caught 6 – 9 months later in the GAB and Victoria where then used to determine the probability of an individual coming from either GAB or Victoria. Where post-core concentrations of elements were outside the pre-edge concentration range for a given season of capture, individuals were assigned to have hatched from an unknown location whose element concentrations are different to those found in the GAB or Victoria for a particular season. Only Sr and Ba were used to generate LDA allocation rules as concentrations have been shown to be influenced by environment variables (Elsdon & Gillanders 2002) more so than Mg and Mn. Magnesium was not used as concentrations are possibly related to changes in growth rate, as well as being used during statolith biomineralisation process (Morris 1991, Arkhipkin *et al.* 2004a, Zumholz *et al.* 2007b). Manganese was not selected as concentrations can be influenced by taxon-specific uptake mechanisms, instrument noise, as well as being more evenly distributed in oceans (Arkhipkin *et al.* 2004a).

When classifying post-core concentrations using pre-edge LDA allocation rules, individuals whose classification confidence was ≥60% were classified as hatched at either Victoria or GAB. Where classification confidence was <60%, individuals were classified as either coming from Victoria or GAB, or an unknown location where post-core concentrations are within the range of element concentrations used to generate allocation rules. Only seasons where significant discrimination between GAB and Victoria were evident from CDA were used in generating LDA allocation rules and subsequent post-core classification. A Jacknife cross-validation (leave-one-out) on the group of individuals used to generate the LDA was then used to determine error rates for the allocation rules. Such a method of juvenile classification assumes no physiological change in the process of statolith deposition over time, i.e. the composition of the statolith is purely due to seawater composition or the effects the environmental variables on composition and not due to differences in deposition process between adults and juveniles.

Using LDA, allocation rules based on elemental concentrations in the pre-edge statolith region was generated from 56 (29 GAB, 27 Victoria) individuals collected during winter 2008, and 55 (27 GAB, 28 Victoria) individuals collected during summer 2008/09. Allocation rules were used to classify post-core concentration to a hatch location for 55 (27 GAB, 28 Victoria) individuals that hatched during winter 2008, and 38 individuals (12 GAB, 26 Victoria) that hatched during summer 2008/09.

4. RESULTS

4.1. Fourier shape analysis

Differences in the relationship between DML and statolith area for both male and female squid (Figure 5-3), coupled with significant differences between the average statolith marginal area (Figure 5-4), suggested that shape needed to be analysed on sexes separately. Such sexual dimorphism was also illustrated when comparing the shape of statoliths of male and female squid caught in the GAB and Victoria (Table 5-3). The OHD between sexes was 8.67×10^{-2} and 8.75×10^{-2} for squid caught in the GAB and Victoria respectively. Statolith shape was also significantly different between small and large statoliths for females and males collected in the GAB and Victoria (Table 5-3). The greatest difference between small and large statoliths was observed for females collected in the GAB (OHD = 14.06×10^{-2}); whereas females caught in Victoria showed less of a difference (OHD = 7.16×10^{-2}). The OHD between small and large statoliths for male squid collected in the GAB and Victoria was 9.36×10^{-2} and 9.18×10^{-2} respectively. A comparison between GAB and Victoria found that both male and female squid had significant differences in the shape of their statoliths when an equal size distribution of statolith areas was selected for analysis (Table 5-3).

4.2. Spatial and temporal element composition variability in *N. gouldi* statoliths

Elements Mg, Mn, Sr and Ba in statoliths varied temporally and spatially (Figure 5-6). Analysing individual elements on the pre-edge region of statoliths revealed significant interaction between location and season of capture for Mg ($F_{location*season}$ = 4.76, df 3,204, p=0.003), Mn ($F_{location*season}$ = 6.61, df 3,206, p<0.001) and Sr ($F_{location*season}$ = 2.69, df 3,195, p=0.047); however, no interaction was evident for Ba concentrations ($F_{location*season}$ = 1.68, df 3,196, p=0.171). Barium showed significant difference between location ($F_{location}$ = 16.35, df 1,196, p<0.001) with concentrations greater in Victoria (5.03 µmol mol^{-1} ±0.05 se) compared with GAB (4.75 µmol mol^{-1} ±0.05 se). Season effects on Ba were also found (F_{season} = 6.42, df 3,196, p<0.001) with concentration lowest during winter (4.73 µmol mol^{-1} ±0.06 se) and greatest during summer (5.05 µmol mol^{-1} ±0.06 se). For Mg, a Tukey's post hoc test was unable to identify which season / location combinations were different from one another; however, the lowest average concentration on the statolith pre-edge was found in squid collected in the GAB during autumn 2009 (145.94 µmol mol^{-1} Ca ±4.2 se) and greatest in Victoria during summer (170.62 µmol mol^{-1} Ca ±4.6 se). Concentrations of Mn in the pre-edge of the statolith was lowest for squid collected in Victoria during winter 2008 (0.32 µmol mol^{-1} Ca ±0.03 se) and greatest for squid collected in Victoria during summer 2008/09 (0.55 µmol mol^{-1} Ca ±0.04). Post hoc tests showed that variation in Mn concentration between seasons was less variable than for squid caught in the GAB (Figure 5-6). Although Sr concentrations displayed significant interaction between location and season, post hoc tests were unable to illustrate location / season combinations were different. For Sr, average element concentration was highest for squid collected in the GAB during summer 2008/09

(8083 µmol mol^{-1} Ca ±91.8 se) and lowest in the GAB during winter 2008 (7594 µmol mol^{-1} Ca ±86.2 se).

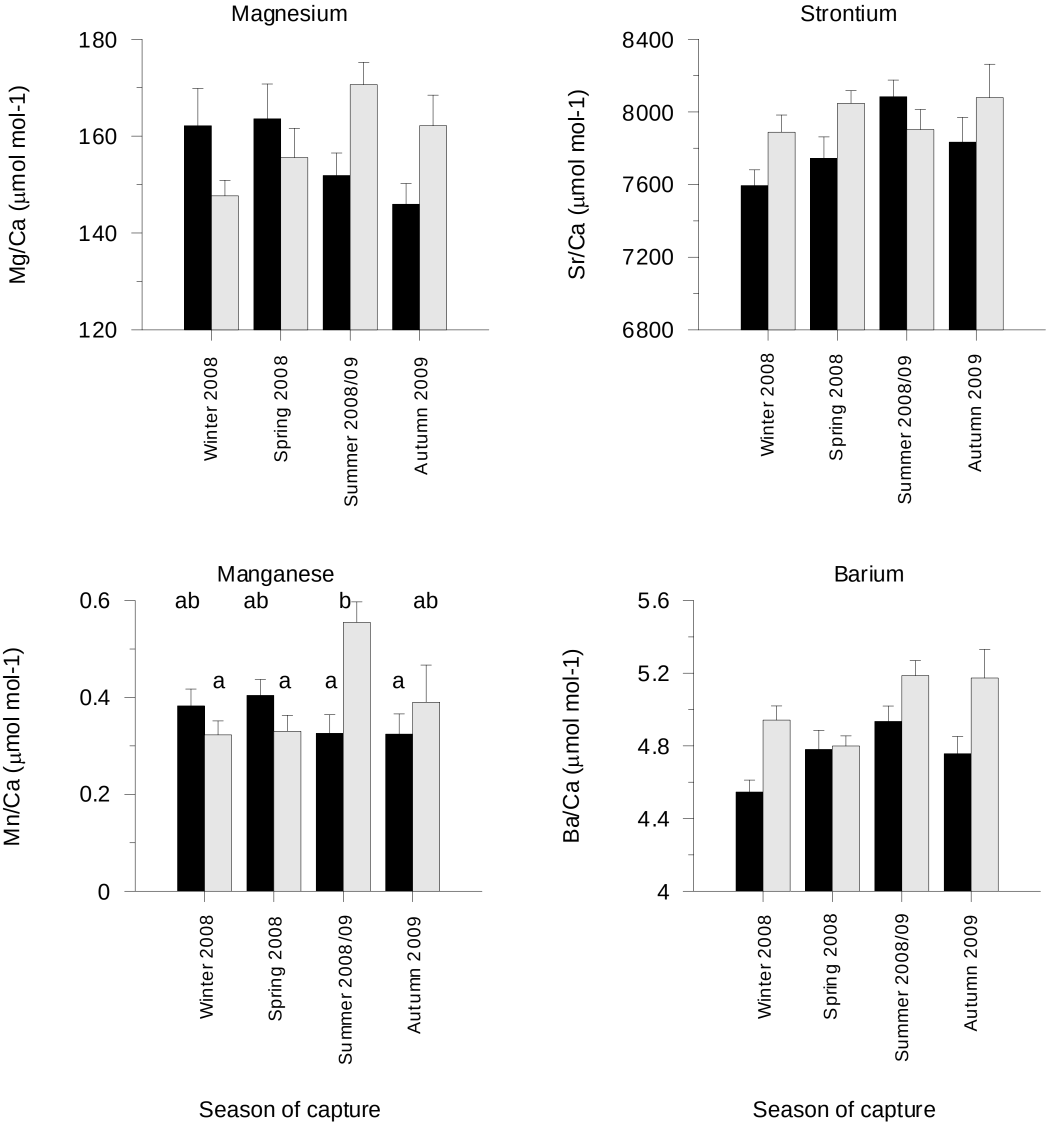

Figure 5-6 Average (±se) concentration of Mg, Mn, Sr, and Ba, for *N. gouldi* statoliths representing 30 days pre-edge from squid collected in the GAB (solid bars) and Victoria (shaded bars) by season of capture. Like letters indicate where mean concentrations were similar.

Comparing the elemental composition of statoliths in the pre-edge region using a MANOVA on all four elements indicated that differences between squid from the GAB and Victoria was not the same for the four seasons that squid were collected ($F_{location* season}$ = 4.16, df 12,543, $p<0.001$). In the CDA plot, 94.5% of variation was captured in the first two axes (Figure 5-7). Spatial and temporal variation in elemental concentration was observed among collections from the GAB and Victoria in winter 2008 and summer 2008/09. In the GAB during winter 2008, higher concentrations of Sr and Ba were observed on the second CDA axis in the pre-edge region of statoliths compared with statoliths from Victoria caught squid by 5% and 8% respectively. During summer 2008/09, distinct differences in concentrations were observed between GAB and Victorian collections in the first CDA axis with Mn, Mg, Ba concentrations highest from squid caught in the GAB by 41%, 11%, and 5% respectively. No spatial difference in elemental concentration was found for squid collected in spring 2008 or autumn 2009. Jacknife cross-validation of elements to their correct original location and season of capture combination was 29% (eight combinations of location and season). However, when season was removed from the analysis 78% of Victoria caught squid were classified to their correct location; whereas only 51% were of squid caught in the GAB were classified to the GAB.

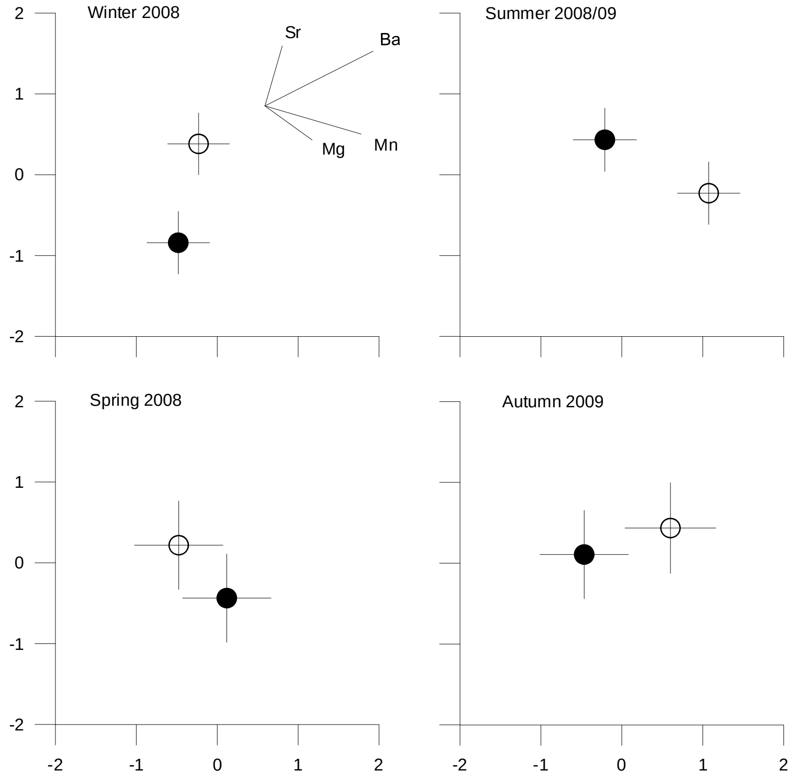

Figure 5-7 Canonical discriminate analysis plot showing the variation in elemental composition of *N. gouldi* collected in the GAB (solid points) and Victoria (open points) during winter 2008, spring 2008, summer 2008/09, and autumn 2009. Error bars represents the 95% confidence intervals around the centroid average for each site. The length and direction of the vectors for each element (Sr=strontium, Ba= barium, Mn = manganese, Mg= magnesium) indicate the correlation between the elements and the axes.

Using Sr and Ba to develop pre-edge elemental LDA allocation rules, jackknife cross-validation correctly assigned 69.4% of individual animals to their correct location of capture compared with 52.7% (±0.25 se, n =10) when location of capture was randomised. Cross-validation was greatest in Victoria where 81.0% of squid were classified to the correct location compared with an average classification rate of 66.6% (±0.40 se, n=10) when location of capture was randomised. Squid caught in the GAB showed greater cross validation variation with 52% of squid allocated to the correct location. However, this value was also greater than the average classification rate of 34.1% (±0.42 se, n =10) when location of capture was randomised. For squid caught in the GAB that hatched during winter 2008 and summer 2008/09, 59% and 100% respectively had post-core Sr and Ba concentrations within the pre-edge concentration range of statoliths from adults caught in the GAB during the same seasons (**Error! Reference source not found.**). Whereas, Victorian squid caught during winter 2008 and summer 2008/09, had 54% and 92% post-core concentrations within the pre-edge concentration range. Such results indicate that independent of capture location, approximately half of squid caught during winter are likely to have hatched at a location other than the GAB or Victoria whereas most squid caught in summer are likely to have come from GAB or Victoria.

During winter 2008 where the predicted allocation confidence was $p \geq 0.6$, 64% of squid caught in the GAB and 89% of squid caught in Victoria had post-core element concentrations similar to those found in Victoria. However, during summer 2008/09, 100% of squid captured in GAB exhibited post-core concentrations similar to Victoria; whereas 67% of squid caught in Victoria had similar post-core concentrations similar to those found in the GAB. Where the predicted allocation confidence was $p<0.6$, approximately 60% of squid caught in the GAB and Victoria during winter 2008 had post-core concentrations similar to pre-edge concentrations found at GAB and Victoria respectively. Compared with summer however, differences were more pronounced with 80% and 73% of squid caught in the GAB and Victoria respectively predicted to have post-core concentrations similar to pre-edge concentrations of statoliths from squid collected in the GAB.

Table 5-4 Number of individuals predicted to have hatched at a given location that were originally caught at either GAB or Victoria. Pre-edge concentrations from animals collected in the GAB and Victoria during winter 2008 and summer 2008/09 were used to generate LDA allocation rules. Post-core concentrations of squid collected in the GAB or Victoria that hatched during the same seasons were allocated to have originated from 1/ an unknown location where post-core concentrations were outside pre-edge concentration range. 2/ Victoria or GAB (where predicted $p \geq 0.6$). 3/ Victoria, GAB or an unknown location whose Sr and Ba post-core concentrations are within pre-edge concentrations with a predicted $p<0.6$.

	WINTER 2008		**SUMMER 2008/09**	
	Victoria	*GAB*	*Victoria*	*GAB*
Number of post-core concentrations to classify hatch origin	28	27	26	12
Number of post-core concentrations within pre-edge concentration range of concentrations from GAB and Victoria	15 (54%)	16 (59%)	24 (92%)	12 (100%)
	Predicted group		Predicted group	
Predicted confidence $p \geq 60\%$	*Victoria*	*GAB*	*Victoria*	*GAB*
Location of capture GAB	7 (64%)	4 (36%)	7 (100%)	0 (0%)
Location of capture Victoria	8 (89%)	1 (11%)	3 (33%)	6 (67%)
	Predicted group		Predicted group	
Predicted confidence $p<60\%$	*Victoria*	*GAB*	*Victoria*	*GAB*
Location of capture GAB	2 (40%)	3 (60%)	1 (20%)	4 (80%)
Location of capture Victoria	4 (67%)	2 (33%)	4 (27%)	11 (73%)

5. DISCUSSION

Analysing shape and element composition of statoliths from *N. gouldi* collected from the GAB and Victoria successfully clarified their stock structure. Using such techniques in unison has provided a mechanism to analyse *N. gouldi* stock structure and migration characteristics that may be applicable to other cephalopod fisheries. Fourier shape analysis indicated significant phenotypic heterogeneity among squid from the two locations, with statolith element composition indicating that squid caught from either Victoria or the GAB originally hatched in various regions of their distribution. Results suggest that although *N. gouldi* caught in GAB and Victoria have different phenotypic characteristics squid from both regions contribute to recruitment. Such results are plausible given that squid caught throughout southern Australia are considered genetically similar (Triantafillos *et al.* 2004). Although the mechanisms of mixing are unclear it is possible that egg and paralarval dispersion, and ontogenetic migrations, are facilitated via southern ocean current systems.

Although the intrinsic shape of otoliths is thought to be genetically defined (Lombarte & Lleonart 1993), many teleosts display spatial and temporal variation in the incremental growth patterns of otoliths (Neilson & Geen 1985) in response to environmental conditions, feeding rate, and water chemistry (Chen *et al.* 2008, Kingsford *et al.* 2008, Neat *et al.* 2008, Tonkin *et al.* 2008). These variables have the capacity to alter physiological allometric growth that affects otolith shape e.g. Atlantic cod *Gadus morhua* (Campana & Neilson 1985). Water temperature greatly influences growth rate and lifespan of squid (Arkhipkin *et al.* 2000b, Hatfield *et al.* 2001, Forsythe 2004) with both positive and negative allometric growth observed (Jackson & Domeier 2003b, Reiss *et al.* 2005). For *N. gouldi,* differences in growth based on hatch season have been observed with summer and autumn animals growing faster than those hatching in winter and spring; however, this variation may also have been a function of productivity, food availability, and temperature (Jackson *et al.* 2003). Water temperature in the GAB is on average 2.35°C ±0.062 se (Modis satellite data from 31/12/2006 – 01/01/2010) warmer than waters off Victoria, which may also be promoting a greater disparity in statolith area and somatic growth (DML). Somatic growth of *N. gouldi* is spatially and temporally variable (Jackson *et al.* 2005), and like teleosts, it is possible that such variation in growth rates affect the deposition of aragonite in statoliths therefore creating variation in statolith shape among individuals. Differences in statolith shapes of *N. gouldi* caught in Victoria and GAB suggest that there is restriction to migration between locations. However, for this difference to become established an individual may need to be at a given location for sufficient time. If this is the case, depletion of stocks from the GAB are unlikely to be replaced by adult squid migrating from Victoria and vice versa, and individuals are more likely to be replaced by *N. gouldi* from immediate areas.

Shape of *N. gouldi* statoliths significantly varied among sexes and squid size, suggesting that these variables need to be considered before using shape for discriminatory purposes. Both males and females displayed variation in statolith area for a given DML indicating a level of disassociation between statolith and somatic growth. Such evidence of a breakdown between somatic and statolith growth is observed for a range of squid species including *Sepioteuthis lessoniana*, *Loligo chinensis*, *Todarodes eblanae*, *T. angolensis*, *L. vulgaris*, and *L. forbesi* (Lipinski *et al.* 1993, Jackson 1995, Martins 1997, Thomas & Moltschaniwskyj 1999), as well as several teleosts (Mosegaard *et al.* 1988, Megalofonou 2006). Disassociation between somatic and statolith growth indicates a high level of variation possibly in response to environmental factors physiological processors, or ontogenetic changes (Lombarte & Lleonart 1993). Differences in statolith shape using Fourier analysis was observed between male and female *N. gouldi* at both locations of capture; however, for teleosts such as *Gadus morhua* and *Scomber scombrus,* sexual dimorphism in otolith shape was not evident (Castonguay *et al.* 1991, Cardinale *et al.* 2004). Compared with longer living teleosts, *N. gouldi* are fast growing ephemerals; consequently it is possible that shape differences are exaggerated over a short period of time. Like statoliths from *Sepioteuthis lessoniana* (Thomas & Moltschaniwskyj 1999) and otoliths from *Gadus morhua* (Cardinale *et al.* 2004), no difference between the shape of left and right statoliths were observed. This is not surprising as they are paired calcareous structures (Morris

1984) that experience identical conditions. Independent of sex and location of capture, statolith shape was significantly different when comparing small and large statoliths indicating that shape changes over time with increasing age and is possibly attributed to differences in statolith growth or ontogenetic migrations. Similar results have been observed when comparing statolith shape in *Gonatus fabrici*. Here, small squid caught in the epipelagic zone had statoliths that were drop-like in shape; whereas larger squid from the meso-bathypelagic zone displayed a consistent shape with changes in internal microstructure (Arkhipkin & Bjørke 2000). Greatest difference between small and large statoliths was from female squid caught in the GAB, which may indicate a greater disparity between the environmental conditions where juvenile *N. gouldi* were located compared to when they were adults.

The composition of *N. gouldi* statoliths within the post-core and pre-edge regions had Mg, Mn, Sr, and Ba present at concentrations suitable for analysing spatial and temporal variability. Information on elemental composition in statoliths found in Oegopsina and Myopsina cephalopods is limited; however, comparisons to *N. gouldi* statolith concentrations from a small number of studies demonstrate similarities. Magnesium in *N. gouldi* statoliths was within the range found in both *L.gahi*, as well as *Gonatus fabricii* statoliths where concentrations were 70 – 170 µmol mol^{-1} Ca and 110 – 590 µmol mol^{-1} Ca respectively (Arkhipkin *et al.* 2004a, Zumholz *et al.* 2007b). Changes in concentration of Mg are considered to be a function of growth rate, as well as ontogenetic stage (Kristensen 1980, Arkhipkin & Bjørke 2000, Arkhipkin *et al.* 2004a) with similar links also found in teleosts (Martin & Thorrold 2005, Hamer & Jenkins 2007). Concentration of Mg^{2+} in the statocyst endolymph is negatively correlated with Ca^{2+} at different times of the day with the higher concentrations associated with the deposition of organic components in the statolith (Morris 1991, Bettencourt & Guerra 2000). Differences in catch rates of *N. gouldi* in Victorian waters may be due to diel migration (Nowara & Walker 1998); however, this would not contribute to variability in Mg concentration on the pre-edge as they were averaged over approximately 30 days of growth. Since the distribution of Mg^{2+} in seawater is relatively uniform throughout oceans as well as being highly physiologically regulated (Zumholz *et al.* 2007b), it is unlikely that differences in Mg within the statolith of *N. gouldi* is a function of differences in elements found in seawater from Victoria and GAB.

Concentrations of Mn found on the pre-edge of *N. gouldi* statoliths were considerably less (range 0.032 – 0.55 µmol mol^{-1} Ca) than concentrations found in *Gonatus fabricii*, *Loligo gahi* and *Todarodes pacificus* (Ikeda *et al.* 1998, Arkhipkin *et al.* 2004a, Zumholz *et al.* 2007b). Using Mn may be subjective as concentrations were sometimes less than the limits of detection of the ICPMS; however, these concentrations can still be useful when analysing trends or spatial and temporal variation (Ben-Tzvi *et al.* 2007, Hamer *et al.* 2009). Like Mg, the distribution of Mn in the ocean is relatively uniform (Donat & Bruland 1995) and for similar reasons, concentrations are thought to be a response to uptake mechanisms rather than environmentally induced (Arkhipkin *et al.* 2004a).

Correlations between Sr and water temperature are used to elucidate ontogenetic migration characteristics in cephalopods. Consequently Sr analysis is suitable to answer stock identification questions where sufficient differences in temperature exist between spatially separated locations. Water temperature can influence Sr concentrations in teleosts; however, both positive and negative correlations with Sr concentration are reported (Elsdon & Gillanders 2003). Arkhipkin (2004a) found a negative correlation between Sr concentrations and water temperature, though positive correlations in cephalopods have been reported (Yatsu *et al.* 1998, Ikeda *et al.* 2002). For example, *Ommastrephes bartrami* distributed in temperate waters had greater concentrations compared with *D. gigas* (Ikeda *et al.* 1996) found in subtropical areas. Ontogenetic migrations may be responsible for variations in Sr concentrations in *N. gouldi*. In New Zealand, female *N. gouldi* migrate to shallow waters (<300 m) during maturation (Uozumi 1998). If similar inshore migrations exist for Australian *N. gouldi*, then it is possible that differences in water temperature among inshore and offshore locations effect Sr concentrations in statoliths. Temperature changes both vertically with depth, as well as spatially, are often related to coastal and oceanographic features such as bays, currents, upwelling as well as ocean floor topography (Elsdon & Gillanders 2003). As water temperature in the GAB decreases from approximately 18°C at 50 m to approximately 10°C at 500 m diel vertical migrations coupled with inshore migration characteristics may influence variation in Sr concentration (Nowara & Walker 1998, Hamer *et al.* 2009). For *N. gouldi* collected in the GAB there was a positive Sr correlation with season of capture (i.e. temperature), with concentrations lowest during winter and highest during summer; whereas in Victoria, such a pattern was less discernable. The influence of water temperature on Sr concentration depends on water salinity in teleosts (Fowler *et al.* 1995, Elsdon & Gillanders 2002); however, for *Sepia officinalis* concentration showed no relation to salinity or temperature (Zumholz *et al.* 2007a). Salinity differs both horizontally and vertically in oceanic systems and is influenced by precipitation, evaporation as well as freshwater input from coastal areas (Dávila *et al.* 2002). The influence of temperature and salinity on deposition rates of Sr is unknown for *N. gouldi*, but it is expected that they will have a similar response to other ommastrephids. Strontium concentrations in *N. gouldi* statoliths were within the range of concentrations found in *Todarodes pacificus*, *Loligo gahi* and *Gonatus fabricii* (Ikeda *et al.* 2003, Arkhipkin *et al.* 2004a, Zumholz *et al.* 2007b). Compared with *Sepia officinalis,* Sr concentrations in *N. gouldi* statoliths were greater (Zumholz *et al.* 2006); whereas gastropod statoliths exhibited smaller concentrations (Zacherl *et al.* 2003a).

Barium concentrations in statoliths are useful in understanding migration and natal origin; however, interpretation can be difficult as localised environmental conditions can be very dynamic compared to other regions. Barium concentrations in both otoliths and statoliths are reflective of oceanographic nutrient concentration and water temperature (Zacherl *et al.* 2003b, Fowler *et al.* 2005, Warner *et al.*

2005, Hamer *et al.* 2006, Zumholz *et al.* 2007a). In ocean systems, these parameters are normally related to upwelling or differences between surface and bottom waters where concentrations increase with depth (Boyle 1988, Lea *et al.* 1989). Although differences among post-core and pre-edge was not analysed in this project, Ba concentrations from *N. gouldi* caught in GAB did have a positive correlation with season of capture (i.e. temperature) though this was not as apparent in *N. gouldi* collected in Victoria. However, the contribution of seasonal upwelling that occurs off the Bonney coastline of Victoria and South Australia during February may complicate interpretation. Summer inshore winds driving coastal upwelling events are indicative of higher nutrients, productivity (Middleton & Platov 2003) as well as temperature. Local upwelling events off western Victoria could be responsible for temporal variability of Ba in ambient waters (Schahinger 1987, Lea *et al.* 1989) compared with GAB. Such variability may influence Ba concentrations in statoliths, therefore reducing its discriminatory power when comparing concentration among GAB and Victoria.

Differences in pre-edge element concentrations between *N. gouldi* caught in the GAB and Victoria were only significant during winter 2008 and summer 2008/09. During winter 2008, greater concentrations of Sr and Ba were found in Victoria compared with GAB indicating that environmental conditions like temperature and productivity associated with upwelling events are likely contributing to differences between regions. However, during summer 2008/09, Mg and Mn were most influential in discriminating between locations with greater concentrations in squid from Victoria. No spatial difference in elemental concentration for squid collected during spring 2008 and autumn 2009 suggests that the combination of environmental and physiological processors are similar in both regions, or alternatively, large scale migration between the two locations occur over a relatively small time during these seasons. Concentration of elements Mg, Mn and Sr displayed significant seasonal interaction between locations suggesting that differences among locations are dependent on the season *N. gouldi* were caught. Barium concentration in statoliths was significantly greater in Victoria (combined seasons) as well as during summer (combined locations). As the Bonney upwelling is a prominent annual oceanographic event occurring during summer and early autumn along the Victorian – South Australian coastline (Butler *et al.* 2002a), greater Ba concentrations in statoliths from *N. gouldi* caught in this region maybe correlated.

In New Zealand, *N. gouldi* are thought to spawn in the upper 100 m of the water column (Mattlin *et al.* 1985, Uozumi 1998) with egg masses identified between 10 – 30 m (O'Shea *et al.* 2004) drifting with ocean currents. Similar egg mass transportation may be occurring in southern Australia facilitated by the Flinders, Leeuwin, and eastern Australian currents. Although these current systems are influenced by topography, canyons, wind and El Niño cycles (Li & Clarke 2004), typical winter flow consists of coastal currents on the shelf running eastwards at approximately 20 cm sec^{-1}, with the Flinders current moving westerly at 10 – 15 cm sec^{-1} at 600 m depth (Middleton & Bye 2007). During summer however, the Flinders current is weaker at depth moving 5 – 10 cm sec^{-1} at 300 – 400m in a westerly direction with coastal currents also moving westerly (Middleton & Bye 2007). If *N. gouldi* in Australia share similar ontogenetic characteristics as New Zealand, then egg masses found on the shelf during winter will drift easterly from the GAB to Victoria; whereas in summer the opposite would occur with egg masses drifting westerly. Such a cyclic pattern may be responsible for genetic homogeneity that was found by Triantafillos (2004). Squid life-history theory suggests that where adults have better chances of survival than their offspring, females release their gametes in multiple, smaller batches through time (Calow 1979). During spawning, *N. gouldi* produce multiple batches of eggs (McGrath Steer & Jackson 2004). This suggestion is also supported via visually identified egg masses (O'Shea *et al.* 2004) containing significantly less eggs compared to counts of 2,176 – 82,395 found in *N. gouldi* oviducts (McGrath & Jackson 2002). As *N. gouldi* populations spawn multiple times throughout the year (Jackson *et al.* 2003) easterly and westerly dispersal of eggs masses are likely to promote genetic homogeneity.

Also, a common ontogenetic migration characteristic of oceanic squid is for juveniles to migrate to deeper water to grow prior to moving to shallow water to spawn. Juvenile Patagonian longfin squid *Loligo gahi* migrate from inshore waters (20 – 50m) to offshore waters off the continental shelf edge (200 – 350m) where they grow, and upon maturation, migrate back inshore to spawn (Arkhipkin *et al.* 2004b, Arkhipkin *et al.* 2004c). *Loligo vulgaris* and *Illex argentinus* also follow similar ontogenetic migratory patterns (Hatanaka 1988, Augustyn 1991). Although it is unknown whether *N. gouldi* follow a similar trend, it is hypothesized that juveniles hatching at different locations of southern Australia, migrate to deeper water and use the Flinders current to move rapidly along the coast. Once favorable conditions are found (e.g. environmental and food availability), they stay resident for a sufficient amount of time that allows for phenotypic variation. Tagging experiments have illustrated that *N. gouldi* are capable of moving at a maximum speed of 0.12 m s^{-1} moving from adjacent bays in Tasmania (Stark *et al.* 2005); however, large-scale migrations were not determined. Although shape analysis techniques have not been used to study phenotypic variability in *N. gouldi* collected in other regions, variation in growth rates have been observed in *N. gouldi* collected from New South Wales, Victoria, Tasmania, and South Australia (Jackson *et al.* 2003), so statolith shape variation may also occur.

For *N. gouldi* caught in the GAB and Victoria during summer 2008/09, it is unlikely that hatching occurred at regions other than Victoria or GAB; however, during winter 2008 approximately half of the squid captured were from locations other than Victoria or GAB. During summer 2008/09 and less pronounced in winter 2008, *N. gouldi* caught in the GAB are more likely to have hatched in Victoria or at a location with similar elemental concentrations and environmental conditions as the GAB. For squid caught in Victoria however, a different pattern was observed compared with *N. gouldi* caught in the GAB. Results suggest that *N. gouldi* caught in Victoria during winter originally hatch in Victoria; whereas squid caught in Victoria during summer hatch in the GAB. Given such phenotypic variation among Victoria and GAB, statolith shapes suggest that *N. gouldi* function as different stocks with spatial integrity. However, *N. gouldi* from both locations contribute to the recruitment and that genetic homogeneity of the species (Triantafillos *et al.* 2004) is a function of egg mass and juvenile drift as a result of seasonal longitudinal ocean currents rather than large scale migration between the two regions.

6. REFERENCES

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6. APPENDIX VI

Ecosystem model based approaches to squid stock assessment

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1. ABSTRACT

Ecosystem models are increasingly providing theoretical frameworks in which to assess the ecological impacts of fishing and other perturbations, allowing more strategic and sustainable fisheries management. In this study we utilise two recently developed ecosystem-based models to investigate the ecological impact of increased fishing pressure on arrow squid in the Great Australian Bight, using Ecopath with Ecosim and in south east Tasmania, using Atlantis. Both model types focus on the biomass flow and trophic interactions between 41 and 44 functional groups, respectively. Model results show that both ecosystems were fairly robust to high levels of fishing on arrow squid populations. We found that the current fishing effort on arrow squid would need to be increased substantially (i.e. by a factor of at least x500 in the GAB system) before noticeable changes occur to their populations. Increased fishing levels would however cause a direct positive effects on principal prey groups (mainly sardines in GAB and myctophids in SETas) and negative effects on predators (New Zealand fur seals in GAB and predatory fish in SETas). Cascading effects of arrow squid removal include those from a changing demographic structure and increased feeding competition on important lower-trophic groups such as zooplankton. The abundance and feeding behaviour of arrow squid, influences the population size and distribution of its predators and the energy flow patterns of the pelagic ecosystem.

2. INTRODUCTION

The global trend in world fisheries is that we are fishing both down (Pauly et al 1998), and through (Essington et al. 2006) the food web. As fisheries remove the larger piscivores, increasing fishing effort is being put on invertebrates and smaller planktivores. The continued search to find new exploitable resources in our oceans may lead to increased pressure on non-traditional target species or an increase in effort in fisheries that are currently only lightly harvested. It is unclear, however, what impacts, both direct and indirect, can be expected from changes of this type. It is therefore important to look at the roles that the lower- and mid-trophic groups, such as that of cephalopods, play in fisheries ecosystems, and to understand how increased fishing pressure on these groups will influence the systems of which they are part.

In contrast to many finfish fisheries worldwide, squid fisheries continue to increase substantially (both in catches and/or number of fleets) (Caddy and Rodhouse, 1998). This trend is reflected in the Southern Squid jig Fishery (SSJF), in Australia over the past 2 decades. The fishery, mainly targets arrow squid, *Nototodarus gouldi* began in 1986 with a single vessel and has now increased to 43 fishing vessels in any one season, with catches up to 435 tonnes a year (Lynch, 2004). Such an increase in squid landings may be due in part to ecosystem impacts of fishing, where squid biomass increases due to a decrease in competition and predation from declining stocks of predatory finfish (Caddy and Rodhouse, 1998; Myers and Worm, 2003a). It has been suggested that a consequence of an increase in squid stocks is that a further increase in landings may be both possible and sustainable (Caddy and Rodhouse, 1998; Xavier et al., 2007).

The central role occupied by squid as both predator and prey in marine ecosystems remains poorly understood, not least because of the general complexity of ecological dynamics and the many indirect interactions between different trophic groups (Gurney and Nisbet 1998). Additionally, it is well known that harvesting effects rarely act linearly on ecological systems and thereby introducing further uncertainties into stock assessments. A novel approach to squid stock assessment is thus proposed which will evaluate the role of squid on ecosystem dynamics using ecosystem modelling approaches. These techniques are particularly relevant for squid populations due to their short life span and the strong environmental-recruitment relationship (Agnew et al. 2002).

Ecosystem models have been developed as tools which can assist in ecosystem management and system level understanding. Whole-of-ecosystem models incorporate both higher and lower trophic levels as well as biological parameters, hydrodynamic features and fisheries components. They therefore allow more comprehensive investigations into the functioning of marine ecosystems under various environmental conditions or fisheries management regimes. They also provide insight into the linkages and processes that occur in both natural and perturbed marine systems beyond that which can be gained from studying a single species or impact.

The concept of ecologically sustainable development has grown globally in recent years, so that now the wider ecological impacts of fisheries on marine ecosystems must be incorporated into fisheries management (Sainsbury and Sumaila, 2003). While the impact of fishing on target species has seen much research historically, the corresponding impact on habitats or ecosystem function within these ecosystems is a more recent and less well understood area of study. Recent reviews of the ecological effects of fishing show that fishing can cause strong indirect ecological impacts (Goñi, 1998a; Hall, 1999b; Pauly et al., 2003a). Cascading effects through trophic webs are difficult to predict, and the unforeseen consequences that can arise from fishing include: the restructuring of trophic linkages; predation or competition release from the removal of target species; changes to the demographic structure of either target or non-target species (eg, sex, size or age ratio changes); and a loss of genetic diversity or change in genetic frequency in target and non-target species.

We use two ecosystem model frameworks to investigate the effects of increased fishing pressure on arrow squid, Nototodarus gouldi, the role they have as both prey and predator, and quantify the inter-reliance of squid stocks in different management areas. To achieve this, the Ecopath with Ecosim (EwE) model framework was used to examine squid-stocks in the Great Australian Bight (GAB) while the Atlantis framework was used to explore squid from waters off south east Tasmania (SETas). Both models provide predictive insight into the effects of fluctuations in fishing effort on the squid biomass in addition to understanding ecosystem resource interactions. Such work will pave the way to future implementation of ecosystem-based management for squid-related fisheries in both the GAB and SETas. Each of these models and their findings are discussed separately in the following document.

3. PART I. GREAT AUSTRALIAN BIGHT, GAB – ECOPATH/ECOSIM

3.1. Study Aims

In this study, Ecopath with Ecosim (EwE) model framework was used to understand the ecological consequences (changes in trophic linkages and biomass flow) of increased fishing pressure on arrow squid stocks located in the GAB. Specifically, we aimed to estimate fishing mortality rates that would cause ecosystem instability and estimate maximum sustainable yields for arrow squid. In addition, we investigated the ability of arrow squid stocks to recover from high-fishing effort followed by a period of reduced fishing. In the process we aimed to determine the importance and function of arrow squid in the pelagic ecosystems in the GAB.

3.2. Methods

3.2.1. Model approach - The Ecopath with Ecosim (EwE) framework

Ecopath is a mass-balanced ecosystem modelling framework that assumes steady stat in the system (Chrisensen & Pauly, 1997, Griffiths *et al*. 2010). It is used for evaluating the importance of predator/prey relationships and the changes in ecosystem structure resulting from environmental perturbations (natural or anthropogenic). While Ecopath can be considered a descriptive analytic tool, Ecosim can be used to predict changes in abundance of system components over time. Ecopath model parameterization is based on satisfying two 'master' equations for each model group: one for production and the other for consumption.

EwE has been widely used in a diverse range of aquatic ecosystems world-wide. For the Great Australian Bight a paired Ecopath with Ecosim model was recently developed to provide an ecosystem perspective of the South Australian sardine fishery (Goldsworthy *et al*. *in preparation*). In the present study, we have used the existing model, modified some components and run various scenarios of dynamic (time-series) changes to the arrow squid fishery. The 41- functional group GAB-Ecosim model was calibrated using time series from 1991 – 2008, allowing 18 years of catch and effort data to 'drive' the model. A detailed description of the model dynamics is outlined in Goldsworthy *et al*. and includes the models input parameters and data sources. Here we will endeavour to briefly describe the biophysical realm, trophic connections, time series and fisheries components enclosed in the model framework. We will also list the modifications and changes we have made to improve the arrow squid component of the model.

3.2.2. Biophysical realm

The model domain covers a region of about 154,084 km^2 in the GAB, extending from the head of the bight, South Australia to Portland, Victoria between longitude 132ºE and 139.7ºE (Figure 6-1). Although the model is not spatially defined, it does broadly characterize a range of habitats that includes continental shelf waters to 200m depth. Any relevant habitat dependency is defined for each biological group by the allocation of a model area fraction (0-1), which acts to restrict the spatial domain of groups that are only associated with particular habitat types. The model does not consider the Gulf St. Vincent or Spencer Gulf but does assume interaction between the coastal and oceanic ecosystems.

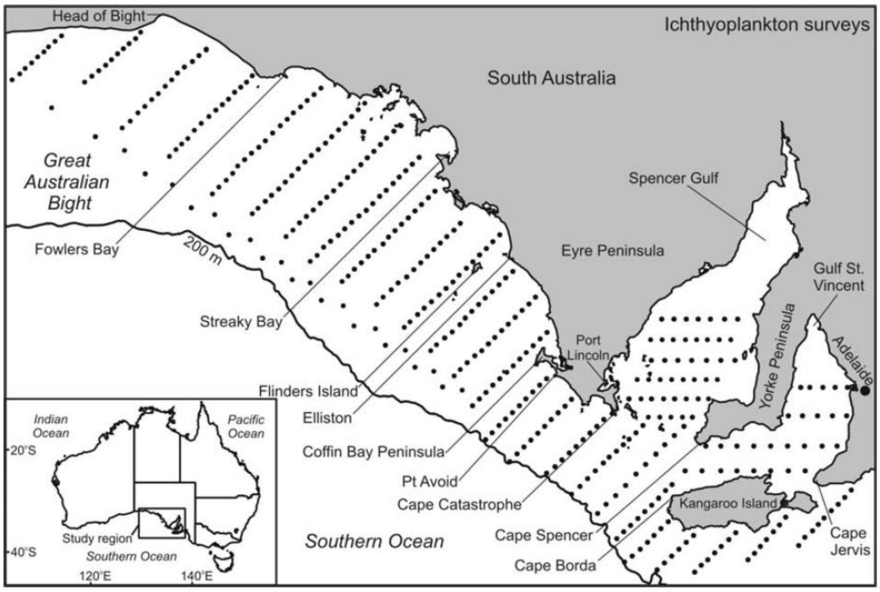

Figure 6-1 Map area of the model domain covering 154,084km^2. Dots in horizontal lines refer to stations and transects during the SARDI Aquatic sciences ichthyoplankton survey conducted between 1998 and 2007.

As it is well established that pelagic primary productivity in the region is highly influenced by seasonal coastal upwelling, particularly in eastern GAB (Ward *et al*. 2006), forcing functions of wind stress were included in the model by the addition of mean monthly upwelling anomalies. Sea surface temperature (SST) was also included as proxies for trends in primary productivity.

The species of the GAB ecosystem were assigned to 41 functional groups based on species similarity in terms of diet, habitat, foraging behaviour, size, consumption and rates of production. The trophic groups accounting for the highest proportion of system biomass include that for small zooplankton (34.5% of total biomass), detritivores (29.6%) and benthic grazers biomass (10.4%). Biomass datasets were available for most model groups from estimates for Spencer Gulf in Currie *et al.* (2009) and Currie and Sorikin (2010). Biological data for most trophic groups were largely collected from multiple stations and transects during the SARDI Aquatic sciences ichthyoplankton survey conducted between 1998 and 2007. Where specific data was not available, the Ecopath model was used to estimate parameters such as *biomass (B,* t km^{-2}*), production per unit of biomass (P/B), consumption per unit of biomass (Q/B) and Ecotrophic efficiency (EE).* Known and estimated values for these parameters are listed in Appendix A.

The model treated migratory groups as part of the system, but assumed that they obtained part of their food from outside the system. This was specified as the 'import' component of food in the diet matrix (Appendix B) which was estimated by assuming that the proportion of oceanic species in the diet was obtained outside of the model area. The GAB model was parameterised with a mixed trophic controlled (V=2, biomass controlled by neither predation nor resources) rather than a top-up or bottom-down.

Arrow squid were a defined as a separate functional group to 'southern calamari' (*Sepioteuthis australis*) and 'other squid' (mainly *Sepia* spp.). For arrow squid P/B and Q/B were 1.95 and 3.9, respectively (Bulman *et al*. 2006, Froese & Pauly 2008). Diet data for arrow squid was sourced from the literature (Bulman *et al*. 2006; Currie and Sorokin, 2010) and supplementary data was added from results in chapter ---- in this report. The updated GAB-Ecosim model estimated that the trophic level of arrow squid as 4.1 and that they accounted for 0.21% of the total systems biomass. The omnivory index of arrow squid was fairly high at 0.62 whereas the respiration per unit biomass (R/B) value was 1.17.

3.2.3. Fishing model

Fishery data on landings, discards and effort were obtained and broken down into 11 fisheries (fleets) operating within the GAB ecosystem listed in

Table 6-1. The eastern GAB region supports some of Australia's most valuable fisheries, including four main Commonwealth and five main South Australian (State) managed fisheries (Wilson *et al*., 2009b; Knight and Tsolos, 2010). The main Commonwealth fisheries that operate are the GAB Trawl (GABT), South East Trawl (SET), southern bluefin tuna (SBT) and shark gillnet fishery (Wilson *et al*., 2009b). Annual fisheries landings and effort data for all fleets between 1991 – 2008 were obtained from the Australian Fisheries Management Authority, CSIRO and SARDI Aquatic Science. Landed species were assigned to their functional group and biomasses summed at the functional group level (t km^{-2}). Time-series of annual catches and catches per unit effort (CPUE) were estimated for most functional groups. The gear effort for all fisheries drove the model.

3.2.4. Additional inputs and modifications to the GAB-Ecosim model.

As the aim of our study was to investigate the ecological implications surrounding the arrow squid fishery, we made the following changes to the existing GAB-Ecosim model:

- Added the Southern squid jig fishery (SSJF) as arrow squid are taken throughout the year as targeted catches. Although the SSJF is almost exclusively a fishery for arrow squid, it occasionally catches Southern calamari and other squid.
- updated the total landings (t/km^2) of arrow squid to include those caught by SSJF

added of CPUE data for arrow squid from the SET and SSJF fisheries. All additional or changed data were taken from AFMA log sheets, supplied on the 10/2010. This included increasing the known catch data for these fisheries into 2009. The estimated mortality rates of predators, natural causes and selected fisheries are reported in

- Table 6-1.
- adjusted the density-dependent predator-prey switching power of arrow squid from 0 to 0.2 to account for their capacity to opportunistically adjust their diet in response to changes in prey abundance and composition change in the area.
- undertook model projections with various estimates of *P/B* and *Q/B* for arrow squid to test their projection flexibility and stability.
- adjusted the model diet matrix, particularly that for arrow squid. Originally arrow squid were described as highly cannibalistic. However, based on stomach content data derived from stomach analysis a greater inclusion of ‘mesopelagic fish’ including fish from the myctophidae and Nansenea families were added. We also made changes to the proportions of arrow squid consumed by New Zealand fur seals and other dominant predators.
- added annual fishing mortality rates, F, for the arrow squid during the 19 year model period were calculated from reported catches (AFMA, 2010) and estimated biomass calculated by the re-parameterized GAB-Ecosim model. The annual estimated biomass and mortality constant (F) are reported in Table 6-2.
- removed biomass accumulation forcing functions for New Zealand and Australian fur seals when running model projections on changing fisheries mortality constants (F) over 50 years.
- respecified temporal changes in fishing fleet effort in the model run interface.

- changed flow control, biomass accumulation and some minor reassessment of dietary relationships iteratively to improve the fit of the model to the time series data and the expected trajectories of the predictions.

Table 6-1 Total mortality rates of arrow squid attributed to fishing, predators and other natural means

Mortality	SSJF	SET	GABT	TOTAL
fleet fishing	5.24E-04	5.20E-05	2.39 E-04	0.000815
other natural	-	-	-	0.195
predation	-	-	-	1.754185

Table 6-2 Fishing mortality rates, F, for arrow squid calculated from fishery catch statistics and applied to the first 19 years of the updated Ecosim simulations.

Year	Estimated biomass (t/km^2)	Arrow squid F-value
1991	0.2202	0.000225
1992	0.1592	0.000225
1993	0.1005	0.00072
1994	0.09673	0.000795
1995	0.09447	0.000599
1996	0.09572	0.00079
1997	0.09061	0.001574
1998	0.1155	0.001003
1999	0.08465	0.001956
2000	0.1066	0.001394
2001	0.1008	0.001702
2002	0.1180	0.000841
2003	0.1221	0.002198
2004	0.1304	0.000192
2005	0.1298	0.001949
2006	0.1391	0.001355
2007	0.1473	0.001613
2008	0.1461	0.000615
2009	0.1470	0.001114

3.2.5. Model scenarios

The aim of this study is to provide an ecosystem perspective of changes associated with projected increases of arrow squid fishing in the GAB. The balanced and updated GAB-Ecosim model was used to investigate scenarios of altered fishing regimes by forcing changes in annual fishing mortality rates (*F*) using the dynamic Ecosim routines and the resulting changes in biomass are assessed. The model was run from 1991 – 2008 using referenced fleet effort time series data, after which the annual fishing fleet effort for each fishery was set to a constant value (the average fishing rate for 2007 and 2008). Known SSJF effort data for arrow squid was used for 2008 and 2009. A mortality time-series for squid was derived for the remainder of the model projections (31 years) to drive the squid dynamics.

The following fishing scenarios were investigated for arrow squid:

1. all fishing of arrow squid ceased from 2009 (F=0);
2. continue fishing at the last known rate (F= F_{2009});
3. increase fishing mortality rates between 2010 and 2050 to:

a. B_{75} or F=0.6

b. B_{50} or F=1.5

c. B_{25} or F=2.2

Relative changes in biomass of all modeled groups through the projection period (of 50 years, 1991 to 2040) for the depletion category for arrow squid (B_0, B_{25}, B_{50}, B_{75}) were plotted as bar graph. Additional scenarios were run to explore the effects of a decadal increase of fishing SSJF effort (x 2000, x 5000 and x 10,000) and investigating the ability of arrow squid stocks to recover by a cessation of all fishing effort (>2020). During these scenarios, only SSJF fishing effort was increased as it seemed impractical to increased effort of the multispecies trawl fisheries (SET and GABT) which were left at 2009 levels.

Lastly, we estimated maximum fishing mortality relative to natural (predation) mortality (F_{max}/M) and biomass depletion relative to the Ecopath base biomass ($B_{unfished}/B_0$). Each fishing scenario was evaluated considering recruitment and predation mortality.

3.3. Results

Model projections for arrow squid suggest severe depletion at exploitations rates (F) > 2 and extirpation at F=10 (Figure 6-2). B_{75} occurs at F=0.6 and B_{25} occurs at F = 2.2 (Table 2). The maximum sustainably yield (F_{msy}) appears to be less than F = 1.8 when 0.14 t km^2 (or 69%) of arrow squid biomass were taken (Figure 6-3). Current fishing rates on arrow squid have little effects on their overall biomass, largely because the contribution from predation mortality rates (per year) were higher (1.75) than that of fishing (0.0008) (Table 6-3).

For most prey and predator groups there are a few (positive or negative) changes in biomass of greater than 20% resulting in the depletion of arrow squid (Figure 6-4). Negative impacts included that on NZ fur seals which are the biggest predator of arrow squid, consuming 18% of their biomass. Negative impacts were found for octopus, inshore small planktivores and blue mackerel (*Scomber australasicus*), all having a relative change in biomass of slightly greater than -20% when 50 % of the biomass of arrow squid was removed (B_{50}). In contrast, a 50 % biomass decrease in arrow squid had a positive impact (of greater than 20%) including dominant prey species of arrow squid (i.e. Jack mackerel, sardines and mesopelagic fish). Other trophic groups that showed positive changes included all the seabirds (i.e. Terns, gannets and petrels), common dolphin, other tunas-kingfishes and small demersal invertebrate feeders. Most of these changes were typically small (<10%) and resulted from an indirect response in the reduction in predation pressure from arrow squid (and NZ fur seals) on sardines, jack mackerel and small zooplankton.

To determine the ability and speed of stocks to recover to pre-increased harvesting levels the response of arrow squid to various scenarios of increased squid fishing effort (x 2000, x5000, x10,000) followed by annual relaxations of fishing (effort = 0) were analysed (Figure 6-5). Recovery of the stock in the medium- to long-term could be possible under an F=5 (or B_5) scenario. For these simulations forced biomass accumulation functions were left on NZ seals. In all projections, arrow squid (as parameterised in the GAB-Ecosim model) demonstrate the ability to recover completely within 2 years and in fact demonstrate a level of increase from the current rates. Within 30 years of non-continued effort on the SSJF fishery arrow squid stocks returned to normal. Stocks recovered in an unexpected ways, where the highest return yield occurred at the highest fished rate. This was largely due to the switching power parameter put on NZ fur seals, which depends on various biomass thresholds to be activated/deactivated.

Table 6-3 Comparative changes of the biomass of arrow squid caused by increasing mortality rate (F)

BIOMASS (t km^2)	REMAINING BIOMASS (%)	F (mortality rate, per year)
0.202	100	0
0.158	85	0.6
0.092	50	1.5
0.055	25	2.2
0.011	5	5
0	0	20

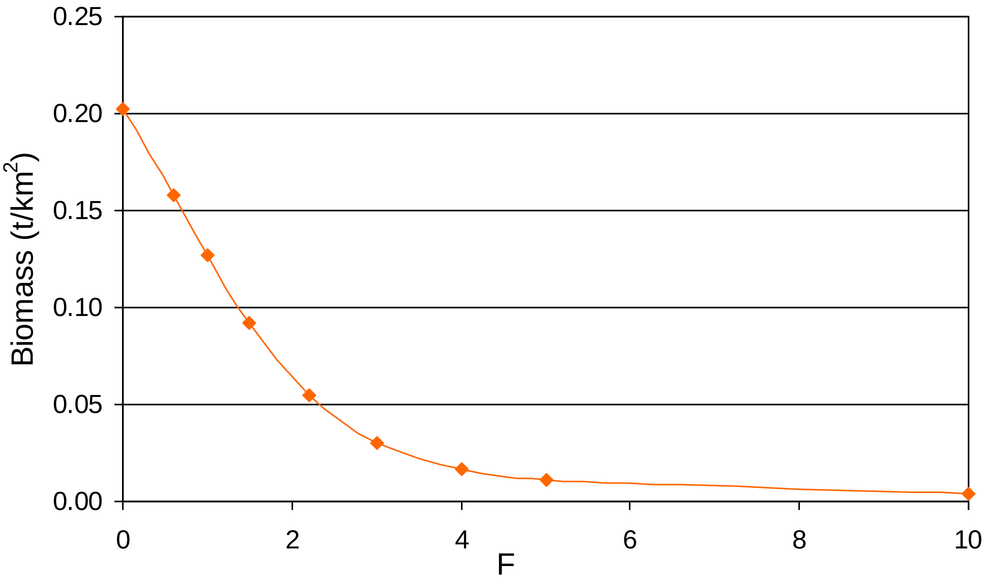

Figure 6-2 Biomass of arrow squid against increased fishing mortality (F)

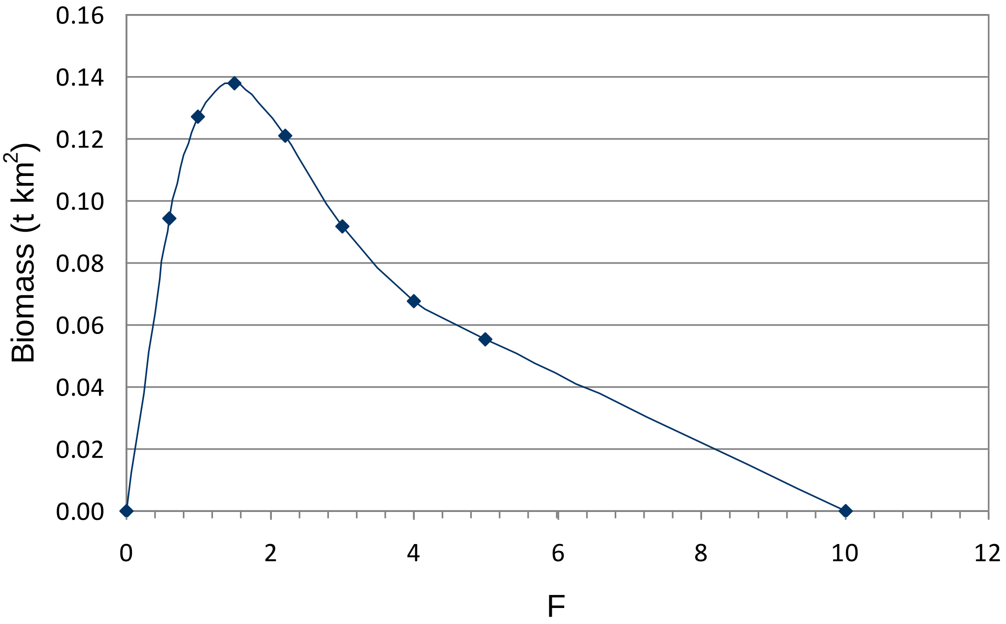

Figure 6-3 Yield of arrow squid against increasing fishing mortality rate (F)

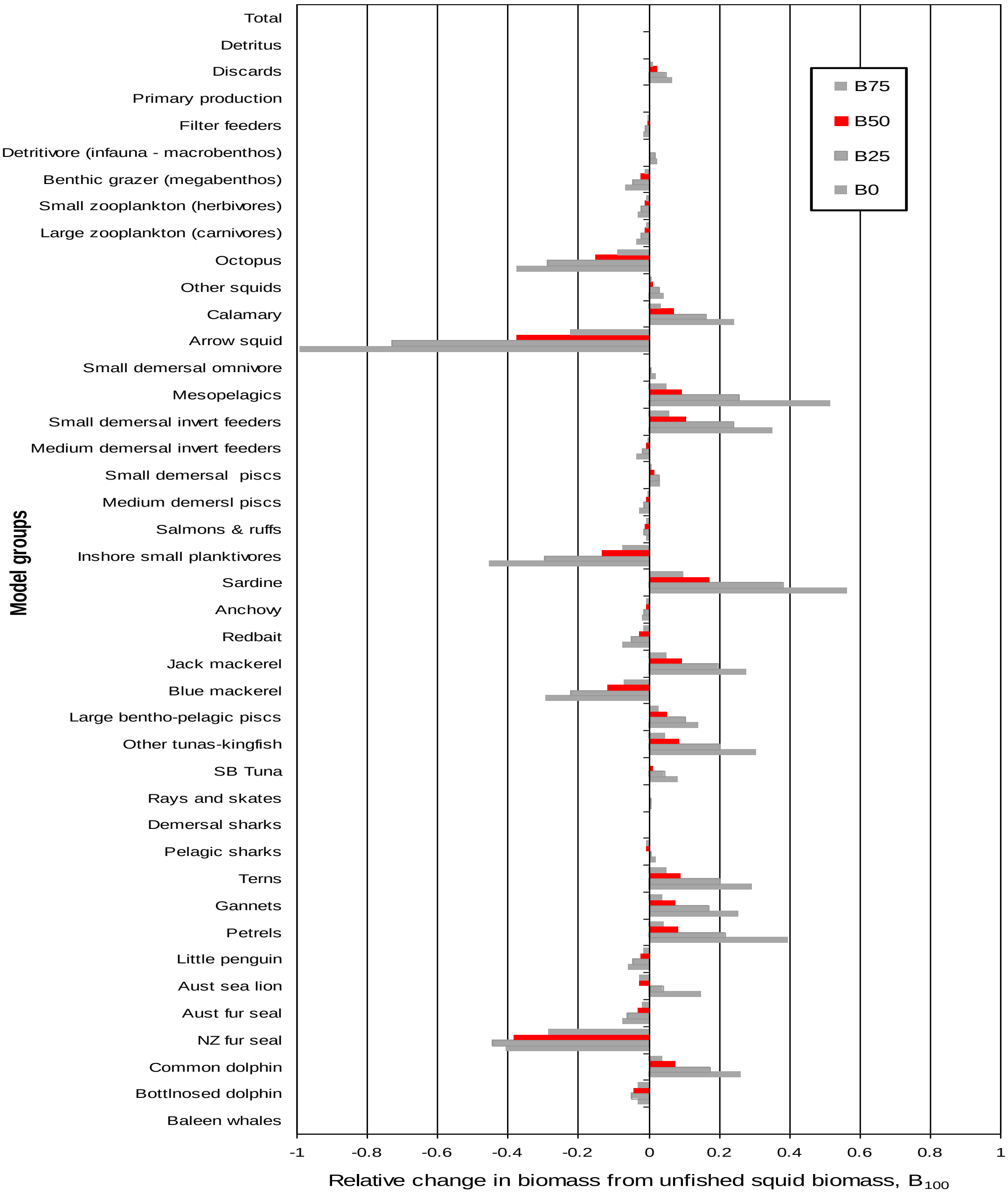

Figure 6-4 Relative changes in trophic groups with increased fishing mortality on arrow squid.

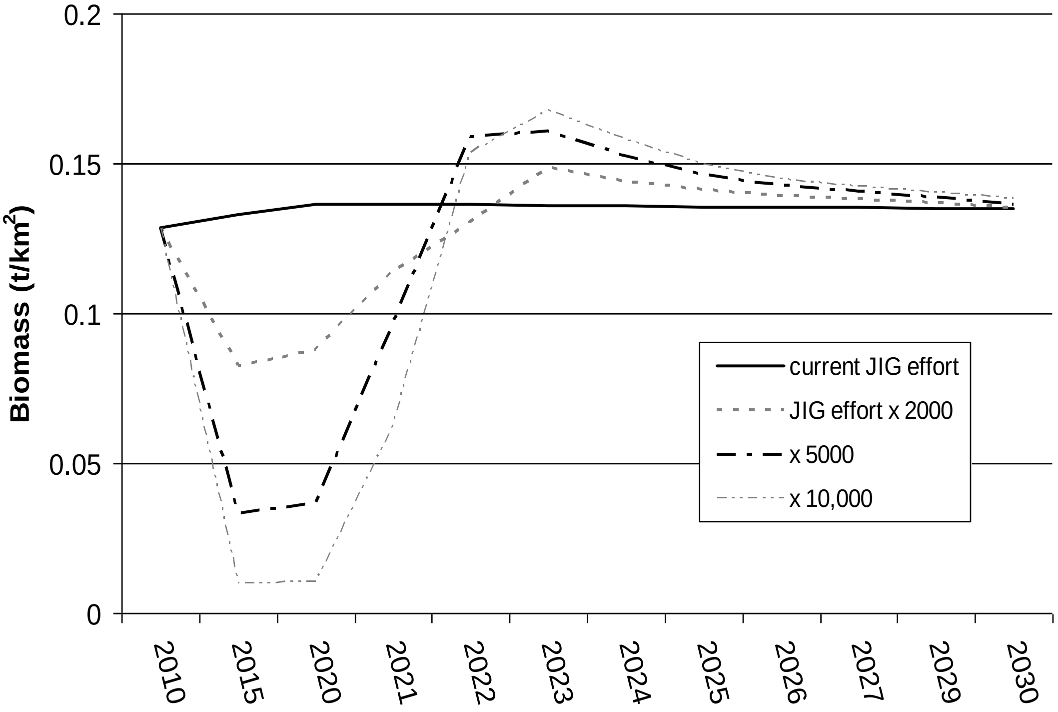

Figure 6-5 Yield of arrow squid against 10-year scenario increases of SSJF fishing effort and expected recovery periods (SSJF effort = 0, 2021-2032). The model used for these simulations parameterized a forcing function on NZ fur seal population between 1991 to 2008.

3.4. Discussion

With the current parameterization of the GAB-EwW model, a large increase in the current fishing mortality of arrow squid is required to cause considerable affects to the ecosystem dynamics. As expected, the most apparent effects were on dominant prey and predators of arrow squid. Prey species that increased in biomass included sardines, mesopelagic fish and jack mackerel. As these mid-trophic fish groups are also important in the diet of other predators, increases in the biomass of some high-order groups occurred. For example, increases in sardines accounted for an increase in seabirds (terns, gannets and petrels), large bentho-pelagic piscivores, tunas and kingfish and Sothern blue tuna. At current fishing rates, tuna (particularly blue fin tuna) numbers have been in a constant state of decline; however, when squid are removed from the system in high numbers tuna respond positively. This is largely due to the huge reduction in competition for epipelagic fish prey groups. Such positive effects on certain trophic groups are not surprising as squid have twice the energy requirements of fish (O'Dor & Webber 1986) consuming in excess of 30% (Jackson *et al*. 1998), and for juveniles as much as 72%, of their own body weight per day to support their high growth rates (Clarke 1996).

Negative impacts from arrow squid removal included those on its major predator (NZ fur seals) and those that decreased in biomass due to increased food competition by those trophic groups that increased. For example, octopus and blue mackerel decreased in biomass, largely due to their heavy reliance on small zooplankton (herbivores) (88% and 76% of their total diets, respectively) which are heavily consumed by mesopelagic fish (93% of their total diet), other squid (88%) jack mackerel (74%) and sardines (67%). Likewise, the decrease in inshore small planktivores, largely contributed to a decrease in benthic grazers of which accounted for 86% of their total diet. Benthic grazers are consumed by a range of trophic groups including those that increased in biomass due to arrow squid removal (sardines and jack mackerel). These are good examples of increased feeding competition on resources and the possible cascading affects that removal of important trophic groups may have on an ecosystem. It also highlights which trophic groups are likely to replace the trophic niche of arrow squid. This includes the abundant epipelagic fish groups which share similar habits (Dunning 1993) and trophic levels to arrow squid. Similar findings have been observed in the central Gulf of California where Humbolt squid (*Dosidicus gigas*) consume and compete for resources with various fish (Rosas-Luis *et al*. 2008).

As indicated by model projections, in the GAB ecosystem, New Zealand Fur seals have unique relationship with arrow squid, one of which strongly determines each others projected biomass. This is largely because in the model arrow squid account for 20% of the diet of NZ fur seals and 18% of the arrow squid biomass consumed by predators is taken by NZ fur seals. To reduce the reliance of arrow squid by NZ fur seals, we parameterized a level of dietary switching (switching power = 2) to allow for a more compensatory and flexible system. Consequently, when arrow squid numbers dropped below a certain biomass (due to increased fishing), NZ seals were shown to search for alternative foods, leading to a more rigid and realistic system. This is particular important to include as currently natural populations of NZ fur seals numbers are increasing in biomass within the GAB system (Ward *et al*. 2006), which will no doubt place a great deal of increased pressure on arrow squid. Indeed in the original GAB-Ecosim model of Goldsworthy *et al., (in preparation)*, a function that forced the biomass of NZ fur seals to increase by 0.005062 t/km^2/yr was included. This was not run in our model, as forced increase of NZ fur seal biomass drastically reduced the ability of arrow squid to recover to pre-1991 levels.

As expected, arrow squid have a rapid ability to recover from high fishing pressure and presumably also to other environmental perturbations. Squid have long been known for their high productivity contributing to their unique life-history parameters (fast growing, low age-at-maturity, reproductive flexibility, high fecundity, and high survival of young). Consequently, squid can sustain large increases of biomass despite their short life-span when mortalities are reduced (Jackson *et al*. 2002). These rapid fluctuations cause a large degree of instability in system dynamics as predators and other mid-trophic groups may be unable to adjust to the speed at which arrow squid biomass changes. This was demonstrated in the increase fishing and recovery scenarios (Figure 6-5) where higher fishing effort contributed to the recovery of larger biomasses due to the inability of predators (mainly NZ fur seals) to ‘switch’ from their preference for arrow squid to other prey items quickly. However, our model assumptions about recruitment influence our conclusions about recovery times and the effects of fishing on the system and that species likely respond differently to climate forcing at the inter-annual and inter-decadal time scales. This may be particularly true for arrow squid as although they produce a large number of eggs, their recruitment tends to be variable, regulated mainly by environmental factors (Jackson *et al*. 2006). Although we attempted to capture recruitment variability due to predation, the climate related variability is large and could show that the system may not respond in a way that our model suggests. Thus, future ecosystem simulations should explicitly consider the sensitivity of model results to different assumptions about climate effects on recruitment.

While model results in this study indicated that there is room for expanding commercial pressure on arrow squid, several other factors impede this realization. Over the last two decades, effort and total catch from the southern Australian arrow squid fishery has widely fluctuated (Jackson *et al*. 2002). The causes of this variability are unknown, but a range of hydrographic (upwelling events) and biological processes (migratory responses to prey availability, reproductive success and recruitment) are likely to be responsible (Jackson *et al*. 2002). In addition to variable catches, the market value for arrow squid remains low in the Australian market which has discouraged fishers from investing in the fishery. However, as we continue to increase catches of top-order fish and more recently mid-order fish, significant regime shifts of the ecosystem are may occur, many of which are highly likely to favour an increase in the biomass of arrow squid (and other cephalopods). Such events have already been documented in existing ecosystems (Rosas-Luis *et al*. 2008). An increase in a generalist and highly consumptive predator such as arrow squid may have dramatic effects on prey populations such as eliminate cyclical interactions, reducing then abundances (prey densities) and driving species to extinction as suggested by Smout *et al*. (2010). The occurrence of these effects depends on the form of the predator’s functional and numerical responses.

As with any model, the GAB-Ewe model is limited by how the key ecosystem components are parameterised. Squid have been acknowledged by many as being as difficult group to parameterise, mainly due to variable growth rates and lack of data on squid abundances. A major shortcoming for this study was that there are no known or estimated values for the biomass of arrow squid in the GAB. A depletion analysis study in GAB estimated arrow squid at 2,049,077 kg (Triantafillos 2008) which is comparable to that estimated by the model (0.2162 t/km^2). As the model estimates are sensitive to initial biomass estimates and parameters, the model may have over- or underestimated the response to increased fishing mortality rates (F). Other missing or erroneous parameters for arrow squid that might improve this model are mortality rates, foraging and dietary information, and seasonal recruitment data. Currently this model does not y capture the spatial aspects of foraging and fishing removals that may be important in explaining trophic interactions and arrow squid fisheries. Greater inclusion of environmental parameters in addition to consideration of fluctuating immigration or advection rates of prey groups should also be considered to improve the model. Given the uncertainty in the parameters, predictions cannot provide an absolute assessment of changes of arrow squid stocks to the GAB ecosystem nor forecast the exact future of the relevant fisheries (if increased pressure was realised). However, despite a level of error in the model, the general trends are probably a reasonable indication of the dynamic changes in the GAB system.

Overall, this study highlights the important role squid play in regulating energy flow patterns in the pelagic ecosystem. Assuming the model to be a reasonable representation of the GAB system, it could be inferred that greater effort be placed on the broader temporal management in the GAB ecosystem rather than managing the effort applied on targeting arrow squid. This is particularly true as arrow squid are tightly bound within the trophic foodweb and respond to changing environmental conditions (productivity, temperature, prey availability). In contrast to the management practices imposed on many fish stocks, managers of arrow squid fisheries must also consider the ecological effects of increasing squid biomass due to decreasing mortality pressure from fisheries, predators or natural means because those effects as ecological affects are likely to more dramatic.

4. PART II. SOUTH EASTERN TASMANIA - ATLANTIS

4.1. Study Aims

In this section, we address questions focused on increased fishing pressure on stocks of pelagic squid (predominantly arrow squid, *Nototodarus gouldi*) located off south eastern Tasmania. We employ the Atlantis ecosystem framework to investigate the impact of different fishing scenarios, our goal being to determine effects in terms of ecosystem impacts or changes in trophic structure resulting from increases in fishing pressure on squid stocks off south eastern Tasmania. In addition we examine the effects of removing myctophid fish as common prey of squid. Our overall goal is to provide strategic insights into the consequences and potential ecological impacts that are associated with increased fishing pressure on pelagic squid in the south east of Tasmania.

4.2. Methods

4.2.1. The Atlantis framework

Atlantis is a deterministic, biogeochemical ecosystem modelling framework (Fulton *et al.*, 2004). It tracks nutrient flows through the main biological and detritus groups within temperate marine ecosystems. The primary processes considered in Atlantis are consumption, production, migration, recruitment, waste production, habitat dependency, predation and (natural and fishing) mortality. The outputs of the model consist of deterministic time series for each biological and spatial component in the modelled ecosystem.

4.2.2. Biophysical realm

The model domain covers approximately 265, 000 km^2 of the waters off south eastern Tasmania (Figure 6-6), and includes a diverse range of habitats. Soft sediment habitats, including sand, mud and seagrass form the dominant component of the area's inshore environment. Rocky reefs and kelp forests also play important roles in the ecology of the region. Offshore the model incorporates both shelf and open ocean environments. Ecologically, the area is highly diverse and contains a high proportion of endemic species. While the overall primary productivity is not as high as in extremely productive ecosystems such as the Benguela system (which is approximately 3 times higher) (Carter, 1982; Harris *et al.*, 1987), it is nevertheless classified as a Class I, highly productive (>300 gC/m2-yr) ecosystem based on SeaWiFS global primary productivity estimates.

The model is spatially defined both vertically and horizontally, using a seven layer, 11 box geometry. We used a bioregion approach to resolve a spatial domain that we consider to be the minimum area necessary to represent the inshore / offshore regions inhabited by the groups of interest in this study (i.e. pelagic squid and myctophids). Within box spatial heterogeneity is also represented, with a fixed proportion of each habitat type (soft, reef, canyon and flat) allocated to each box. Any relevant habitat dependency is defined for each biological group, which acts to restrict the spatial domain of groups that are associated only with particular habitat types. By incorporating vertical stratification, we were able to incorporate the vertical migrations of biological components, and represent hydrodynamic and biological processes that vary with depth.

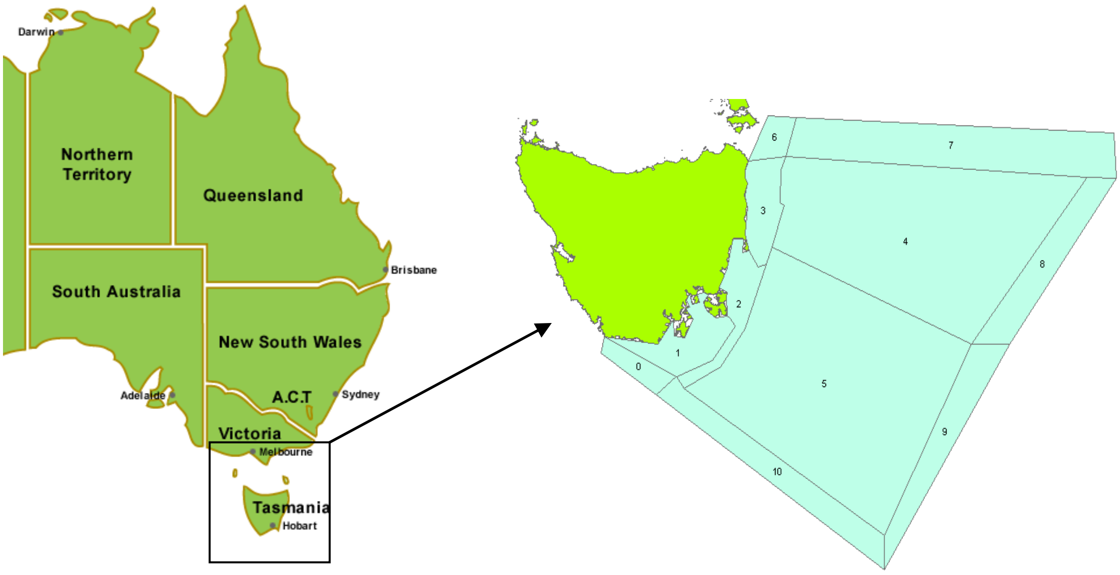

Figure 6-6 The spatial area of south eastern Tasmania that is covered by the model domain. Boxes 0 and 6 – 10 are boundary condition boxes, while 1 – 5 are dynamic boxes.

4.2.3. Biology

The biological groups included in the south eastern Tasmanian (SETas) model were made up of functional groups (aggregate groups of species with similar size, diet, predators, habitat preferences, migratory patterns and life history strategy) (Appendix C). The biological components provide a representation of the entire foodweb, inshore and offshore, pelagic and demersal and from bacteria and phytoplankton up to top predators. The majority of the invertebrate and all the primary producer groups are represented using biomass pools, while the squid and vertebrates are presented as age structured stocks. In addition to these living biological groups, pools of ammonia, nitrate, silica, carrion, labile and refractory detritus are also represented dynamically.

Parameters for initial abundance estimates were obtained from the published sources listed in Appendix C. Data for other biological parameters such as, seasonal distribution, reproduction, growth and habitat preference, were obtained from a variety of sources and re-parameterised from ecosystem models that encompassed the study domain (Fulton *et al.*, 2007a; Fulton *et al.*, 2007b).

All the fish groups in the model are represented as age structured groups, and defined in terms of the size of each individual in each age class. Squid are treated slightly differently. Because of the plasticity documented in arrow squid life history traits, especially individual growth rates, and age and weight at maturity (Jackson and Moltschaniwskyj, 2001a; Pecl, 2001a; Jackson and Moltschaniwskyj, 2002; McGrath Steer and Jackson, 2004), we have represented squid populations as two linked biomass pools (namely juveniles and adults), rather than using the more detailed age structured representation used for the vertebrate groups. This allowed us to track growth and reproduction at the population level (in terms of mg N m^{-3}), which is useful for stock management, without delving into the Pandora's box of individual variation in squid populations. Therefore data for individual growth and reproduction rates was not required in this model. The percentage of N per weight of squid was estimated at 10%, based on work by Villanueva *et al* (2004).

4.2.4. Trophic Connections

The potential trophic connections between groups are given in Appendix D. This matrix represents the potential accessibility of a prey item to a predator, rather than a definitive predation rate. Whether predation actually occurs depends on whether the predator and prey coincide temporally and spatially (given mobility, habitat preferences and habitat state), the total amount of forage available (summing across prey groups) and whether the prey is of an appropriate size to be caught and consumed by the predator. Some vertebrate predatory interactions with invertebrates are further divided by age class to represent the strength and rapidity of ontogenetic diet shifts that occur in these groups.

The diet matrix defines the links between the different functional groups. Thus, the value 0 means that there is no potential trophic relationship between one group and another, while any other value defines the availability of each food source to each consumer (i.e. the maximum potential proportion of the stock of a given food source that is accessible by a given consumer at any one time). Data for the trophic connections in the diet matrix are listed in Appendix C, along with the literature sourced. The final values used were the result of estimates from these sources modified through model calibration so that the resultant realised diet composition matched the available data, and the time series trajectory generated by the model matched trajectories of available time series of observations.

4.2.5. Fishing model

While Atlantis has the capacity to incorporate dynamic fishing fleets, this study is a strategic investigation of increases in fishing pressure, therefore we simply enforce a constant fishing mortality rate on each fished group. This has the effect of increasing the proportion of the population that is landed, and removes some of the noise associated with variations in fishers' behaviour that a dynamic fishing model can impose. A fishing mortality (F value) was estimated for each fished group by setting F to the proportion of the total population of each group that was taken as catch. Fishing pressure was imposed based on estimates of the current rates of fishing by both federal and state fleets (Anon, 2004; Smith and Waytes, 2004). The final values used were modified from the reported catch values in the calibration process, in order to allow a stable biomass (i.e. no evidence of numerical instability) that simultaneously resembled biomass trajectories that were observed in the system over the past 10 years.

4.2.6. Model calibration

Time series trajectories of both biomass and abundance of many groups was constructed from data provided by the Tasmania Aquaculture and Fisheries Institute (Barrett *et al*., 2007). These time series showed biomass trajectories for reef species over 10 years, from 1992 – 2002. We used these time series to calibrate the trajectories of the reef groups in our model. For the groups where no series data was available (e.g. the off-shore pelagic groups) we parameterised the model to obtain a stable system state with biological parameters that were within the values provided in the literature.

4.2.7. Scenarios

The major aims of this study were to investigate the impacts of an increase in the fishing pressure on squid stocks in the waters of south eastern Tasmania.

Table 6-4 outlines the specific fishing scenarios that were employed to investigate the above situations. The range of scenarios covered current fishing rates, high fishing rates and extreme fishing pressure that caused a collapse of the squid and myctophid stocks, so that they were effectively removed from the system. This range of scenarios was designed to explore the impact on the surrounding ecosystem, rather than the impact on the fished stocks themselves. In each of the scenarios all parameters were identical, other than the level of fishing pressure applied. All scenarios ran for the first 10 years without a change in fishing level to allow for model ‘burn-in’, then for a further 30 years with the perturbations imposed. Fishing on squid was across both adults and juveniles, fishing on myctophids was from age class 2 (from 2 years old).

Table 6-4 The scenarios that were examined in this study

No.	*Scenario name*	*Specifications*
1	base fishing rate	Current fishing effort: - annual squid catch rate is 0.01 of total biomass - no fishing pressure applied to myctophids
2	high squid pressure	Annual squid catch rate is increased to 0.5 of total biomass
3	squid stock collapse	Fishing pressure on squid increased to 0.9 of total biomass
4	high pressure on myctophids	Annual myctophid catch rate is 0.5 of total biomass
5	myctophids stock collapse	Annual myctophid catch rate is 0.6 of total biomass

4.3. Results

Under very high fishing pressure on squid off South-East Tasmania, the broad system structure did not change substantially. The functional groups that were influenced by the loss of squid are shown in Figure 6-7. Note, as Atlantis is a deterministic model any changes seen in the scenarios where fishing regimes were altered are a direct result of this alteration, not a result of stochastic events. Only groups that increased or decreased by at least 4% were reported in the results.

In regard to the impacts on the targeted groups, the squid population showed a considerable degree of density dependence, making them more robust to high levels of fishing pressure than were the myctophids. When fishing pressure on squid was increased to 50% of the biomass (high squid pressure scenario), the total biomass of squid (i.e. catch and unfished biomass combined) increased by approximately 30% from the base fishing rate scenario. Model results found that up to 85% of the squid biomass could be fished before the population became unviable.

As expected, high levels of fishing pressure imposed on squid reduced the respective biomass of these groups. The results of each scenario showed a proportional response in respect to the level of fishing pressure and the impact on other ecosystem components (i.e. any group that was heavily impacted from the collapse of squid was also impacted by a lesser reduction in numbers of these groups, simply by a reduced degree). As such, only the results from the scenarios where the squid stock completely collapsed will be presented here.

Small pelagic fish (FPS) showed a small increase in biomass of 4% as a result of the reduction in squid numbers. This increase was displayed as a higher abundance rather than any increase in size of individuals, and was caused by predation release from the squid after their collapse. Banded morwong (FVV) showed the greatest impact from the removal of squid stocks of 49%, despite being only a minor prey of them. No change in the size or condition of banded morwong was seen, the increase was due to an increase in abundance, particularly of the juveniles as a result of predation release from the squid. Pipefish, gobies, damselfish etc (FDP) suffered an indirect reduction in abundance of 4% as a result of the collapse of the squid stocks. This occurred due to the switching behaviour of some of the piscivorous fish; namely, FVS (medium sized pelagics such as barracouta, pike, etc), FDS (morwong, bream, whiting etc), SHD (benthic sharks) and SHP (pelagic sharks). The switch meant these predators consumed a larger proportion of these small fish due to the lack of squid prey available.

The non-migratory mesopelagics (FMN) also benefited from a reduction in squid numbers, increasing in abundance by 7% due to a release from predation. Demersal sharks (SHD) dropped in size by 4% in response to the reduction in squid as prey, but showed no change in abundance. This reduction in size is more pronounced in the juveniles than the adults (the weight of the juveniles drops by 6%, whereas the adult weight drops by only 2%), indicating that squid is a more important prey item for the juveniles of this group.

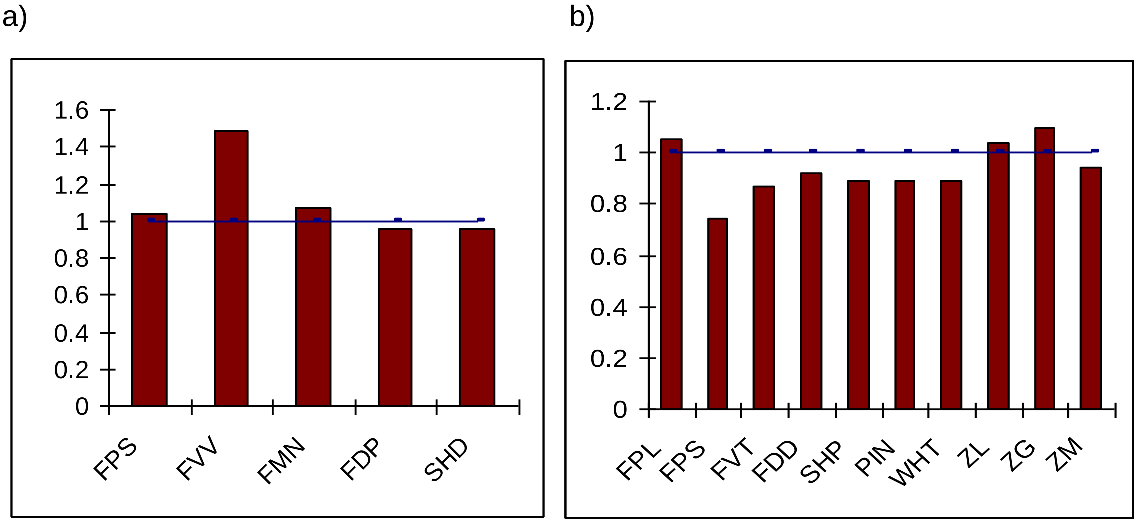

Figure 6-7 Proportion of initial biomass of impacted groups after 30 years in response to a) squid removal and b) myctophid removal.

4.4. Discussion

The SETas-Atlantis model found that a high removal of squid from the SETas ecosystem will have a minimal effect on the biomass of most trophic groups. However, some restructuring of trophic linkages is associated with the removal of squid from the system. The most obvious is an increase in predation on the small reef fish (damselfish, gobies etc) which occur when squid are removed and carnivorous fish focus on alternative prey sources. In general however, the SETas ecosystem appears to be robust to increased pressure on squid. Although squid have a high biomass and play a substantial role as both predator and prey in this marine ecosystem, their generalist nature in both of these roles means their removal does not cause a significant change to the ecosystem. No known predators consume squid to the exclusion of other prey items (Smale, 1996). Thus, a reduction in squid numbers simply leads to an alteration in proportion of prey items for any one predator, as other groups move in to fill the niche left by the squid. Similarly, as squid are themselves predators of many fish species, the reduction in their numbers actually benefits many fish; both directly from a release from predation, and indirectly, by increasing the prey available (i.e. by a reduction in competition).

We found that up to 85% of the squid biomass could be fished before the population became unviable. This pattern is likely to be due to high reproduction rates, short life spans and high levels of cannibalism found in the group. It seems that a limited increase in pressure on squid populations was beneficial to the stock, as cannibalism is reduced. This benefit, however, is not unlimited, and as fishing pressure is increased eventually the biomass drops as a result of the removal of spawning stock.

As a major prey of pelagic squid we tested what effects of its removal in the SETas foodweb. It became evident that removal of myctophids would cause a more substantial alteration to trophic linkages within this ecosystem than would the removal of pelagic squid. Our results are similar to those obtained by Shannon *et al* (2000) and Cury *et al* (2000), who showed that heavy exploitation of small pelagic fish in a highly productive system can lead to an increase in their prey and a decrease in their predators, causing a disruption in the trophic flow in the system. There is a danger in targeting the critical link between higher and lower trophic groups in any ecosystem. This link however can differ from system to system. While anchovies, sardines and herrings perform this role in system such as Southern Benguela, it appears that southeastern Tasmania is particularly dependant on myctophid fish as it is missing the magnitude of small pelagics of these other systems.

The removal of myctophids reduces predation on krill and gelatinous zooplankton, which has cascading effects throughout the trophic web. Larger planktivorous competitors such as the mackerel group benefit from the increased food supply. Smaller planktivores however, such as sardines and anchovies, suffer a much stronger pressure from predators such as pinnipeds, small chondrichthyans and large piscivorous fish. This reduction in sardine and anchovy abundance further reduces pressure on zooplankton, creating a positive feedback loop, where less and less of the system's productivity is being passed to the higher trophic levels. The pinnipeds and large piscivorous fish in turn suffer from the vastly reduced prey source with the removal of the myctophids, and reduction of other small planktivores. The impact of the reduction in biomass of the piscivorous fish and pinnipeds then extends through the foodweb to the highest trophic groups in the system. The reduction in orca and pelagic shark biomass is the result of a reduction in these prey groups. So, from zooplankton up to orcas, a clear reduction in the trophic flow in this system can be seen.

The results presented here support the view that the ecosystem as parameterised here fits under the wasp-waisted system structure, that is myctophids are one of the few links between plankton and higher trophic groups (Cury *et al*., 2000; Shannon *et al*., 2000). Shannon *et al* (2000), found that the ecosystem impacts of high fishing pressure on small planktivorous fish depend on whether the system is bottom-up controlled (i.e food resources limit population numbers) or top-down controlled (i.e. predation limits population numbers). This duality is seen because in bottom-up controlled systems small pelagic planktivores are limited by their food source making their populations density dependant. Increasing the mortality of these fish has the dual effects of reducing density dependence, whilst also allowing competing (similarly food limited) planktivorous species to increase at a more rapid rate and fill the newly available niche. It therefore does not lead to the loss of the important link between plankton and higher trophic groups seen in a top-down controlled system.

The levels of primary production from phytoplankton in this region suggest that the ecosystem is top down controlled. However, the high fluctuation of productivity on a seasonal basis (Harris *et al*., 1987) may lead to temporary, seasonally-driven bottom-up control of the system. In this model phytoplankton was parameterised so that it did not become limiting. However, other parameterisations of the model where phytoplankton biomass was significantly reduced (thereby creating a bottom up controlled system) produced different results, specifically a reduction in impact from the removal of myctophids. Future work could address the impacts of fishing in a system where phytoplankton biomass fluctuated, causing the system to switch seasonally between top-down and bottom-up control.

It must be remembered that this is a simplified representation of the south eastern Tasmanian ecosystem and the fisheries that occur there. Fishing pressure in this model study is uniform, both spatially and temporally, and doesn't take into account switching behaviour of fishers, or reduced fishing pressure when stocks become limited. Similarly, the ecosystem is assumed to be in a relatively stable state in the control run (i.e. no groups are in a state of serious decline or uncontrolled growth). This stable control state allows a more clear examination of the impacts to the system under the treatment scenarios, than would a system that is already seeing the collapse of some of the fisheries. It is assumed that, although this is may not be an exact representation of the current state in south east Tasmania, the stability would have been a feature of the system prior to heavy fishing. Therefore the results are representative of a state that has been perturbed by fishing and provide a strategic insight into the kinds of impacts that would be expected from a reduction in biomass of the key groups examined. Further work is required to incorporate a more realistic fishing effort in the model. There is evidence that tuna stocks are rapidly diminishing from this area (Galeano *et al*., 2005). A more detailed representation of the fisheries could clarify whether these results are maintained when large piscivores such as tuna are heavily fished. Nevertheless, although the quantity of impact may be highly uncertain, the direction of the changes is realistic and informative.

An increase in landings of squid stock is unlikely to have much an impact on the ecosystem as a whole, although the alternative parameterisations of the model would need to be explored to be certain of this result. While it is beyond the scope of this study to consider what a sustainable level of fishing on squid would be, a high level of pressure does not appear to cause a large shift in the trophic structure or ecosystem dynamics of this system.

5. PART III. COMBINED GAB AND SETAS MODEL SUMMARY

Model results show that both (GAB and SETas) ecosystems were fairly robust to high levels of fishing on arrow squid populations. We found that the current fishing effort on arrow squid would need to be increased substantially (i.e. by a factor of at least 500 in the GAB system) before noticeable changes occur to their populations. This is largely because in the models 99.9% of the current (2009) mortality for arrow squid is attributed to predation or natural mortality whereas fishing levels have remain low. Thus, at low to medium levels of fishing, the population dynamics of arrow squid remain more responsive to the biomass changes of their dominant predators. This was particularly evident in the GAB system where a high proportion of arrow squid are consumed by New Zealand fur seals. Although, due to the generalist nature of arrow squid and their high food intake, their removal is likely to benefit many mid-trophic groups, directly from a release from predation, and indirectly by increasing prey availability (by a reduction in competition). This in turn is likely to maintain biomass levels of predators that would otherwise be affected by arrow squid removal, particularly where the high-order predator has a high capacity to alternate or 'switch' between prey items quickly.

Model projections of high fishing pressure caused a direct positive effects on principal prey groups (mainly sardines in GAB and myctophids in SETas) and negative effects on predators (NZ fur seals in GAB and predatory fish in SETas). Cascading effects of arrow squid removal include that from a changing demographic structure and increased feeding competition on important lower-trophic groups such as zooplankton. Due to the abundance and feeding behaviour of arrow squid, it was not surprising that they influence the population size and distribution of its predators and the energy flow patterns of the pelagic ecosystem. However, using the SETas model we also demonstrate that high levels of fishing on dominant prey groups (myctophids) of arrow squid are likely to demonstrate an even greater negative impact on ecosystem structure (than the removal of just arrow squid). As the GAB ecosystem is largely supported by mid-trophic fish such as sardines and anchovies as opposed to myctophid fish, we suspect that similar results would be shown if these epipelagic fish were removed in large numbers from the GAB.

We found that arrow squid biomass is likely to respond rapidly from increased fishing pressure, because of their rapid generation time and semelparous life-history. This was demonstrated by the GAB-Ecosim model, where after simulating a decade of increased SSJF fishing effort, arrow squid declined (even as much as 10% of their initial biomass) but once fishing ceased (to 0 effort) stocks completely recovered in 2 to 3 years. Due to the speed in which recovery occurs, the model projected that there may be a time-lag in the capacity of predators to switch between other prey sources and arrow squid. As the model included the broader ecological affects, it was shown that similarly to most cephalopod species, arrow squid biomass can respond to changes in the biomass of available predators and prey. As recruitment rates determine model projections for arrow squid recovery, long-term sustainability of squid stocks depend on the ability of management to respond to fluctuations in recruitment which may be driven by uncertain factors ranging from environmental conditions to anthropogenic fishing pressures.

Overall, results from both models demonstrate that there is much scope to sustainably increase targeted fishing effort for arrow squid. Indeed, unless current fishing effort is multiplied by a factor of 500, an increase in arrow squid landings is unlikely to have much an impact on the ecosystem dynamics of these systems as a whole. Currently Australia imports large quantities of squid as market interest along with market value is low for Australian fisherman. However, given the viability of such ventures in addition to potential positive affects on other more valuable fisheries (ie. Tuna and oily epipelagic fish such as sardines) due to reductions in predation and competition, it seems increasing fishing effort on arrow squid would actually have greater benefits to marine systems than leaving populations to increase. Although, if such ventures were undertaken, managers must closely monitor abrupt changes in the demographics of key predators and prey groups to successfully maintain ecological sustainability. This is essential to prevent large and often difficult to predict regime changes to the broader ecosystems off the GAB and SETas.

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APPENDIX A

Inputs and outputs from the GAB-Ecosim model in 1991. Taken from Goldsworthy et al. FRDC report. Those values in bold text represent values estimated from the model.

	Group Name	Trophic level	Biomass (t/km^{-2})	P/B (year^{-1})	Q/B (year^{-1})	EE
1	Baleen whales	3.01	0.0389	0.020	5.0970	0.000
2	Bottlnosed dolphin	4.61	0.00611	0.080	16.566	0.000
3	Common dolphin	4.66	0.0039	0.090	20.511	0.000
4	NZ fur seal	4.67	0.00453	1.183	47.525	0.944
5	Aust fur seal	4.52	0.00047	1.156	28.819	0.982
6	Aust sea lion	4.90	0.00422	0.792	29.444	0.006
7	Little penguin	4.71	0.0007	1.290	85.600	0.413
8	Petrels	4.09	0.0031	1.000	147.100	0.070
9	Gannets	5.00	0.0000308	1.000	138.300	0.000
10	Terns	4.51	0.00000635	1.000	89.900	0.000
11	Pelagic sharks	4.90	0.0459	0.200	1.200	0.900
12	Demersal sharks	3.92	0.3072	0.180	1.800	0.325
13	Rays and skates	3.68	0.4594	0.350	2.700	0.014
14	SB Tuna	4.49	0.1452	0.200	1.600	0.900
15	Other tunas-kingfish	4.48	0.0769	0.200	1.200	0.900
16	Large bentho-pelagic piscs	4.67	0.4518	0.337	3.315	0.830
17	Blue mackerel	3.23	0.2190	0.370	3.500	0.847
18	Jack mackerel	3.22	0.7951	0.470	3.300	0.900
19	Redbait	3.38	0.5021	0.740	2.800	0.900
20	Anchovy	3.63	1.2720	0.700	5.040	0.510
21	Sardine	3.36	1.5175	1.600	5.040	0.302
22	Inshore small planktivores	3.93	0.447	1.010	7.300	0.900
23	Salmons & ruffs	4.51	0.226	0.440	5.400	0.900
24	Medium demersal piscs	3.47	0.302	0.485	5.400	0.810
25	Small demersal piscs	2.66	1.467	0.853	5.366	0.392
26	Medium demersal invert feeders	4.00	0.078	0.860	5.400	0.959
27	Small demersal invert feeders	3.53	0.1467	1.090	5.500	0.900
28	Mesopelagics	3.07	0.3274	1.005	6.673	0.900
29	Small demersal omnivore	3.77	0.1700	0.840	16.000	0.904
30	Arrow squid	4.06	0.2162	1.950	3.900	0.900
31	Calamary	4.50	0.0818	1.950	3.900	0.900
32	Other squids	3.14	0.1112	2.500	5.850	0.900
33	Octopus	4.16	0.2940	2.500	5.850	0.900
34	Large zooplankton (carnivores)	2.20	1.257	20.000	70.000	0.800
35	Small zooplankton (herbivores)	2.00	34.537	5.000	32.000	0.800
36	Benthic grazer (megabenthos)	3.24	10.736	1.600	6.000	0.800
37	Detritivore (infauna - macrobenthos)	2.52	30.205	1.600	6.000	0.800
38	Filter feeders	2.80	1.554	1.600	6.000	0.800
39	Primary production	1.00	14.900	745.000	0.000	0.106
40	Detritus	1.00	10.0000			0.009

APPENDIX B

Fleet Landings (catch t/km^{-2}) – GAB-Ecosim model

Group name	SASardine Fishery	SAMS-Line Fisheries	SAMS-Net Fisheries	SBT Purse Seine	SBT Pole & Bait	SET (Trawl)	GABT (Trawl)	SSJF (Jig)	Demersal shark	SG Prawn Fishery	GSV Prawn Fishery	WC Prawn Fishery	Total
Pelagic sharks	0	9.13×10^{-5}	0.00811	0	0	0	0	0	1.54×10^{-5}	0	0	0	0.00822
Demersal sharks	0	0.00166	0.00821	0	0	2.16×10^{-5}	2.26×10^{-5}	0	0.00628	0	0	0	0.0162
Rays and skates	0	0.000229	6.00×10^{-5}	0	0	0	2.23×10^{-6}	0	0	0	0	0	0.000292
SB Tuna	0	0	0	0.0214	9.23×10^{-6}	0	0	0	0.000242	0	0	0	0.0216
Other tunas-kingfish	0	0	0	0.0135	9.17×10^{-5}	1.38×10^{-5}	1.95E-07	0	0.000251	0	0	0	0.0138
Large bentho-pelagic piscs	0	0.00137	0.000447	0	0	0.00127	0.000336	0	1.00×10^{-7}	0	0	0	0.00342
Blue mackerel	0	0	0	0	0	0	1.95E-07	0	0	0	0	0	1.95×10^{-7}
Jack mackerel	0	0	0	0	1.03×10^{-6}	0	6.49E-08	0	0	0	0	0	1.09×10^{-6}
Sardine	5.72×10^{-5}	0	0	0	0	0	0	0	0	0	0	0	5.72×10^{-5}
Salmons & ruffs	0	9.05×10^{-5}	0.00469	0	0	0	0	0	5.00×10^{-7}	0	0	0	0.00478
Medium demersal piscs	0	0.00265	0.00173	0	0	0.000595	0.00105	0	0.000183	0	0	0	0.00621
Small demersal piscs	0	0	0	0	0	8.47×10^{-6}	0	0	0	0	0	0	8.47×10^{-6}
Medium demersal invert feeders	0	0	0	0	0	8.47×10^{-6}	0	0	3.30×10^{-6}	0	0	0	1.18×10^{-5}
Small demersal invert feeders	0	0	0	0	0	0	0	0	6.00×10^{-7}	0	0	0	6.00×10^{-7}
Arrow squid	0	0	0	0	0	5.18×10^{-5}	1.09×10^{-5}	1.14×10^{-4}	0	0	0	0	1.76×10^{-4}
Calamary	0	0.000966	0.000749	0	0	0	3.25E-08	1.20×10^{-6}	0	0.000151	1.26×10^{-7}	1.48×10^{-10}	0.00187
Other squids	0	0	0	0	0	0	3.25E-08	1.0×10^{-8}	0	0	0	0	3.25×10^{-8}
Octopus	0	0	0	0	0	0	0	0	1.00×10^{-7}	0	0	0	1.00×10^{-7}
Benthic grazer (megabenthos)	0	0	0	0	0	0	1.45×10^{-6}	0	3.00×10^{-7}	0.00215	1.80×10^{-6}	2.11×10^{-9}	0.00216
Sum	5.72×10^{-5}	0.00706	0.0240	0.0348	0.000102	0.00197	0.00142	1.14×10^{-4}	0.00698	0.00230	1.93×10^{-6}	2.26×10^{-9}	0.0787

APPENDIX C

Functional groups in SETas-Atlantis, their initial biomass values and source of data

Group Code	Group composition	initial biomass (t)	source
PL	Diatoms	3040732	((Harris et al. 1987, Bax & Williams 2001))
PS	Picophytoplankton	16567450	(Harris et al. 1987, Dandonneau et al. 2004)
ZG	Salps, coelentrates	3746517	(Bulman et al. 2002b)
ZL	Krill (mainly, also chaetognaths etc)	904516	(Young et al. 1996)
ZM	Copepods	655168	(Young et al. 1996)
ZS	Small zooplankton	3748595	(Fulton *et al.* 2007)
BC	Carvivorous infauna	1246740	(Bax & Williams 2000)
BD	Benthic deposit feeders	103895	(Bax & Williams 2000)
BFD	Deep benthic filter feeders	183317	(Bax & Williams 2000)
BFF	Shallow filter feeders	12236	(Edgar & Barrett 1999, Bax & Williams 2000)
BFS	Urchins	1224	(Edgar & Barrett 1999, Bax & Williams 2000)
BG	Grazing gastropods (abalone etc)	2447	(Edgar & Barrett 1999, Bax & Williams 2000)
BMD	Crustacea	329971	(Edgar & Barrett 1999)
BMS	Octopus, seastars, carnivorous gastropods, etc…	19578	(Edgar & Barrett 1999, Barrett et al. 2007)
BO	Meiobenthos	2077899	(Fulton *et al.* 2007)
MA	Macroalgae	244724	(Barrett et al. 2001)
SG	Seagrass	2447	(Barrett et al. 2001)
CEP	Pelagic squid (mainly arrow squid)	74930	(O'Sullivan & Cullen 1983a, Gales *et al.* 1993, Lynch 2004)
FDE	Mullets, luderick, garfish…	71102	(Edgar & Barrett 1999)
FVV	Banded morwong	283	(Edgar & Barrett 1999)
FDP	Syngnathidae, Gobiidae, damselfishes	2298	(Edgar & Barrett 1999)
FDS	flounder, gurnard, wrasse, snapper, whitings, latchet, flatheads…	48125	(Edgar & Barrett 1999)
FVB	southern hula fish, leatherjacket,…	3237	(Edgar & Barrett 1999)
FDD	Dories, whiptails, hapuku, cardinalfish…	27506	(Bulman et al. 2002b)
FBP	Purple wrasse	949	(Edgar & Barrett 1999)
FDC	Blue throat wrasse	557	(Edgar & Barrett 1999)
FDF	Blue eye trevalla, warehou	601	(Fulton *et al.* 2007)
FPS	pilchard, sardine, anchovy	2186	(Bulman et al. 2002b)
FPL	Mackerels	22098	(Bulman et al. 2002b)
FVS	bonito, yellowtail kingfish, teraglin, barracouta, mulloway, aust salmon…	42573	(Bulman et al. 2002b)
FMM	Myctophidae	149047	(Bulman et al. 2002b)
FMN	Sternoptychids (hatchetfish), cyclothene (lightfish)	125328	(Bulman et al. 2002b)
FVD	Flying fish, sauries, redbait	80070	(Bulman et al. 2002b)
FVT	Tunas and billfish	5898	(Young et al. 1996, Bulman et al. 2002b)
SHB	dogfish	65313	(Fulton *et al.* 2007)
SHD	Benthic sharks	51756	(Fulton *et al.* 2007)
SHP	Large Pelagic sharks	10264	(Bulman et al. 2002b)
SSK	Skates and rays	1500	(Fulton *et al.* 2007)
WHB	Baleen whales	1366	(Fulton *et al.* 2007)
WHS	Dolphins	497	(Fulton *et al.* 2007)
WHT	Orcas	756	(Fulton *et al.* 2007)
PIN	Australian fur seal	536	(Fulton *et al.* 2007)

APPENDIX D

Trophic links in the SETas Atlantis model. A red box indicates a potential trophic link between predator (rows) and prey (columns).

7. APPENDIX VII

Intellectual Property

All information arising from this research will be publically available both via this final report and through the scientist literature.

8. APPENDIX VIII

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