FRDC FINAL REPORT

ARROW SQUID IN SOUTHERN AUSTRALIAN WATERS – SUPPLYING MANAGEMENT NEEDS THROUGH BIOLOGICAL INVESTIGATIONS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NON-TECHNICAL SUMMARY

1999/112 Arrow squid in southern Australian waters – supplying management needs through biological investigations

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OBJECTIVES

1. Undertake extensive statolith age studies to determine validated age, growth rates and life spans throughout the fishing region both spatially and temporally.
2. Assess rates and timing of maturity, and the effect that the maturation process has on muscle growth and body condition of arrow squid.
3. Identify squid stocks using genetic tools to determine if there is a single or multiple stocks and whether the Australian stock is separated from the New Zealand stock.

OUTCOMES ACHIEVED TO DATE

- Assessed the genetic structure of arrow squid across southern Australia which showed all squid are a single species with limited population structuring.
- Determined that *N. gouldi* is a multiple spawner and that fuel from reproduction comes from food rather than at the expense of body condition
- Described spatial and temporal patterns in growth, maturity and reproduction over 2 years and found pronounced variability in spatial and temporal size and age.
- Determined the reproductive strategies and gonad investment over time and space and found marked differences between female and male strategies.
- Described the temporal patterns in growth and the number of cohorts present on fishing grounds at Portland over the course of a year.
- Documented how investment in gonad and body tissues change over time and differs between male and females.
- Identified the cost of reproduction at a cellular level by muscle fibre dynamics and found that maturation results in minimal change to the muscle fibre structure in *N. gouldi*.

This report presents the findings of a FRDC funded project to identify important biological features of the Australian arrow squid *Nototodarus gouldi*. The research involved both field and laboratory based research from 1999 to 2003. A major part of the research was undertaken as a PhD project by Belinda McGrath-Steer. This project has been successful with a number of published/submitted papers and a PhD thesis that has been completed, reviewed and accepted with minor changes. Although much of the work was carried out by Dr. George Jackson and Belinda McGrath-Steer, other collaborators on the project that were co-authors on published papers include Dr. Gretta Pecl, Dr. Simon Wotherspoon, Dr. Lianos Triantafillos, Dr. Alistair Hobday, and Mr. Mark Adams.

We used standard allozyme electrophoresis to determine the genetic structure of the arrow squid population across southern Australia from northern New South Wales to Western Australia. This analysis revealed that *N. gouldi* represents a single species in Australian waters. While this study indicated that there was considerable genetic mixing across all sites investigated, there was some suggestion of population sub-structuring on the Australian east coast. This indicates that there may be more than one stock in Australian waters. This has significant implications for management of arrow squid. Currently it is managed as a single stock, which may not be the case. Further more detailed research is needed to identify how many separate stocks may exist in Australian waters. We were not able to obtain specimens from New Zealand to see how genetically similar the Australian and New Zealand stocks of *N. gouldi* are.

Very little was known regarding the mode of reproduction of *N. gouldi*. Prior to larger scale work it was necessary to establish how this species reproduces (ie., whether it spawns once and then dies or whether eggs are released in batches over time). Squid were chosen from Tasmanian waters, as this area was the most accessible for the collection of large numbers of fresh individuals needed for the reproductive study. It was found that egg production and gonad growth did not take place at the expense of somatic growth, ie., when the ovary increased in size during maturation this did not result in a loss of body condition. Furthermore, examination of ovary oocyte size distributions along with examining relationships between ovary and oviduct weights, all pointed to *N. gouldi* producing eggs in multiple batches. Evidence for multiple spawning was also indicated by the presence of stretched empty oviducts showing that some females had already spawned.

A major focus of this study was a broad-scale sampling study across four locations covering four states (Ulladulla NSW, Port Lincoln SA, Lakes Entrance Vic and Tasmania), over two seasons, and repeated over two years. This revealed complex variation in size, age and maturation between these different sites over time. Furthermore, the growth pattern over the two years also differed, indicating that the squid were displaying considerable flexibility in their growth over time. Life spans were completed in less than a year indicating that each season the fishery is exploiting a new generation of squid. Squid hatched in summer/autumn grew consistently faster than squids hatched in winter/spring. Furthermore squid in the 1999/2000 season also grew faster than did squid in 2000/2001. There were some marked differences across sites. The Ulladulla squid were generally smaller and younger, with smaller gonads (and may be a small morph of the species), while Tasmania and Lakes Entrance generally had larger and older individuals with larger gonads. Port Lincoln squid were variable and intermediate. Interestingly, during spring 2001, both Tasmania and Port Lincoln had individuals that were much smaller than those of the other seasons at these sites and were more like squid from Ulladulla. Trends in maturity were also marked, with ages of mature individuals ranging over 100 days from youngest to oldest. Sea surface colour (SSC) was able to help to explain variability in growth of winter-hatched female squid but not for males.

Our broad-scale sampling study over time and space was also used to more closely examine the variability in the reproductive strategies of *N. gouldi*. Since we now know that we are dealing with only a single species, any changes observed represent how this species responds to different conditions encountered over the course of the work. Reproductive strategies were investigated by examining changes in relative investment in both ovary and somatic tissues over both a smaller temporal-scale (bi-annual) and a larger spatial-scale (geographical). The only variation in female strategies found on a broad-scale was between high and low latitude sites, with female squid caught from lower latitude sites showing higher levels of gonad investment in comparison to their higher

latitude counterparts. Males on the other hand showed both broad-scale spatial and temporal changes as well as small-scale temporal changes in reproductive traits, with spring caught males having greater levels of investment than in autumn; in addition males from low latitude had higher gonad investment than those from higher latitudes. Patterns of repro-somatic investment had implications for spawning strategies as females with higher gonad investment apparently released eggs simultaneously, whereas females with low gonad investment possibly spawned eggs independently of one another.

To obtain better temporal resolution on growth and reproduction in *N. gouldi*, we obtained nine monthly samples over a period of a year from fishing grounds off Portland, Victoria. The seasonal patterns in growth were somewhat paradoxical and there were marked differences between sexes. Only the spring-hatched males had slower growth rates than the other three seasons that were all statistically similar while the spring and summer-hatched females grew significantly faster than the autumn and winter-hatched females. The monthly age data allowed us to explore cohort structure off Portland. Our information indicated the presence of at least four cohorts present over the course of the year that indicated the fishery was targeting a dynamic, changing population of squid.

In terms of gonad investment, females caught during the cool season had lower gonad investment and higher somatic investment than warmer-caught females. While males also had less gonad investment during cool periods, the investment in the soma followed a similar pattern to the gonad that contrasted to the observations for females.

Muscle fibre dynamics of *N. gouldi* were investigated relative to reproductive development to quantify the cellular cost of reproduction. There were changes in the proportion of large and small muscle fibres, a decline in the proportional zones of mitochondria-rich fibres throughout the mantle, and a decrease in the width of muscle blocks at the anterior end. This suggested that there was a decline in available energy for muscle growth with maturation. As these cellular changes could not be identified at the whole animal level, the cost of reproduction to mantle tissue is likely to be small. The significance of this means that generally, there is not going to be marked differences in mantle consistency or integrity between immature and mature/spawning females.

Acknowledgements

We are grateful to a number of people who helped make this project possible. We are particularly appreciative to the many people who have helped with the collection and compilation of material. From the University of Tasmania (Utas) we are especially indebted to Ralph Mitchell, Mike Steer, Sean Tracey, Jacqueline Parker, Tertia MacArther and Julian Finn for squid collection and dissection and Chester Sands for providing genetics assistance. We are indebted to Jeremy Lyle for providing access to the facilities at the TAFI Marine Research Laboratories, and to Katrina Phillips (Utas) who created superb maps.

For the collection of specimens from right around the south and east coast of Australia we are extremely grateful to Ian Knuckey and the many fisheries observers of the Marine and Freshwater Research Institute of Victoria and Protec Marine, Port Lincoln. In particular, thanks must go to arrow squid fisher Lisle Elleway, who provided an enormous amount of anecdotal information, encouragement and ship space to this project.

Many colleagues assisted with scientific expertise and assistance with scientific expression, analysis and writing, these include Natalie Moltschaniwskyj, Gretta Pecl, Jayson Semmens, Katrina Phillips and Julian Finn.

BACKGROUND

Arrow squid (*Nototodarus gouldi*) is one of the largest under-exploited fisheries in southern Australian waters. On a global scale squid stocks are being increasingly targeted as finfish are over-fished (O'Dor et al. 1997). Next to the South American/Falkland Islands squid fisheries, the Australian arrow squid is possibly the next greatest squid resource in southern temperate waters. Currently there is a growing interest in squid fishing in southern Australian waters. The recent steps by AFMA to commence discussions on squid management issues underpin the increasing importance of squid as a fishery target. There is now the unique opportunity with this fishery to obtain basic biological information needed by managers before the stocks come under increasing pressure. There is a considerable lack in understanding of many of the most important aspects of southern arrow squid biology. We don't know how long they live, how variable are their life spans, when they reproduce and what are the important reproductive features of the stock? Furthermore, is there a single stock or multiple stocks, and is the Australian stock genetically separated from the New Zealand *N. gouldi* population? In reference to oceanic squids (including arrow squid), Dunning (1988) noted that a more thorough understanding of squid life histories and population dynamics is an essential prerequisite to responsible management of the existing and potential commercial fisheries for these species.

Use of statolith ageing techniques has revolutionised our understanding of squid age, growth and population dynamics. We now know that life spans are measured in days not years (eg. Jackson & Choat 1992, Jackson 1994). Managers therefore face the unique problem of dealing with a completely new population each fishing season. Moreover, squid are known to show extreme plasticity in growth depending on the season of hatching (eg. Jackson et al. 1997). It is now time to obtain specific information on arrow squid in Australia. Statolith techniques in association reproductive and genetic studies will provide a substantial amount of data over the short-term. This data will also be paramount for the future management of the fishery over the long-term.

NEED

The majority of Australian fishery stocks are at or near full exploitation and several have been over-exploited (FRDC 1996). Arrow squid are an exception to this trend. There are potentially very large stocks in southern waters but exploitation is currently low. However, there is an increasing interest in exploiting Australian arrow squid stocks and pressure on these stocks will increase as finfish stocks continue to decline. Australian fishery managers are in a unique position to obtain basic biological data on these stocks *before* they are subject to considerable pressure. Squid stocks are notorious for having huge natural fluctuations, which can have a great impact on commercial operations. By understanding the basic biology of the organism (age, life spans, rates of maturation and population genetics) it will help to identify and perhaps separate the influences of fishing pressures as opposed to natural population fluctuations. This application presented the unique opportunity to collect essential information to provide management with crucial baseline data before any major exploitation of the resource.

This project **fits squarely within FRDC's strategic priority** of Program 1: **Resources Sustainability**. This work will therefore provide needed data for priority areas of *knowledge of wild fish resources for sustainable management, general biology and genetics* and *stock definition* (FRDC 1996).

OBJECTIVES

This study was structured to collect basic biological data on southern arrow squid to provide managers with baseline data for developing future squid fishery policies. The project incorporated three approaches:

(1) Extensive statolith age studies to determine validated age, growth rates, life spans throughout the fishing region both spatially and temporally.
(2) Assessment of rates and timing of maturity, and the effect that the maturation process has on muscle growth and body condition.
(3) Identification of squid stocks using genetic tools to determine if there is a single or multiple stocks and whether the Australian stock is separated from the New Zealand stock.

Our objectives were predominantly met except for two parts in objective 1 and 3 above. The validation experiment was inconclusive due to poor increment resolution in the post-stained region. We were also not able to secure any specimens from New Zealand for comparative genetics work.

GENERAL INTRODUCTION AND OVERVIEW

The management of squid fisheries continues to pose unique problems due primarily to the rapid growth, short life spans and rapid turnover of squid populations. Thus, squid fisheries essentially exploit a new generation each year. Many aspects of squid biology are unique and are very different to other marine organisms, including finfish. Thus, new and unique approaches to fishery management policy are required to deal appropriately with squid fisheries. As the arrow squid fishery continues to expand and deal with 'boom' and 'bust' years, there is the continual need to develop fishery policy suited to the particular biology of the species under exploitation. This is because not all squid species display the same basic life-history characteristics. However, at the commencement of this study, very little was understood about the basic biology of the exploited species *Nototodarus gouldi*. This project was therefore aimed at providing answers to a number of pressing questions regarding genetics, age, growth, maturation parameters, and changes in tissue integrity with maturity.

Since there were a number of discrete questions we were answering, we grouped our research effort into discrete studies, rather than producing one large single investigation, incorporating all the work undertaken. Therefore, we have structured this report as a series of complete studies, each with all the relevant information to eliminate the need to refer to other areas of the report for clarification. Each chapter is presented as a complete scientific study and is in the same format as a published scientific paper. Many of the chapters are already published or under review as scientific papers.

CHAPTER ONE

AN ALLOZYME INVESTIGATION OF SPECIES BOUNDARIES AND STOCK STRUCTURE IN AUSTRALIAN POPULATIONS OF THE ARROW SQUID *NOTOTODARUS GOULDI* (CEPHALOPODA: OMMASTREPHIDAE)

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Adapted from: ICES Journal of Marine Science (2004) in press

INTRODUCTION

The arrow squid, *Nototodarus gouldi* (McCoy 1888), is an oceanic and neritic squid, endemic to southern Australia and northern New Zealand. For most of its distribution, *N. gouldi* inhabits the waters <500 m on the continental shelf and slope, and is most common at depths from 50-200 m (Uozumi 1998). In Australia, this species has been shown to occur between latitudes 27°13'S and 43°40'S, in seawater temperatures ranging from 11°C to over 25°C (Dunning 1998). Throughout these waters, and those off the northern and central New Zealand coast, *N. gouldi* is the dominant ommastrephid squid and is subject to commercial and recreational harvesting (Dunning & Forch 1998). Arrow squid are also a key component of offshore ecosystems as they are eaten in large numbers by numerous predatory species (Coleman 1984; Gales *et al.* 1994).

Little is known of the early life history of this species. Reproduction is presumed to be typical of ommastrephids, where a large pelagic egg 'balloon' is produced and released in mid-water, possibly in the region of a pycnocline (Sakurai *et al.* 2000). Hatchlings have been collected in late spring to summer over a broad area of the southern Australian continental shelf, from 28°S in southern Queensland to 34°S in the western Great Australian Bight, as well as off central New South Wales from mid-summer to midwinter (Dunning & Forch 1998).

Prior to this study, the only information concerning the stock structure of this species in Australian waters was a preliminary allozyme study by Richardson (1983), which found no evidence of genetic differentiation in south-east Australia. However, more extensive genetic and morphological work in

New Zealand waters has since revealed the presence of two species of *Nototodarus*, with a second species, *N. sloanii*, occurring around the South Island and south to the Auckland Islands Shelf (Smith *et al.* 1987). With increasing fishing pressure on *N. gouldi*, there is a growing need to understand the stock structure of this species in Australian waters. It is presumed that arrow squid should display high levels of gene flow throughout its range, given its mode of spawning via pelagic egg 'balloons', the pelagic nature of the juvenile stages, and the mobile habit of adults. However, given its extensive distribution in Australia, a consequential association with several different current systems, and the presence of two species in New Zealand within a much smaller geographic region, there is clearly a need to comprehensively examine the genetic structure of arrow squid throughout their entire Australian range.

This project is part of a larger study of *N. gouldi* in Australian waters, aimed at describing the important biological characteristics of this species. Preliminary data to date indicate that this species has a life cycle spanning ~ 1 yr or less, with highly variable growth rates that are heavily influenced by environmental parameters (Jackson et a. submitted). A reproductive study of this species in Tasmanian waters has indicated that *N. gouldi* is a multiple spawner, with energy for reproduction being acquired from food rather than at the cost of somatic condition (McGrath & Jackson 2002). Given the general unpredictability of marine environments and a short life span, it is not surprising to find that recruitment in this species is highly variable on a spatial and temporal scale. This variability is reflected in catch and fishing effort, which also fluctuates widely between years and regions (Nowara and Walker 1998). Since the fishery targets a new generation of squid every year, there is concern that the combination of a year of high fishing effort coinciding with low recruitment could lead to over-fishing. For this reason there is the need to collect and monitor important biological parameters of the population of *N. gouldi* in Australian waters.

Molecular genetic studies have demonstrated that cryptic species are a common occurrence in squid (Augustyn and Grant 1988; Brierley *et al.* 1993; Izuka *et al.* 1996; Yeatman and Benzie 1994; Triantafillos and Adams 2001). Consequently, a molecular systematic assessment of species boundaries deserves to be one of the first steps in any serious study of squid biology. Such a study should first identify whether the various populations are conspecific prior to undertaking any assessment of population structure. Of the numerous molecular techniques available, allozyme electrophoresis remains one of the most appropriate for an initial investigation of both species boundaries (Avise 1994; Hillis *et al.* 1996; Richardson *et al.* 1986) and broad population structure (Ihssen *et al.* 1981; Ryman and Utter 1987). Allozyme data have already proved useful for assessing intraspecific differentiation in a number of economically-important cephalopod species (*e.g.* Katugin 1995; Triantafillos and Adams 2001; Kassahn *et al.* 2003). The present study uses allozyme

electrophoresis to clarify the taxonomic status of Australian *N. gouldi* and to provide insight into its population genetic structure throughout southern Australia.

MATERIALS AND METHODS

Sample collection

Samples of *Nototodarus gouldi* were collected using a variety of techniques from five sites along the coast of southern Australia between January 2000 and December 2002 (Fig. 1.1; Table 1.1). Distances between sites ranged from 700 to 4300 km. Tasmania was represented by a spatial replicate sample set, with the replicate (Storm Bay) collected on the same day, from a location less than 15 kilometres from the initial site (Wedge Island).

Animals from Bunbury, Wedge Island and Storm Bay were frozen whole at the site of capture after being stored on ice for up to 48 hours. Tissues obtained from these animals displayed consistently lower levels of enzyme activity and did not stain for a couple of the less-important polymorphic markers. Animals from Kangaroo Island, Ulladulla and Iluka were collected fresh and a small piece of tentacle tissue (~1g) immediately removed from each individual and placed in liquid nitrogen. Tissues samples were returned to the laboratory and stored at –80°C, pending genetic analysis.

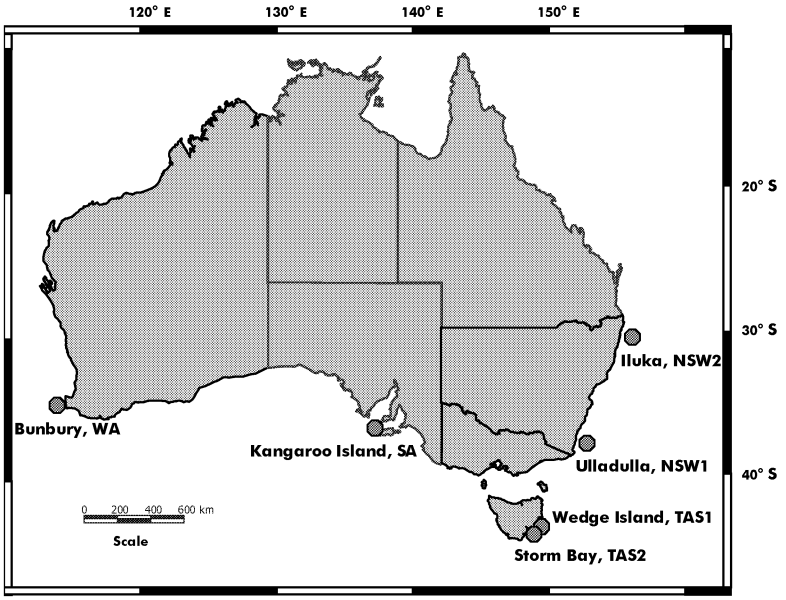

Figure 1.1: Map of Australia showing the collection sites of the *Nototodarus gouldi* examined in this study.

Allozyme electrophoresis

The allozyme study was carried out in two stages, according to the rationale outlined by Triantafillos and Adams (2001). Initially, a large number of allozyme loci were screened in an overview study of 30 individuals, comprising seven animals each from Bunbury, Kangaroo Island, Ulladulla and Iluka, and two animals from Wedge Island. Having determined that enough polymorphic loci were present to permit an assessment of population structure, a second stage of allozyme analysis was undertaken. Here, a large number of animals from all sample sites (Table 1.1) were genotyped at the polymorphic loci plus at a selection of monomorphic loci which could be reliably typed as "double-stains" without increasing the number of gels run (for technical details, see Richardson *et al.* 1986). Based on the overview study, the optimum sample size was set at 50 individuals per sample set, although this number was not always available.

Table 1.1 Details of sample sites used in the electrophoretic study. *n* is sample size.

Sample set	Code	Date of capture	Method of capture	*n*
Bunbury, Western Australia	WA	1 Oct. 2002	Demersal fish trawling	18
Kangaroo Island, South Australia	SA	30 Jan. 2000	Dab netting	51
Wedge Island, Tasmania	TAS1	13 Jan. 2000	Jigging	32
Storm Bay, Tasmania	TAS2	13 Jan. 2000	Auto jigging	7
Ulladulla, New South Wales	NSW1	4 Feb. 2000	Fish trawling	50
Iluka, New South Wales	NSW2	2-4 June 2002	Prawn trawling	45
			TOTAL	203

Allozyme electrophoresis was conducted according to the principles and methodology of Richardson *et al.* (1986). Tissues were homogenized by sonication in two volumes of homogenizing solution (deionised water containing 0.2% 2-mercaptoethanol and 0.2mg mL^{-1} NADP). The following 37 enzymes displayed zymograms of sufficient activity and resolution to permit allozymic interpretations in the overview study:- aconitase hydratase (ACON, EC 4.2.1.3), acid phospatase (ACP, EC 3.1.3.2), aminoacylase (ACYC, EC 3.5.1.14), adenosine deaminase (ADA, EC 3.5.4.4), alcohol dehydrogenase (ADH, EC 1.1.1.1), adenylate kinase (AK, EC 2.7.4.3), fructose-bisphosphate aldolase (ALD, EC 4.1.2.13), aldehyde dehydrogenase (ALDH, EC 1.2.1.5), alkaline phosphatase (AP, EC 3.1.3.1), arginine kinase (ARGK, EC 2.7.3.3), carbonate dehydratase (CA, EC 4.2.1.1), enolase (ENOL, EC 4.2.1.11), esterase (EST, EC 3.1.1), fructose-bisphosphatase (FDP, EC 3.1.3.11), fumarate hydratase (FUM, EC 4.2.1.2), glyceraldehyde-3-phosphate dehydrogenase (GAPD, EC 1.2.1.12), lactoylglutathione lyase (GLO, EC 4.4.1.5), aspartate aminotransferase (GOT, EC 2.6.1.1), glycerol-3-phosphate dehydrogenase (GPD, EC 1.1.1.8), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), isocitrate dehydrogenase (IDH, EC 1.1.1.42), cytosol aminopeptidase (LAP, EC 3.4.11.1), malate dehydrogenase

(MDH, EC 1.1.1.37), "malic" enzyme (ME, EC 1.1.1.40), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), nucleoside-diphosphate kinase (NDPK, EC 2.7.4.6), purine-nucleoside phosphorylase (NP, EC 2.4.2.1), dipeptidase (PEP-A, EC 3.4.13; substrate val-leu), tripeptide aminopeptidase (PEP-B, EC 3.4.11; substrate leu-gly-gly), proline dipeptidase (PEP-D, EC 3.4.13; substrate phe-pro), phosphoglycerate mutase (PGAM, EC 5.4.2.1), phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglycerate kinase (PGK, EC 2.7.2.3), phosphoglucomutase (PGM, EC 5.4.2.2), pyruvate kinase (PK, EC 2.7.1.40), L-iditol dehydrogenase (SORDH, EC 1.1.1.14), and triose-phosphate isomerase (TPI, EC 5.3.1.1). The nomenclature for referring to loci and allozymes followed Adams *et al.* (1987).

Data analyses

The allozyme data were analysed for population structure using the computer program GENEPOP, version 3.1b (Raymond and Rousset 1995). All *P* values were adjusted using the sequential Bonferroni technique (Rice 1989) to compensate for multiple tests, starting with an initial significance level of 0.05. F-statistics were calculated using the program FSTAT version 2.8 (Goudet 1995, 1999). Genetic differentiation among sample sets was estimated using Nei's unbiased measure of genetic distance (Nei 1978), under the assumption that the loci found to be monomorphic in the overview study were invariant in all sample sets.

RESULTS

Allozyme variation

A total of 48 putative allozyme loci were examined in the overview study. Of these, thirty-nine were monomorphic across all 30 specimens, while nine loci displayed electrophoretic variation consistent with the presence of two or more co-dominant alleles. The remaining 173 specimens were then screened for these nine polymorphic loci plus four invariant loci (*Ak*, *Est2*, *PepA*, and *PepB*), chosen anecdotally for their potential to display allelic variation (Table 1.2).

Species boundaries

There was no evidence of cryptic species within *N. gouldi* for the sites sampled in this study. No fixed differences were present between any localities and indeed all sample sets displayed very similar allele frequencies at the nine polymorphic loci (maximum difference in allele frequency = 34% for allele *Sordh*^{d}; see Table 1.2). This high degree of genetic similarity was reflected in the Nei Distances between sample sets, which ranged from 0.000 to 0.003.

Population structure

The allozyme data (Table 2) were analysed for a range of population genetic measures as provided by GENEPOP. These were (1) deviation from Hardy Weinberg expectations for each locus/sample set combination, (2) linkage disequilibrium between genotypes at different loci, and (3) differences in allele frequency for pairwise comparisons of sample sets at each locus. No significant deviations from Hardy Weinberg expectations were found for any locus or site, and homogeneity tests revealed no evidence of linkage between any two loci in any sample set. As such there is no evidence that sample sets were not representative of single, panmictic populations at each locality. Importantly, these analyses also confirmed that each genetic marker could provide an independent test of between-locality stock structure in *N. gouldi*.

Table 1.2: Allozyme frequencies (expressed as a percentage) in arrow squid for the six sample sets at nine polymorphic loci. Maximum sample sizes shown in brackets for each site. (*** = samples displayed no activity at this locus). The loci *Ak*, *Est2*, *PepA*, and *PepB* remained invariant after screening all individuals.

Locus	Allele	WA (18)	SA (51)	TAS 1 (32)	TAS 2 (7)	NSW 1 (50)	NSW 2 (45)
Acp	*c*	47	69	58	50	64	56
	b	53	28	42	50	36	43
	a		3				1
Acyc	*g*				7		1
	f	85	78	72	79	68	70
	e	6	8	17	7	14	4
	d	9	12	6	7	14	22
	c					1	
	b			5		3	2
	a		2				1
Est1	*b*	97	94	98	100	93	93
	a	3	6	2		7	7
Fum	*c*	***	95	***	***	97	96
	b					1	
	a		5			2	4
Got1	*c*					1	
	b	100	99	100	100	99	100
	a		1				
Got2	*b*						1
	a	***	100	***	***	100	99
PepD	*d*		1				
	c	3	1				
	b	97	95	100	100	99	100
	a		3			1	
6Pgd	*b*	3				1	1
	a	97	100	100	100	99	99

Locus	Allele	WA (18)	SA (51)	TAS 1 (32)	TAS 2 (7)	NSW 1 (50)	NSW 2 (45)
Sordh	*g*		2			4	
	f		1			1	
	e	19	17	19		9	24
	d	6	4	9	40	6	8
	c	72	76	69	50	78	66
	b	3		3	10		2
	a					2	

An initial comparison of allele frequencies between the two replicate samples sets from Tasmania revealed no significant differences at any locus, and as a consequence these were pooled to form a combined sample set (TAS). Thereafter, pairwise comparisons of allele frequencies between all sample sets revealed only two departures from homogeneity, both involving the Iluka sample set ($0.01 < P < 0.05$ for *Acyc*, NSW2 vs TAS; $0.01 < P < 0.05$ for *Sordh*, NSW2 vs NSW1). No attempt was made to further explore the nature of any population substructuring, given the small genetic distances and overall similarities in allele frequency encountered.

F-statistics

F-statistics were calculated for the five major sample sets (WA, SA, TAS, NSW1, and NSW2) using the genotypic data for all nine polymorphic loci. The F_{IS} values for both analyses did not differ significantly from zero, supporting the assumption of panmixia within sample sets. A marginally-significant positive value was obtained for F_{ST} ($F_{ST} = 0.009$; 95% confidence intervals 0.001 to 0.012; $P<0.05$), supporting the assertion that there was significant genetic divergence among samples sets. This value remained marginally-significant when the data were re-analysed after the removal of the Iluka sample set, suggesting that whatever genetic divergence may exist in the metapopulation is not just a function of a northern (i.e. Iluka) versus southern (i.e. the other sites) genetic dichotomy.

DISCUSSION

Cryptic species

The allozyme data presented herein provide no evidence for the presence of cryptic species in *Nototodarus gouldi* among over 200 squid from six localities spanning its Australian distribution. This finding is consistent with studies of most other oegopsids. Indeed, of the 20 or so oegopsid squid examined using molecular techniques to date, the presence of cryptic species has only been suggested in *Martialia hyadesi* from the Patagonian and Antarctic Polar front (Brierley *et al* 1993) and a sub-species of *Berryteuthis magister* from the north Pacific (Katugin 2000). By comparison, cryptic speciation is much more prevalent in myopsid squid (e.g., Izuka *et al.* 1994, 1996). Highlighting this contrasting pattern for the two groups is the fact that cryptic species have been found in all five loliginids

examined so far from Australia (*Photololigo chinensis*, and *P. edulis*, Yeatman and Benzie 1994; *Sepioteuthis australis*, Triantafillos and Adams 2001; *Loliolus noctiluca*, Citroen 2001; and *Sepioteuthis lessoniana*, Triantafillos, unpublished data). These differences probably reflect the more neritic habitat of loliginids, their near-shore benthic spawning habits, and their larger hatchlings, which would be less likely to drift in the pelagic environment than their smaller ommastrephid and other oegopsid counterparts. Taken together, these biological attributes presumably provide loliginds with greater opportunities to form localized, genetically-isolated populations, some of which may occasionally become full species (without necessarily undergoing morphological change) given sufficient time.

Population structure

Population genetic analysis of the allozyme data revealed no evidence of within-site heterogeneity at any of the localities sampled but did infer that the entire Australian metapopulation is not panmictic. Pairwise comparisons of allele frequencies and F-statistics revealed marginally-significant differences in allele frequencies at two loci between Iluka and one other site. This suggests that the Iluka sample in northern NSW may represent a separate stock when compared to sites further to the south and west. Moreover, F-statistics also suggested additional between-site heterogeneity among these other sites.

Beyond these inferences, the data provide no support for any obvious population substructuring within *N. gouldi*. Allele frequencies at every site were broadly similar for all polymorphic loci for localities separated by distances of up to 4,300km. Such data are consistent with a single, randomly mating stock across southern Australia and compatible with the predicted effects of recruitment via sexually produced offspring with high dispersal ability. Similar patterns of genetic homogeneity have been observed in other oegopsid squid. For example, only a low level of population differentiation was found in the circum-polar sub-Antarctic squid *Moroteuthis ingens* on a global scale (Sands *et al.* 2003). This outcome was explained in terms of eggs and hatchlings being transported long distances in circum-polar currents and jet streams associated with frontal zones.

Our results however should be viewed cautiously, for two reasons. Although allozyme analysis can provide valuable insight into species boundaries and therefore is an appropriate starting point for a molecular systematic assessment, its utility for detailed population structure analysis is usually constrained by an inability to generate both adequate numbers of genetic markers overall and adequate numbers of alleles per marker (Hillis *et al.* 1996). Such is the case herein for arrow squid, where only three of the nine polymorphic loci were sufficiently variable (*ie.* the combined frequency of rarer allele(s) > 10%; see Richardson *et al.* 1986) to be useful indicators of population structure. Low levels of allozyme diversity have been found in other squids such as *Loligo pealei* (Garthwaite *et al.* 1989),

Loligo opalescens (Reichow & Smith 2001), and appear to be characteristic of squid in general (Ally and Keck 1978; Brierley *et al.* 1995). Caution should also be exercised due to the low numbers of individuals in two sample sets (Western Australia and Tasmania), which reduce the chances that any real differences in allele frequency can be shown to be statistically significant. Taken together, these caveats increase the probability of a type II error i.e. that genuine population substructuring will remain undetected due to the null hypothesis of panmixia being falsely accepted (Richardson *et al.* 1986).

Implications for management of arrow squid fishery

The results of this study have clear implications for the management of arrow squid. At present, they are managed in Australia as if all individuals are members of a single, interbreeding stock. The results presented here indicate the possibility of more than one stock, particularly along the east coast. If indeed there is a discrete stock in the north-eastern region of the distribution of *N. gouldi*, management strategies need to be modified and so that these stocks can be managed separately. Given the likelihood of increased harvesting of this ecologically-vulnerable species, there is clearly a pressing need to extend this study further to determine exactly how many stocks are present in the Australian region. Without this information, there is some concern that localised stocks may become depleted and result in a recruitment failure. This, combined with heavy fishing pressure has already contributed to the collapse of the fisheries for *Illex illecebrosus* and *Todarodes pacificus* in the North-West Atlantic and North-West Pacific Oceans, respectively (Dawe and Warren 1993).

Conclusions

Despite some general limitations, our allozyme analyses demonstrate that further molecular investigation of fine-scale population structure is warranted for *N. gouldi*. As shown elsewhere, the two most suitable approaches here are likely to be (a) microsatellite analysis and (b) analysis of mitochondrial DNA sequence data (Adcock *et al.* 1999a,b, Shaw *et al.* 1999, Reichow & Smith 2001). As an Australian cephalopod example, Kassahn *et al.* (2003) have demonstrated the utility of microsatellite and mtDNA data for exploring fine-scale population structure within each of the two major subpopulations of the cuttlefish *Sepia apama* identified by allozyme analysis.

Given that its distribution encompasses both Australia and New Zealand, any future study of population structure in *N. gouldi* should ideally include some New Zealand populations to assess what degree if any the New Zealand stock is genetically connected to the Australian stock. There is the possibility that the New Zealand squid might be genetically associated with one of the Australian east coast stocks. The biological status and potential connection of the arrow squid stocks between these two countries clearly needs resolving. Future sampling would ideally also be structured to consider any

degree of temporal genetic differences (e.g., Katugin & Mokrin 2001). While this study presents preliminary work, research needs to now take the next step with more powerful genetic analyses and larger spatial and temporal samples sizes from both Australian and New Zealand sites. There is also the likelihood of increased fishing pressure on Australian populations of arrow squid which underscores the urgency of obtaining better genetic resolution for this species.

CHAPTER TWO

EGG PRODUCTION IN THE ARROW SQUID *NOTOTODARUS GOULDI* (CEPHALOPODA: OMMASTREPHIDAE), FAST AND FURIOUS OR SLOW AND STEADY?

Authors: Belinda L. McGrath-Steer and George D. Jackson

Adapted from: *Marine Biology* 141: 699-706

INTRODUCTION

Flexibility is an inherent characteristic of sexual maturation and spawning in many squid species (e.g. Boyle et al. 1995; Melo and Sauer 1999; Pecl 2001). Increasingly, maturation and spawning studies show that squid are no longer restricted to terminal spawning modes, and subsequent to the pivotal study by Harman et al. (1989) on the reproductive biology of *Stenoteuthis oualaniensis*, there has been a growing body of literature describing alternate squid reproductive strategies. We now know reproductive processes in squid are inherently linked to the environment (Arkhipkin et al. 2000; Pecl 2001; Jackson and Moltschaniwskyj 2001), individual energy reserves (Harman et al. 1989; Moltschaniwskyj and Semmens 2000) and behavioural influences (as suggested by Maxwell and Hanlon 2000). Even at the species level they have been found to vary greatly depending on the location and season from which they were caught (Boyle et al. 1995; Arkhipkin et al. 2000 and Pecl 2001). It is no longer feasible to simply class species into the categories of either terminal or multiple spawners, but rather, individuals are likely to fall somewhere along a continuum between these two strategies (Mangold 1987).

The underlying reproductive strategy of a species can be described as a set of trade-offs between life-history traits and competing physiological processes (Stearns 1992; Rochet 2000). For example, the process of sexual maturation in some squid species has been shown to reduce somatic growth by either directly utilising mantle muscle as an energy store (e.g. *Todarodes pacificus,* Ikeda et al. 1993; *Moroteuthis ingens*, Jackson and Mladenov 1994) or, by diverting energy away from mantle growth (e.g. *Photololigo sp.,* Moltschaniwskyj 1994). In addition, the regulation or compensation of these traits may also vary in response to environmental pressures, so individuals can adopt various tactics within an overall strategy depending on their surroundings (Rochet 2000). It is likely that this inherent flexibility within the

reproductive strategies of squid, is a function of the rate and timing of energy reserve use during maturation (Moltschaniwskyj and Semmens 2000).

As there is only a finite amount of energy available for both reproductive and somatic activities, these processes must essentially compete for a portion of available reserves (Calow 1983). This type of trade-off is most obvious in females, who must invest considerably more energy into reproductive processes such as vitellogenesis and the development of large accessory reproductive organs, compared with males (Stearns 1992). At the whole animal level, the energy required for sexual development may be derived from three sources. Firstly, somatic tissue maybe broken down and used as an energy source (O'Dor and Wells 1978; Jackson and Mladenov 1994; Arkhipkin 1993); secondly, energy may be acquired directly from a food source (Collins et al 1995; Moltschaniwskyj and Semmens 2000); or thirdly, energetic needs maybe met from a combination of both food and storage (as suggested for *Illex*, Laptikhovsky and Nigmatullin 1993). The rate at which these reserves are used will determine the underlying reproductive strategy an individual will express.

Species that invest large amounts of energy into reproduction towards the end of their life cycle essentially trade somatic growth for reproductive output, thus compromising body condition once maturity is reached (Calow 1979). Consequently, the female releases all her eggs in one or two large batches. Alternatively, individuals may allocate smaller amounts of energy towards maturation throughout the majority of their life (Calow 1979). Sexual development in this type of strategy is a longer drawn out process and may result in repeated spawning (e.g. see Lewis and Choat 1993; Moltschaniwskyj 1995; Pecl 2001). By investigating the patterns and timing of energy allocation between competing processes, it is possible to determine the overall reproductive strategy used by a species.

In this study, the process of egg production and release is examined in the temperate shelf squid *Nototodarus gouldi*. *N. gouldi* is found throughout southern Australian waters, and the northern waters of New Zealand (Uozumi 1998). This species forms Australia's largest squid fishery, however despite its distribution and commercial importance, little is known of its life history or ecology. The level of energy allocation and investment between somatic and reproductive organs were examined to identify trade-offs between these competing processes in order to understand the underlying reproductive strategy used by *N. gouldi*. In addition, patterns of oocyte size and storage were investigated to assess what type of spawning trait *N. gouldi* is likely to adopt.

MATERIALS AND METHODS

Collection methods and Processing

A total of 188 female *N. gouldi* were collected from Storm Bay, Tasmania (~ 43°10′S; 147°35′E). All individuals were captured by commercial jigging vessels over the summer months of December 1999 and January 2000 and frozen within 6 hours of capture. An additional sample of 13 mature squid was also taken in February 2000 from Storm Bay and preserved fresh for macroscopic inspection of the ovaries and oviducts.

Total body weight and dorsal mantle length (ML) were measured for each specimen. Females were then dissected and assigned a maturity stage (1-juvenile, 2-immature, 3-preparatory, 4-maturing, 5-mature, 6-spent) using a modification of Sauer and Lipinski's 1990 paper to suit our species. The weights of the ovary, oviducts, nidamental glands, oviducal glands, digestive gland, mantle muscle and fin were also recorded. As somatic activity and maintenance of semelparous species may be compromised during sexual maturation (Calow 1983), each individual was examined for signs of muscle or gonad regression. To identify at what level of maturity individuals were when mated, any indication of spermatophore deposition around the buccal membrane was noted. To compare the proportion of weight allocated towards sexual maturation in comparison to somatic weight, a gonado-somatic index (GSI) was calculated for each individual as:

Total reproductive weight /(Total body weight) x 100

Where total reproductive weight = Weight of the ovary + oviducts + oviducal glands + nidamental glands.

Evidence of recent feeding was assessed using a stomach fullness index (0, empty to 5 completely full).

Macroscopic inspection of ovaries and oviducts

Ovaries and oviducts of 13 fresh mature females were fixed in a formalin acetic-acid calcium-chloride solution (10ml of 37% formaldehyde, 5ml glacial acetic acid, 13g calcium chloride-dehydrate and 100ml water) for 2 weeks, weighed then transferred to 70% ethanol for storage. The number of eggs in the fixed oviducts were estimated by counting eggs within a 100 mg sub-sample and scaling this number up for the whole oviduct weight. The size of ovulated and non-ovulated oocytes was also determined by measuring the diameter of 100 randomly selected oocytes from both the middle of the ovary and the oviducts of each specimen, using an ocular micrometer in a stereomicroscope. Damaged or deformed oocytes were not measured.

Statistical analysis

To examine the relationships between somatic and reproductive investment, four geometric regression equations were calculated (after Green 2001), using mantle length as the independent variable and either the gonad weight, mantle weight, fin weight or digestive gland weight as the dependant variable. For each of these regression equations standardized residuals were calculated for each individual. As the four tissue weights were regressed against ML, the value of each residual gives an indication of the relative condition of each tissue without the bias of body size. That is, the residuals of animals with heavier tissues sit above the predicted value (or regression line) for their length, while those animals with lighter tissues have residuals that sat below the predicted value for their length. For this study, individuals with heavier tissues for their length and thus higher residuals were considered to have tissues in better condition.

To determine if individuals with higher gonad condition had poorer somatic condition, the ML-gonad residuals were correlated separately with each of the ML-mantle, ML-fin and ML-digestive gland residuals. This would identify any trade-off which maybe occurring between somatic and reproductive growth. For example, if a large amount of energy was being allocated towards gonad growth at the expense of mantle muscle growth, a negative correlation between the ML-gonad: ML-mantle residual pairs would be expected.

To assess if mature oocytes were accumulating in the oviduct for a single release, correlations between the oviduct weight and individual size were performed. If oocytes were being stored in the oviduct in preparation for a single spawning event, oviduct weight would increase until all oocytes had completed development and thus would be highly correlated with body size (Harman et al. 1989). All figures in text following ± refer to standard errors.

RESULTS

Although a wide size range of female *N. gouldi* were investigated in this study (182 – 430mm ML), neither juvenile (stage 1) or spent animals (stage 6) were recorded. Both the ovary and the nidamental gland weights were highly correlated with body size ($r = 0.95$, $n = 188$, $p < 0.001$ and $r = 0.935$, $n = 186$, $p < 0.001$ respectively), suggesting that in *N. gouldi* the process of sexual maturation is likely to be size-dependant. Despite this however, for each maturity stage females were found over a wide range of lengths and weights (Fig 2.1).

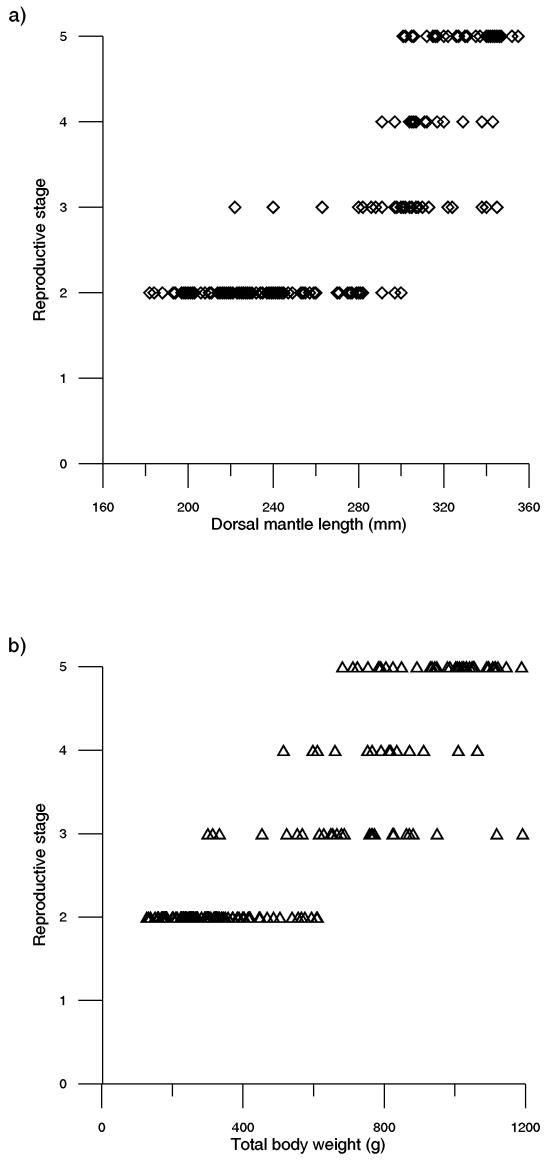

Figure 2.1: *Nototodarus gouldi.* Length and weight ranges for each maturity stage.

Energy allocation

The ML regressions performed against the gonad, mantle, fin and digestive gland weights' showed relatively strong relationships, with all coefficients of determination above 0.82 and little variation in residual values for each equation (Table 2.1). No significant correlations were found between the ML-gonad residuals and the ML-mantle ($r=0.01$, $p>0.05$. $n=178$); ML-fin ($r=0.07$, $p>0.05$, $n=177$) or ML-digestive gland ($r=0.07$, $p>0.05$, $n=175$) residuals, indicating changes in the condition of the gonad were not related to changes in either the mantle, fin or digestive gland condition (Fig 2.2). Female *N. gouldi* were found to have a relatively low gonado-somatic index (Fig 2.3) with mature (stage 5) individuals varying from 5.55% to 15.9% with an average value of 9.29% (± 0.40).

Table 2.1: *Nototodarus gouldi.* Geometric mean (type II) regression statistics for dorsal mantle length with a) the gonad, b) mantle weight, c) fin weight and d) digestive gland weight.

	Slope	95% Confidence intervals	Intercept	r^2
a) gonad	9.744	0.101-0.089	-23.152	.85
b) mantle	2.816	0.356-0.328	-4.6270	.93
c) fin	3.025	0.328-0.296	-5.8011	.89
d) digestive gland	4.733	0.203-0.177	-10.0891	.82

Spawning mode

A significant difference in oviduct egg size and weight was found among individuals. Individual oviduct egg size varied from 624.7 (± 28.2) to 1386.5 (± 9.3) μm, and oviduct egg weight ranged from 0.484 ($\pm$ 9.58 $\times$ 10^{-6}) to 0.776 ($\pm$ 7.22 $\times$ 10^{-6}) mg. Although oviduct egg size and weight varied considerably between individuals, very little variation was observed within an individual. The total estimated oviduct egg number for 13 mature females examined macroscopically ranged from 2,176 to 82,395 eggs. This estimation of batch fecundity, although taken from a small sample size (n=13), does incorporate nearly the whole range of oviduct weights encountered for *N. gouldi* (n=188) (with only 4 of the frozen oviducts not analysed occurring outside this range), and therefore gives a reasonable assessment of possible batch fecundity in this species. Female size and oviduct weight were not significantly correlated ($r = 0.256$, $n = 54$, $p > 0.05$)(Fig 2.4). In addition six of these thirteen mature females were found to have stretched flaccid empty oviducts (as described by Pecl 2001). These females were checked for signs of mantle deterioration, ovary depletion and body lesions, however all squid were in similar condition to mature animals without stretched oviducts and thus were not considered spent.

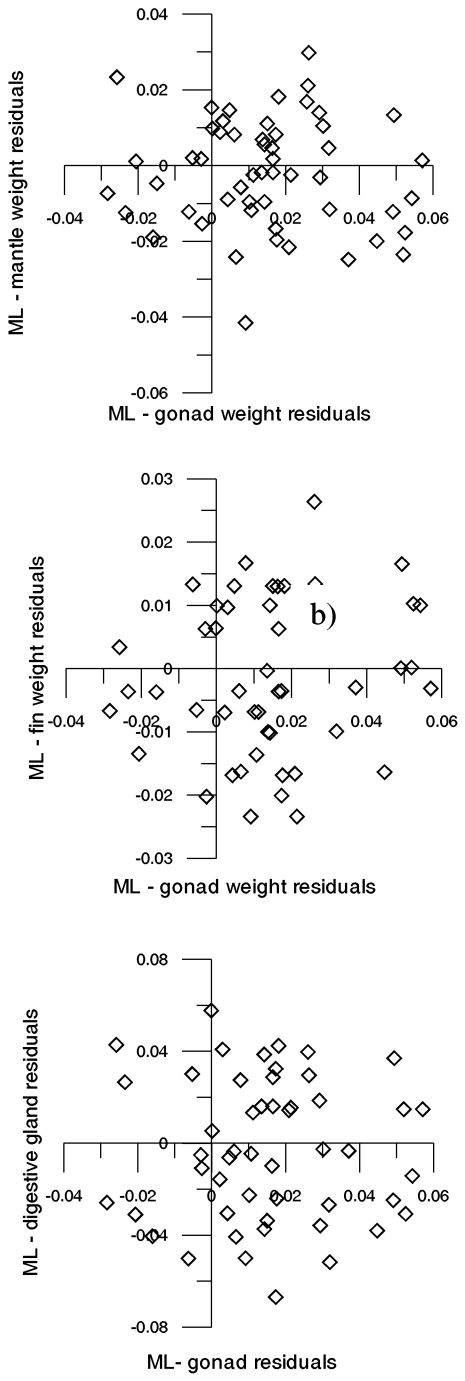

Figure 2.2: *Nototodarus gouldi*. Residuals from the ML-gonad regression with a) ML-mantle weight residuals, b) ML-fin weight residuals and c) ML-digestive gland weight residuals. Positive values represent heavier structures than the model predicts and negative values indicate structures lighter than the model predicts. As a trade-off is most likely observed in maturing (stage 4) or mature (stage 5) animals, only these maturity stages are represented in the figures. Values in parentheses are dorsal mantle length.

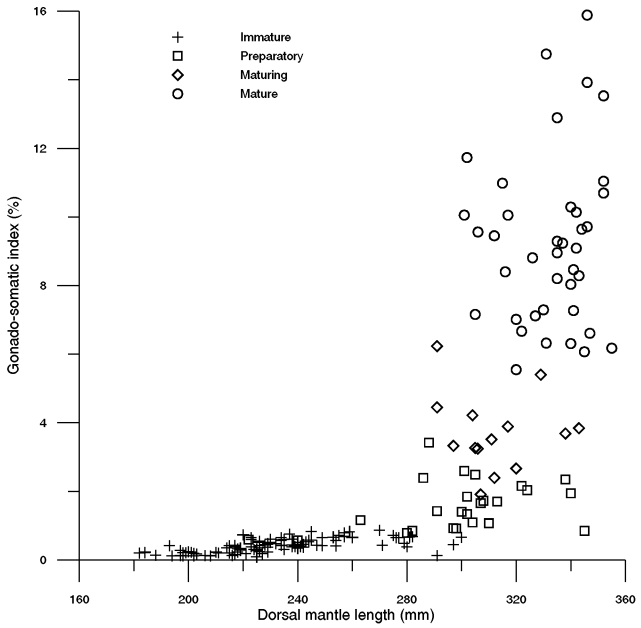

Figure 2.3: *Nototodarus gouldi.* Changes in Gonado-somatic index with squid length. Maximum value obtained was 15.9% with mature animals reaching a mean of 9.29% (±0.40SE).

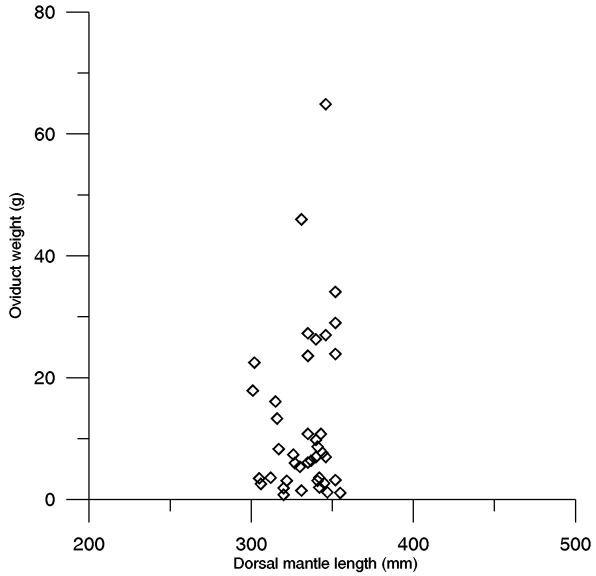

Figure 2.4: *Nototodarus gouldi.* Oviduct weight as a function of dorsal mantle length in mature.

Apart from one individual, the oviduct weight did not exceed the weight of the ovary (Fig 2.5). Individual females showed varying modal peaks in ovarian oocyte size distributions (Fig 2.6), with an obvious bimodal distribution occurring for eight females. Further, smaller oocytes made up the majority of eggs in the ovaries of eight individuals. Thus, even at full sexual maturity, female *N. gouldi* continue to produce and develop oocytes in discrete batches.

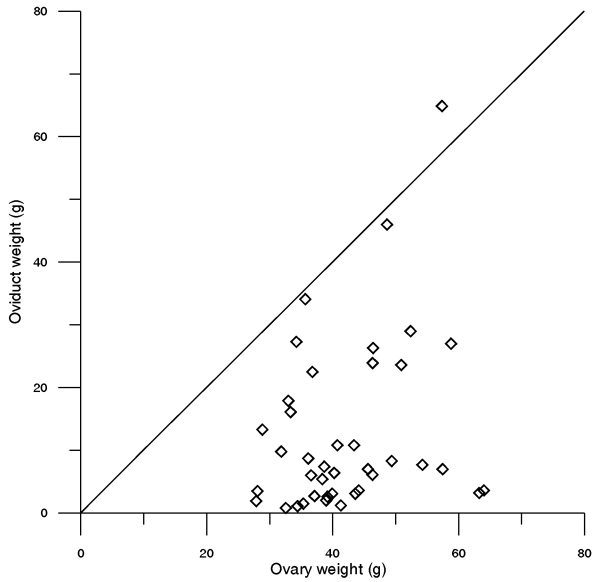

Figure 2.5: *Nototodarus gouldi*. The relationship between ovary weight and oviduct weight for mature females. Straight line indicates a slope of 1.

One of the two individuals that did not have a higher proportion of smaller oocytes in the ovary had a stretched empty oviduct (Fig 2.6b). As the ovary weight of this female was comparable to the ovaries of similar sized females (50.33 g), and had one of the lowest oviduct weights (8.31 g), this squid had probably already spawned and was possibly nearing another spawning event. The second animal that did not display the trend had two peaks of oocyte sizes in its oviduct (Fig 2.6c), one at 200-300 microns and the other at 700-900 microns with each peak possibly indicating a separate batch of developing oocytes.

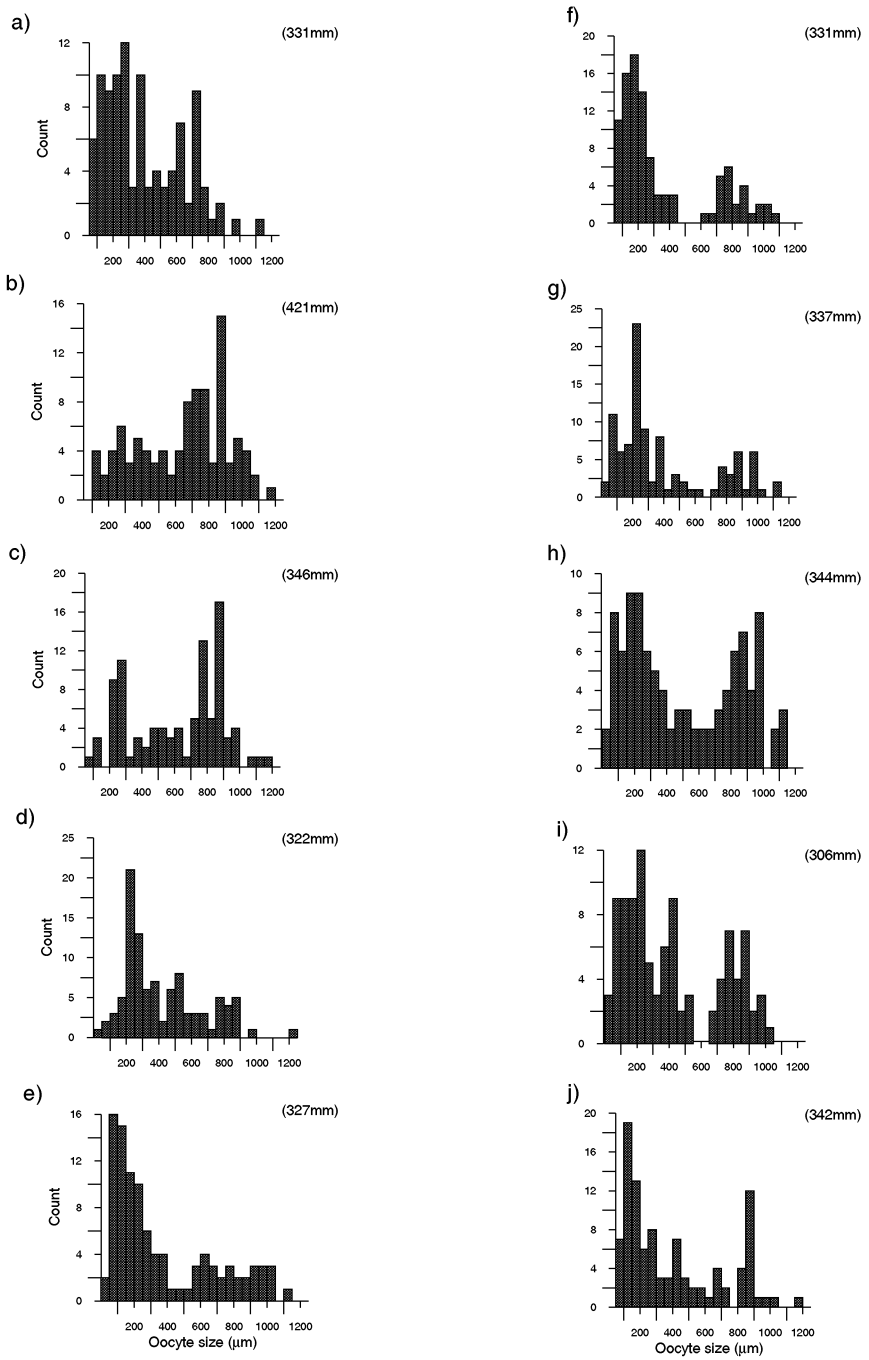

Figure 2.6*: Nototodarus gouldi*. Size frequencies of ovarian oocytes for 10 mature females (stage 5).

Signs of mating were observed only once females had reached the preparatory maturity stage (stage 3), with 48% of these females mated. In addition, 85% of maturing females (stage 4) were mated, while all mature females (stage 5) had mated. Although stomach fullness did vary between maturation stages (Fig 2.7), animals were continuing to feed while sexually developing because 53.8% of maturing and 66.7% of mature animals had some food in their stomachs.

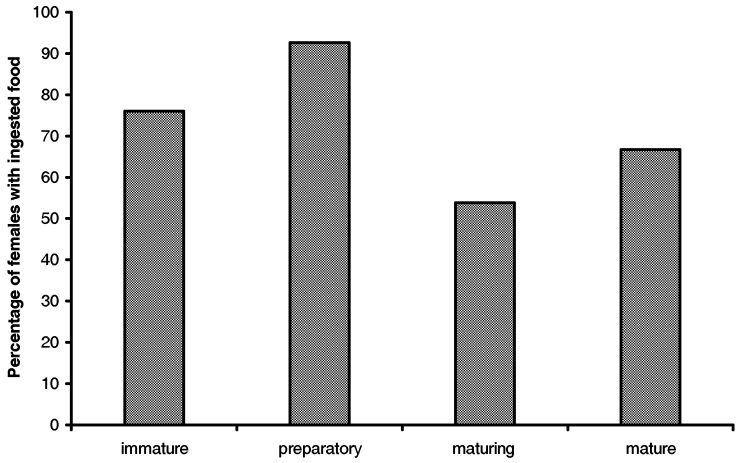

Figure 2.7: *Nototodarus gouldi.* The percentage of female with some food still remaining in their stomachs at capture

DISCUSSION

Nototodarus gouldi from Tasmania undergo reproductive development without any obvious changes in relative mantle, fin or digestive gland mass. If a trade-off between somatic and reproductive growth were to occur, an increase in gonad size would be coupled with a decrease in somatic tissue, thus providing evidence of an energy transfer from one process to another. There was little indication of this mechanism occurring in *N. gouldi.* Individuals that had a large gonad for their size did not compensate for this increased ovarian growth by either utilising somatic tissue as an energy store or by diverting energy away from somatic growth. This observation provided little evidence of reproduction occurring at an expense to somatic growth, since both processes occur simultaneously and it is probable that individuals continue to grow after sexual maturity has been reached. This conclusion is further supported by the relatively low gonado-somatic index calculated for mature squid (9.29% ± 0.40%). This indicates only low levels of energetic investment are being allocated towards reproductive growth at any one time. This is in strong contrast to those ommastrephid squids thought to be semelparous (e.g. 20% for *Illex argentinus,* Rodhouse and Hatfield 1990; 23% for *Illex illecebrosus* ovulated eggs alone, O'Dor 1983; and 50% for *Todarodes pacificus*, Ikeda et al. 1993). Instead, *N. gouldi* has a GSI similar to multiple spawning squids such as *Stenoteuthis oualaniensis* (average GSI of mature females of 8.8%, Harman et al. 1989); *Sepioteuthis lessoniana* (average GSI of mature females 12.7%, Pecl 2001) and *Sepioteuthis australis* (average GSI for winter caught females 9.0%, Pecl 2001).

The low energetic requirements of maturation in *N. gouldi* combined with little indication of a significant trade-off, provides strong evidence that egg production is a slow and steady process with eggs probably released in small batches. Supporting evidence for a multiple spawning mode is

provided by the lack of a significant correlation between oviduct weight and the mantle length. This observation indicates there are large variations in the total number of ova found in the oviducts of mature animals. This feature has commonly been suggested as a clear indication that oocytes are not accumulated in the oviduct in preparation for a single spawning event (see Harman et al. 1989; Moltschaniwskyj 1995; Gonzales and Guerra 1996), but rather ova are stored for release in small batches. Furthermore, the ovary weight of all females except one was consistently heavier than oviduct weight, suggesting that during oocyte development the ovary was not being depleted of eggs as they moved into the oviduct nor were eggs accumulating in the oviduct in large quantities.

Ovarian oocytes are found over a broad range of sizes which indicates oocytes are not being produced and developed synchronously, as would be expected with a semelparous spawning strategy (Harman et al. 1989). Instead the ovaries of *N. gouldi* continue to produce oocytes once maturity is reached. As a number of size modes were observed for 80% of the females examined, it is likely that female *N. gouldi* are developing eggs in batches. Although some semelparous species have been shown to contain ovarian ova in all stages of development when spent (e.g. *Loligo opalenscens*, Knipe and Beeman 1978), the current study does show that the production and development of oocytes is still possible once individuals become sexually mature (e.g. *Illex coindetii*, Gonzales and Guerra 1996).

As mature ova would continue to be stored in the oviducts unless spawning intervenes (Harman et al. 1989), partially filled oviducts would be characteristic of an intermittently spawning squid (Mangold 1987). Although all mature females contained oviducts at various weights, we also observed stretched empty or almost empty oviducts in 46% of fixed animals (n=13). This observation provides direct evidence of a previous spawning event, and further supports the opinion that *N. gouldi* is a multiple spawner.

The lack of a reduction in somatic condition in maturing and possibly spawning *N. gouldi* is in stark contrast with most other ommastrephid squids except *Stenoteuthis ovalaniensis* (Harman et al. 1989). Species such as *Illex illecebrosus*, *Illex argentinus, Illex coindetii, Todarodes sagittatus* and *Todarodes pacificus* all experience some decline in somatic growth during the later stages of sexual development (Hamabe, 1963; Ikeda et al 1993; Laptikhovsky and Nigmatullin 1993; Arkhipkin et al. 2000). Many of these species appear to allocate energy in the direction of high reproductive effort at the expense of growth or maintenance of the soma, with feeding ceasing after the first spawning event so body tissues are increasing drawn upon to fuel reproduction (as suggested for *Illex* by Laptikhovsky and Nigmatullin, 1993). For *N. gouldi* the presence of food in the stomachs of mature individuals indicates *N. gouldi* continue to feed and meet the energetic costs of egg production primarily through a direct acquisition

strategy, as found in *Loligo gahi* (Guerra and Castro 1994) and *Photololigo* sp. (Moltschaniwskyj and Semmens 2000).

Conclusions

The overall reproductive strategy of *N. gouldi* exhibits little evidence of a trade-off between somatic and reproductive processes, a continued maintenance of the soma and only small amounts of energy allocated towards maturation over a large part of the life-span. By investigating the mechanisms of reproductive development, researchers are able to better understand where individuals will fit along the terminal-multiple spawning continuum. As we now know there is no general pattern of maturation and spawning in squid, future investigations need to address the degree of flexibility within a strategy. This approach would provide some insight into what type of reproductive tactic an individual will express in response to its surroundings.

CHAPTER THREE

VARIATION IN AGE, GROWTH AND MATURITY IN THE AUSTRALIAN ARROW SQUID *NOTOTODARUS GOULDI* OVER TIME AND SPACE – WHAT IS THE PATTERN?

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Adapted from: Marine Ecology Progress Series 264: 57-71

INTRODUCTION

The study of squid age, growth and population dynamics continues to be of topical interest. The short life spans and rapid turnover in populations make them ideal marine models for studying the dynamics and how environmental influences may modify growth. Rapid growth rates along with physiological strategies aimed at maintaining "life in the fast lane" (Jackson & O'Dor 2001) place squid in a unique position to respond to environmental or climatic changes. The spawning population of squid reflect the conditions only experienced during the current year, because of the short generation time. It has been suggested that the life styles of squid promote faster changes in gene frequency than many other organisms (more like insects than vertebrates) and that because of this, squid should be able to track changes in climate or biological conditions more efficiently (O'Dor 1998). There have also been observations of large inter-annual fluctuations of squid ranging from 'plagues' (Rodhouse 2001) to population crashes (O'Dor 1992). Parallels in squid life histories have been made to terrestrial weeds (O'Dor 1998) and even locusts (Rodhouse 2001). Indeed, recent work (Jackson & Domeier 2003) have proposed that squid are effective ecological indicators, with phenotypic variability in *Loligo opalescens* rapidly changing in response to upwelling and productivity variation in the California Current as a result of short-term El Niño/ La Niña influences.

There is now a substantial body of work that has demonstrated the marked influence of temperature or season on size, growth rates and life spans of both loliginid (Jackson et al. 1997, Jackson &Moltschaniwskyj 2001, 2002, Forsythe et al. 2001, Hatfield 2000, Hatfield et al. 2001, Macy & Brodziak 2001) and ommastrephid (Dawe & Beck 1997, Arkhipkin et al. 2000, Arguelles et al. 2001) squids. Oceanographic conditions can also be an important influence on the successful growth,

abundance and survival of squid (Waluda et al. 2001, Agnew et al. 2002, Garrison et al. 2002, Kang et al. 2002). In high productivity or high plankton biomass waters, standing stocks of chlorophyll are expected to be higher; this may propagate through the food web and support higher standing stocks, growth rates and/or size of higher trophic level predators like squid. Furthermore, the abundance and location of squid can also influence the distribution of higher trophic predators (Jaquet & Gendron 2002). Studies of squid population dynamics thus need to consider the spatial and temporal biological and oceanographic influences when making assessments on squid growth and reproduction.

This study focused on the biology of the Australian arrow squid *Nototodarus gouldi*. There was little known on the growth dynamics of this species in Australia although some surveys have previously documented aspects of the distribution and size composition in southern Australia (Machida 1983, Smith 1983, Dunning & Förch 1998) along with the diet (O'Sullivan & Cullen 1983). Unlike many other ommastrephid species, *N. gouldi* is predominantly a continental shelf species and is the dominant ommastrephid squid in continental shelf waters off southeastern Australia south of 27° S and around the North Island, and northern regions of the South Island of New Zealand (Dunning 1998, Dunning & Förch 1998). It is most abundant in a depth range of 50-200 m and can even enter shallow waters and estuaries particularly during summer (Winstanley et al. 1983). The reproductive strategy of *N. gouldi* appears to be multiple spawning with no evidence of somatic degradation due to gonad growth (McGrath & Jackson 2002). Extensive work has been previously carried out on the growth and biology of both *N. gouldi* and the New Zealand endemic *N. sloanii* in New Zealand waters (Uozumi 1998).

This study was aimed at filling gaps in our knowledge of the spatial and temporal population dynamics of *N. gouldi* in southern Australian waters. Aspects of the variation in size, age, growth and maturity rates were explored using a statolith-based ageing technique.

METHODS

Samples were collected from four locations in southern Australia, over two seasonal periods and repeated for two consecutive years. Squid samples were obtained from commercial fishing fleets from the fishing ports of Ulladulla, New South Wales (Ull); Port Lincoln, South Australia (PL); Lakes Entrance, Victoria (LE) and Hobart, Tasmania (Tas), (Fig. 3.1) hereafter referred to as Tasmania. The sampling regime was designed to obtain squid that had grown through the coolest or warmest periods; summer/autumn caught (warm season squid, hereafter referred to as autumn squid) and spring caught

(cool season squid, hereafter referred to as spring squid). Samples were collected from all seasons except for Port Lincoln, where a spring 2001 sample was not obtained.

All samples were random and obtained by commercial bottom trawl except for the summer/autumn Tasmania sample that was a combination of trawl and auto-jig caught squid. Many of the samples for each location and date were pooled from several days of trawling (Table 3.1).

All squid were frozen upon capture and processed at the Institute of Antarctic and Southern Ocean Studies laboratory. Data recorded for each squid included sex, dorsal mantle length (ML, mm), total weight and gonad weight (g). Each squid was also assigned to a maturity stage that was modified from Lipinski (1979). Statoliths were removed, rinsed with water and stored dry at room temperature. A sub-sample of statoliths was selected for age estimates from the complete size range of individuals. Statoliths were mounted in the thermoplastic cement Crystal Bond on a microscope slide for preparation for ageing. Statoliths were ground dry on both the anterior and posterior plane to produce a thin section using 30 μm lapping film and polished using a 5.0 μm lapping film. Total increment counts were taken using a Nikon Eclipse E400 high power microscope (400x) using polarized light. The mean of two counts that varied less than 10% of the mean, was taken as the age estimate in days. Most counts were very close to one another (within 5%).

Initial validation work has provided some evidence of the daily periodicity of statolith increments in *N. gouldi* (Jackson unpublished data). Furthermore, statolith ageing has also been suggested as daily in the congener *N. sloanii* in New Zealand based on ageing of progressive modes (Uozumi 1998). Thus, the statolith increments were assumed daily for *N. gouldi* in this study.

Sea surface temperature / ocean colour

Weekly mean sea surface temperature (SST) off the coast from each of the sampling ports was calculated from the period of back-calculated hatching through the sampling period covered in this study. Data was sourced from the NOAA-CIRES Climate Diagnostic Centre (http://www.cdc.noaa.gov/) (Reynolds et al. 2002). We also explored the relationship between squid growth rates and local productivity as measured by SeaWiFS (Sea-Viewing Wide Field-of-view Sensor) which provides quantitative data on optical properties of the ocean, and from these the standing stock of chlorophyll a (a measure of phytoplankton) are calculated (e.g. Joint and Groom, 2000). It is often assumed that measured standing stock (colour) is proportional to productivity (Joint and Groom,

2000), and thus sea surface colour (SSC) is often used as a proxy for chlorophyll productivity. SeaWiFS measures the colour from the upper 20-30 m of the surface ocean; in very clear waters, it may include deeper portions, while in more turbid waters it will represent a thinner surface layer (Joint and Groom, 2000; Wilson et al., 2002). In addition, the relationship between SSC and the whole water column chlorophyll value is poorly known for most regions of the world (Joint and Groom, 2000). A subsurface chlorophyll maximum is evident in many regions, and if deeper than the depth visible to the satellite, may not be detectable. This results in bias in estimating the water column standing stock of chlorophyll. A further caveat is that it is generally accepted that in coastal waters the chlorophyll a concentration may need to be tuned to local conditions, as the inherent optical properties of constituents such as dissolved organic matter and suspended sediments vary strongly and can bias the apparent chlorophyll concentration. In this study we assumed that the SSC data are representative of the upper surface layer at each site and that they are a reasonable measure of local productivity.

A measure of squid growth rate (the slope of the log-log age-size relationships, Table 3.3) for the sex/site/season combinations was related to a proxy for productivity (SSC). The SSC data were derived from the SeaWiFS 8-day 9 km chlorophyll a product, and average values for each 8-day period for a 1 degree box at each location for the period 1999-2001 were obtained using customized Matlab programs. The 1° boxes were centred so that the edge of the box touched the coast; Port Lincoln (136°E, 35°S), Lakes Entrance (148°E, 38.5°S), Ulladulla (151°E, 35°S), and Storm Bay, Tasmania (147.6°E, 43.7°S). These SSC data were then averaged to provide a monthly time series for each of the four locations and the average SSC value for the months of peak hatching (as determined from back-calculated statolith ages) for each location was calculated. For the purpose of this study we had to assume that hatchling and juvenile squid occur in the same region as the adults. Because there was a difference between locations in SSC and in the growth rate between males and females, the growth anomaly (difference from the mean) for each site and sex, rather than absolute values, was used in the analyses to allow sites to be combined. Similarly, the anomaly from the average SSC values for the months of peak spawning from each location were used.

Statistical analyses

Sexual dimorphism was determined for mature individuals by performing Bonferonni adjusted t-tests to compare both log body weight and log ML between the sexes, for each combination of location, season & year. This was carried out for all samples except Tasmania spring 2000 and autumn 2001 where there were insufficient males to test for sexual dimorphism. Furthermore, we wanted to know if arrow squid were schooling with the same sex or if there were mixed schools. This was determined by using a CHI-square test that determined if the number of males and females in each sample were significantly different than 1:1. Growth, along with weight, age and gonad weight of mature

individuals was compared across locations, sexes, seasons and years using factorial analysis of variance. In each case, location, sex, season and year were all treated as fixed factors. Where the presence of high order interactions was indicated, pairwise comparisons amongst groups were computed using the logical constraints method of Westfall (1997) except where otherwise stated.

Growth was analysed by fitting a separate lines regression model to the log-transformed weight-at-age data to determine the effects of location, season, year and sex on body weight, with age as the covariate. Since growth data are more closely influenced by conditions experienced post hatching and especially during the juvenile stage, individuals were grouped according to the season of hatch. Season of hatch was grouped to 3-4 month periods (e.g., late summer-mid autumn and winter-spring). However, since maturity is more related to conditions at time of capture, analyses using mature individuals were grouped according to time of capture.

Differences in the mean log wt (which equates to the median weight on the original un-transformed data) of mature males and females were analysed separately due to sexual dimorphism in this species. A 3-way full factorial ANOVA with location, season of capture and year as the factors of interest was used to determine differences in body wt for males and females. For females, Tasmanian spring caught 2000 and autumn caught 2001 samples were removed due to less than 5 mature individuals in the samples. Mature animals in this and all subsequent analyses included both stage 5 and stage 4 individuals. The stage 4 squid were considered functionally mature even though there was no evidence yet of eggs in the oviducts of females or spermatophores in Needham's sac of males.

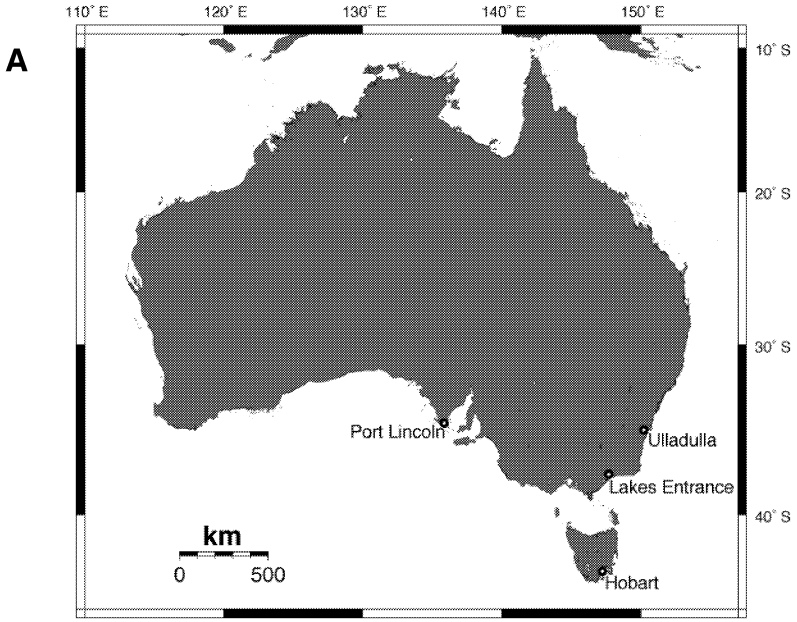
We were interested in exploring the effects of location, season of capture and year on median gonad weight for mature males and females separately. For males, a 3-way full factorial analysis of variance (ANOVA) was used to test differences in median testis wt, and for females a 3-way full factorial ANOVA was used to test differences in median ovary wt. This data were extremely unbalanced due to samples with less than 5 individuals being removed from the analysis, as it was not clear these samples would be truly representative.

The age of mature males and females was analysed together as sex was a factor of interest. To determine the effects of location, season of capture, year and sex on the mean age of mature animals a 4-way full factorial ANOVA with the 4-way interaction removed was used. Female samples from Tasmania spring caught 2000 and autumn caught 2001, as well as Ulladulla -autumn caught 2001 were removed due to less than five mature individuals in these samples. This resulted in 0 df for the 4-way

interaction. When a significant interaction or main effect was obtained from the ANOVA model on mean age, body size or gonad weight, a Tukey's HSD was used to determine where significant differences were occurring.

RESULTS

The squid were obtained from temperate Australian waters between approximately 35° S and 43° S (Fig 1a) that were subject to marked seasonal fluctuations in SST. There was a general decrease in water temperature from Port Lincoln/Ulladulla to Lakes Entrance and Tasmania (Fig. 3.1b). There was no overlap in SST between Tasmania and Lakes Entrance and the other sites. However, of the two warmer sites, Ulladulla was more variable with considerably cooler winter temperatures but warmer summer temperatures compared to Port Lincoln. There were no marked differences in the seasonal trend of SST among the years of the study.



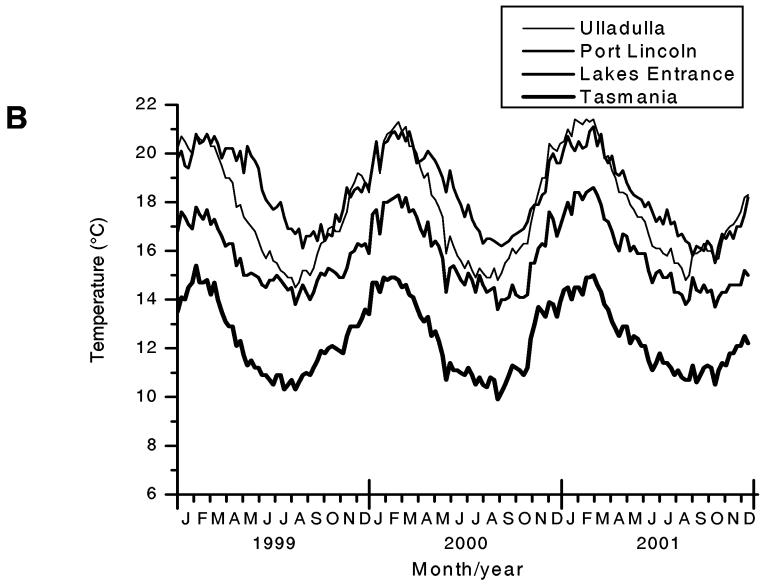


Figure 3.1: The map of Australia showing ports for where squid were collected (A) and the seasonal trend in sea surface temperature (B) for each of the port locations

Sexual dimorphism and sex ratios

At all locations and times except Ulladulla in the autumn of 2001, significant differences were found between the median ML and median Wt of males and females, with females being considerably heavier than males (Fig. 3.2). The median wt for mature females ranged between 1.27 and 2.46 times heavier than the median wt of mature males, while the median ML for females ranged between 1.1 and 1.37 longer than males. Ulladulla squid were consistently the smallest individuals with the majority of mature individuals < 500 g. This contrasted with Tasmania where most mature individuals were > 500 g. Lakes Entrance squid tended to be larger with the majority also > 500 g, while Port Lincoln squid were intermediate in size, and variable over time, with some samples having median weights > 500 g and other samples having weights < 500 g (Fig. 3.2). Out of a total of 15 samples, the majority (n=9) showed no difference in the F:M sex ratios (Table 3.1). For those samples that did show significant differences in sex ratios, four had a majority of females and only two had a majority of males.

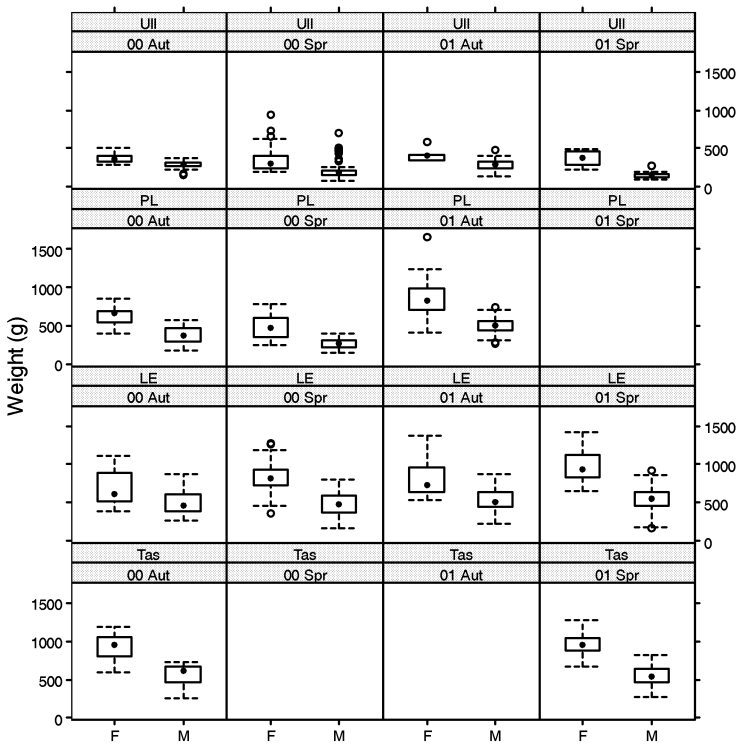


Figure 3.2: The weight distribution at each of the sites for each of the seasonal periods for male and female individuals of *Nototodarus gouldi*. F=females, M=males, Ull=Ulladulla, PL= Port Lincoln, LE= Lakes Entrance, Tas=Tasmania, Spr=spring, Aut=autumn, 00= 2000, 01=2001, circles indicate individuals that are outliers.

Table 3.1: Collection details of *Nototodarus gouldi* including number of individuals aged and the sex ratio, ns = non significant, *p<0.05, ** p<0.01, ***p<0.001.

Location	Collection period	Number of samples/ d of sampling	Method of capture	Total number caught	Total aged	Males caught/ (aged)	Females caught/ (aged)	Sex ratio F:M
Ulladulla	Late summer 2000	6 trawls over 2 d	Trawl	201	77	99 (32)	102 (45)	1:1.03 ns
	Spring 2000	2 trawls over 2 d	Trawl	197	74	79 (35)	118 (39)	1:1.49**
	Mid autumn 2001	1- 3 hr trawl	Trawl	85	63	42 (32)	43 (31)	1:1.02 ns
	Spring 2001	3 d trawling	Trawl	53	53	22 (22)	31 (31)	1:1.41 ns
Port Lincoln	Mid autumn 2000	3 d trawling	Trawl	98	64	54 (32)	44 (32)	1:0.81 ns
	Spring 2000	2 trawls over 2 d	Trawl	172	76	85 (37)	87 (39)	1:1.02 ns
	Mid autumn 2001	3 d trawling	Trawl	169	67	98 (32)	71 (35)	1:0.72*
Lakes Entrance	Late summer 2000	Squid from 3 boats over 4 d	Trawl	128	67	69 (31)	59 (36)	1:0.85 ns
	Spring 2000	1-5 hr trawl	Trawl	237	66	152 (31)	85 (35)	1:0.56***
	Mid autumn 2001	2 trawls	Trawl	83	62	52 (33)	31 (29)	1:0.6*
	Spring 2001	3 trawls over 2 d	Trawl	128	68	86 (33)	42 (35)	1:0.49***
Tasmania	Late summer 2000	2 d jigging	Auto jig	96	53	24 (19)	72 (34)	1:3***
	Spring 2000	3 d trawling	Trawl	168	61	80 (30)	88 (31)	1:1.1 ns
	Mid autumn 2001	3 d trawling	Trawl	150	65	76 (32)	74 (33)	1:0.97 ns
	Spring 2001	4 d trawling	Trawl	148	68	71 (32)	77 (36)	1:1.08 ns
Total				2113	984	1089 (463)	1024 (521)	

Growth

A total of 2113 squid were sampled in this study and of these, 984 were aged using statolith increment counts and used in age and growth analysis (Table 3.1). Growth of *N. gouldi* was rapid and the life cycle appeared to be less than a year as the oldest squid aged was 329 d. The oldest immature female was 275 d from Tasmania while the youngest mature female was 171 d from Ulladulla. The oldest immature male was 229 d from Tasmania while the youngest mature male was 142 d from Ulladulla. Ulladulla individuals were the smallest and youngest while Port Lincoln and Lakes Entrance had the oldest individuals (Fig. 3.3). Since both axes were logged to linearise the data, growth was described by a power curve in all instances (Table 3.2).

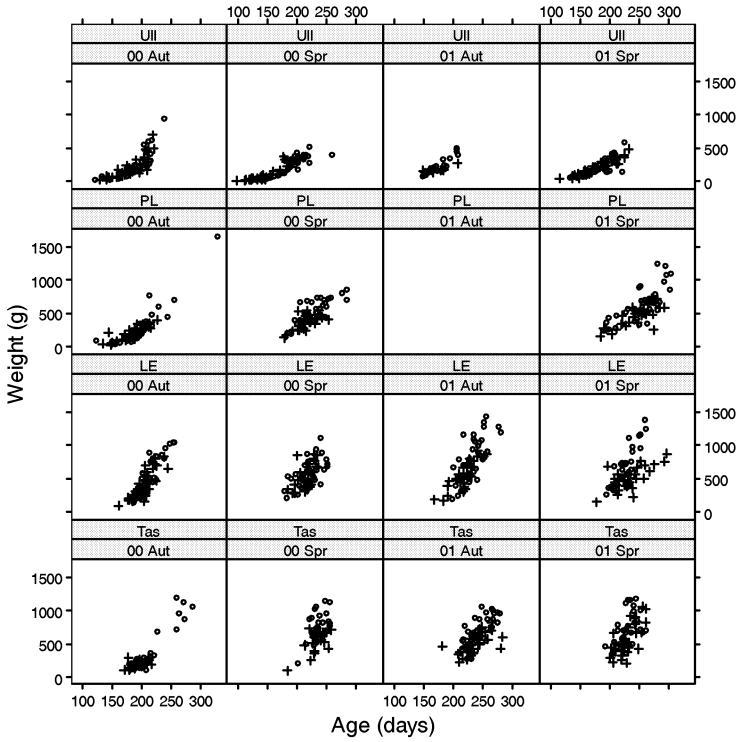


Figure 3.3: The relationship between estimated age and total weight for male and female individuals of *Nototodarus gouldi* for each of the locations and times. Crosses represent males, circles represent females. Ull=Ulladulla, PL= Port Lincoln, LE= Lakes Entrance, Tas=Tasmania, Spr=spring, Aut=autumn, 00= 2000, 01=2001.

Table 3.2: Regression details for the relationship between age and weight for male and female individuals of *Nototodarus gouldi* from all locations, seasons and years sampled in this study grouped according to hatch period, *p<0.05, ** p<0.01, ***p

Location	Hatch period Season/year/sex	Slope	SE slope	Intercept	R^2	N
Ulladulla	Summer-Autumn 1999/00					
	Females	4.992***	0.372	-20.941	0.83	38
	Males	5.307***	0.411	-22.468	0.84	33
	Winter-Spring 1999					
	Females	5.102***	0.262	-21.438	0.90	44
	Males	5.088***	0.316	-21.027	0.90	31
	Summer-Autumn 2000/01					
	Females	4.699***	0.349	-18.905	0.91	19
	Males	1.622**	0.45	-3.209	0.62	9
	Winter-Spring 2000					
	Females	3.799***	0.299	-14.578	0.80	41
	Males	3.692***	0.204	-13.917	0.88	44
Port Lincoln	Summer-Autumn 1999/00					
	Females	4.267***	0.394	-17.184	0.76	39
	Males	4.597***	0.489	-18.841	0.72	36
	Winter-Spring 1999					
	Females	2.57***	0.414	-7.74	0.56	31
	Males	3.218***	0.506	-11.416	0.57	31
	Summer-Autumn					
	Females					
	Males					
	Winter-Spring 2000					
	Females	2.571***	0.358	-7.851	0.62	33
	Males	2.562***	0.35	-8.013	0.64	31

Location	Season/sex	Slope	SE slope	Intercept	R^2	N
Lakes Entrance	Summer-Autumn 1999/00					
	Females	5.595***	0.52	-23.877	0.77	36
	Males	5.241***	0.606	-21.959	0.71	31
	Winter-Spring 1999					
	Females	2.559***	0.547	-7.494	0.41	33
	Males	3.163**	0.869	-10.778	0.32	29
	Summer-Autumn 2000/01					
	Females	4.239***	0.70	-16.513	0.53	34
	Males	3.981***	0.476	-15.173	0.69	32
	Winter-Spring 2000					
	Females	4.451***	0.50	-17.674	0.75	27
	Males	2.149***	0.46	-5.564	0.42	32
Tasmania	Summer-Autumn 1999/00					
	Females	4.773***	0.394	-19.925	0.81	35
	Males	2.813***	0.705	-9.596	0.36	29
	Winter-Spring 1999					
	Females	3.798***	0.88	-14.177	0.41	28
	Males	4.729***	1.056	-19.554	0.54	18
	Summer-Autumn 2000/01					
	Females	3.185***	0.575	-11.043	0.47	35
	Males	2.077***	0.532	-5.157	0.34	31
	Winter-Spring 2000					
	Females	2.81***	0.74	-8.774	0.32	32
	Males	4.101***	0.805	-16.062	0.46	31

The separate lines regression showed a significant four-way interaction of (location x season x year x sex, F= 4.31, df=2,922, p=0.014). Because of the complexity of the interaction and the presence of the missing cell (a sample was not obtained for Port Lincoln in spring 2001), the subsequent analysis relied on a series of pairwise comparisons of slopes within gender and location subgroups to discern patterns of growth. Our pairwise analysis of slopes of all locations/times resulted in far too many comparisons to readily interpret. We therefore split the data into sub-groups by location, season and year and made comparisons within these sub-groups. Not all comparisons were significantly different, but for those that were, there was a general trend across all locations of individuals growing significantly faster in 1999/2000 (year 1) compared to individuals that hatched in late 2000/early 2001 (year 2). Furthermore, there was also a consistent trend of summer-autumn hatched individuals growing faster than winter-spring hatched individuals (Table 3.3).

Table 3.3: Summary of growth rates of *Nototodarus gouldi* grouped according to location. The combination of sex, year and hatch season in left column are squid with significantly greater growth rates than squid in the right column, p values refer to pairwise comparisons.

Location	Sex/year/hatch season		Sex/year/hatch season	p value
Ulladulla	Females Yr 1 sum-aut	>	Males Yr 2 win-spr	0.028
	Males Yr 1 sum-aut	>	Females Yr 2 win-spr	0.022
			Males Yr 2 win-spr	0.009
	Males Yr 1 win-spr	>	Males Yr 2 win-spr	0.008
			Females Yr 2 win-spr	0.022
	Females Yr 1 win-spr	>	Males Yr 2 win-spr	0.005
			Females Yr 2 win-spr	0.019
Port Lincoln	Males Yr 1 sum-aut	>	Females Yr1 win-spr	0.018
			Females Yr 2 win-spr	0.005
			Males Yr 2 win-spr	0.010
	Females Yr 1 sum-aut	>	FemalesYr 1 win-spr	0.030
			Females Yr 2 win-spr	0.009
			Males Yr 2 win-spr	0.018
Lakes Entrance	Males Yr 1 sum-aut	>	Females Yr 1 win-spr	0.015
			Males Yr 2 win-spring	0.001
	Females Yr 1 sum-aut	>	Females Yr 1 win-spr	0.003
			Males Yr 2 win-spr	0.000
Tasmania	Females Yr 1 sum-aut	>	Males Yr 2 sum-aut	0.003

Comparisons between locations revealed that Ulladulla females and males grew significantly faster than Port Lincoln and Lakes Entrance females in year 1 winter-spring. In year 2 winter-spring, Ulladulla females and males and Lakes Entrance females grew significantly faster than Lakes Entrance males. There were no significant differences in the growth of summer-autumn hatched squid for both sexes at all locations in year 1. However in year 2, Ulladulla females grew significantly faster than Ulladulla males and Tasmania males. Lakes Entrance females also grew significantly faster than Tasmania males.

There was a significant positive relationship between the growth rate anomaly and the SSC anomaly for female squid from the winter-spring period (regression, $F_{1,6} = 6.20$, $p<0.05$, $R^2=0.51$) but not for the summer-autumn period (Fig. 3.4). There was no significant relationship between the growth of male squid and productivity in either season. When productivity-growth relationships at individual sites were considered all were non-significant, perhaps due in part to small sample size (n=4 samples per site, 3 for Pt Lincoln). As before, however, there was a positive relationship between SSC and growth rate for females (relationship strength for Pt Lincoln > Lakes Entrance > Ulladulla > Tasmania), while for males, the relationship went from weakly positive to negative in the same location order).

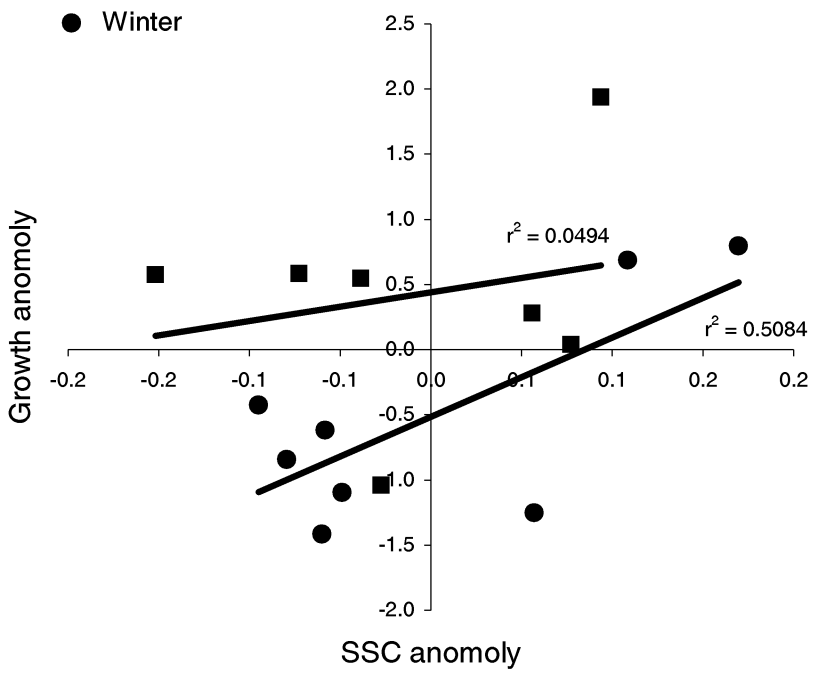


Figure 3.4: The relationship between productivity (sea surface colour) and growth anomalies for females from summer and winter periods (n=8 winter, n=7 summer).

Based on a sub-sample of aged individuals it was possible to determine hatch-periods (Fig. 3.5). The hatching periods for the Port Lincoln squid differed from the other three sites and had hatch peaks in spring during 1999 and in autumn and winter during 2000. Tasmania and Lakes Entrance had similar hatching periods that occurred during winter and autumn in 1999/2000 and spring and autumn in 2000/2001. Ulladulla had hatch periods during spring 1999, autumn and spring in 2000 and autumn in 2001. These hatch peaks would be related to the fact that we had four distinct sample periods during the study (except for Port Lincoln that had 3) that back-calculated to four distinct hatch periods in the study. Therefore, it is likely that hatching occurs throughout the year and the peaks identified in our study are due to distinct sampling periods rather than suggesting distinct spawning periods.

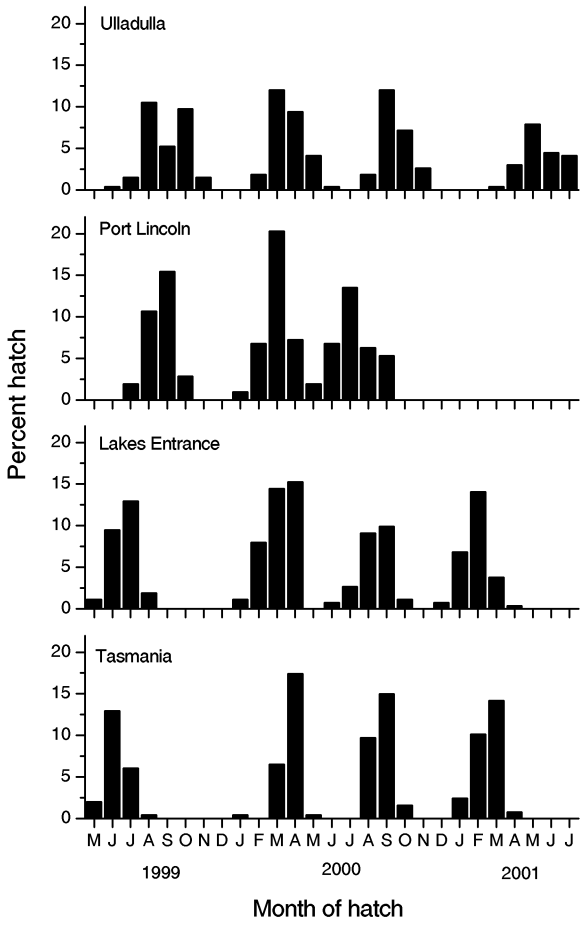


Figure 3.5: The hatch date distribution for all the aged individuals from each of the collection ports.

Reproductive Analysis

Mean size and age of mature individuals

Males

Due to the sexual dimorphism, the median wt of mature males and females were analysed separately. Males had a significant location x season x year interaction (F_W=21.79, df=2,822, p<0.001). There were predominantly two weight groupings for the male squid (Fig. 3.6). The heaviest males were from Tasmania, Lakes Entrance and Port Lincoln for 2001 and Lakes Entrance and Tasmania autumn in 2000, and all these samples were not significantly different. The smaller males were from the spring samples from Port Lincoln and Tasmania in 2000 and both seasons for Ulladulla in both years. All the small males were similar except for Ulladulla spring 2001 males that were significantly smaller than all other samples. The Port Lincoln autumn 2001 males were intermediate in weight and were significantly different from all other samples. The plasticity in weight of mature males is highlighted by the difference between the heaviest and lightest males (Tasmania autumn vs. Ulladulla spring 2000) where the median difference is a striking 290 % (586.3 g, SE 15.0 vs. 150.3 g, SE 30.6). All regions except for Lakes Entrance showed a marked decrease in weight for spring caught males as opposed to autumn caught males.

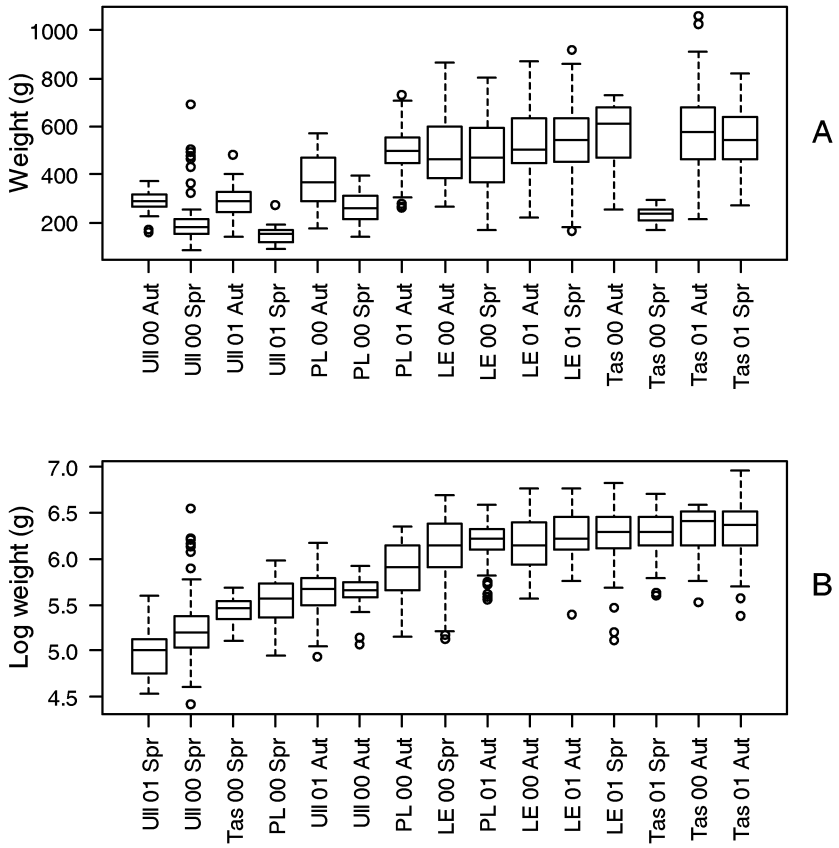


Figure 3.6: The distribution of mature male weights according (A) location and the distribution of logged mature male weights (B) shown sequentially. Ull=Ulladulla, PL= Port Lincoln, LE= Lakes Entrance, Tas=Tasmania, Spr=spring, Aut=autumn, 00= 2000, 01=2001, circles indicate individuals that are outliers.

Females

Weight of mature females only showed evidence of a location x season interaction (F_W=13.03, df=2,301, p=0.0001). However, this was interpreted cautiously as there were missing cells and evidence of higher order interactions for the males. We therefore fit a model to just the Lakes Entrance and Ulladulla locations for which there were no missing cells but also attained the same result of only a location x season interaction (F_W=8.05, df=1,155, p=0.01). We therefore could not detect a yearly difference in weight of mature females. The Ulladulla samples were the smallest in weight and were significantly different from all other sites, while the largest females that were for the most part indistinguishable in weight, were from Tasmania autumn 2000, spring 2001; Lakes Entrance spring 2000, autumn 2000/2001 and Port Lincoln 2001 autumn. Port Lincoln 2000 autumn and Lakes Entrance 2000 autumn were intermediate between the grouping of small and large females (Fig. 3.7).

The only significant difference in seasonal weight of mature females was found in Port Lincoln 2000 where the autumn females were significantly heavier than their spring counterparts.

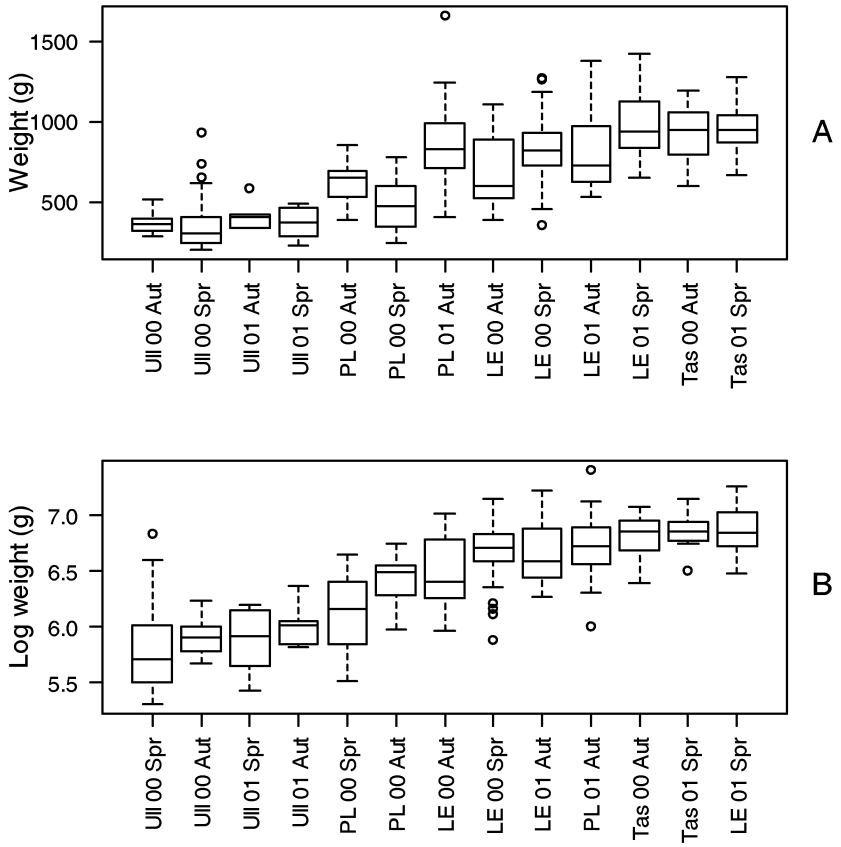


Figure 3.7: The distribution of mature female weights according (A) location and the distribution of logged male weights (B) shown sequentially. Ull=Ulladulla, PL= Port Lincoln, LE= Lakes Entrance, Tas=Tasmania, Spr=spring, Aut=autumn, 00= 2000, 01=2001, circles indicate individuals that are outliers.

Age

There was considerable variation in age of mature individuals for location, year and season (Fig. 3.8a). The Ulladulla 2001 spring male sample had the youngest mean age of 165 d while the Port Lincoln 2001 autumn females had the oldest mean age of 280 d. A grouping of Ulladulla samples generally had the youngest individuals while Tasmania and Port Lincoln formed another group that had the oldest individuals, with the Lakes Entrance squid being somewhat intermediate in age (Fig. 3.8a). Port Lincoln was the most variable of the samples, and although Port Lincoln had the oldest squid, the 2000 spring male sample had a young mean age of 194 d and grouped with the younger Ulladulla samples. Similarly, the Tasmania 2000 spring males were also considerably younger than the other Tasmanian samples. Thus, although there were overall trends, those two young Tasmanian and Port Lincoln samples fell considerably outside the trend in age for each location.

A full factorial model resulted in a significant location x season x year interaction (F_{age}=18.98, df=2,532, p=0.0001). Since sex was not a significant factor, we re-ran the ANOVA with all three-way interactions involving sex removed, but this did not modify our initial findings. We therefore undertook Bonferonni adjusted pairwise comparisons of mean age. The pairwise comparisons revealed that there was a continuum in mean age rather than any clear groupings. When grouped according to increasing mean age, there was just a gradual trend of increasing age from Ulladulla 2001 spring males to Port Lincoln 2001 autumn females (Fig. 3.8b).

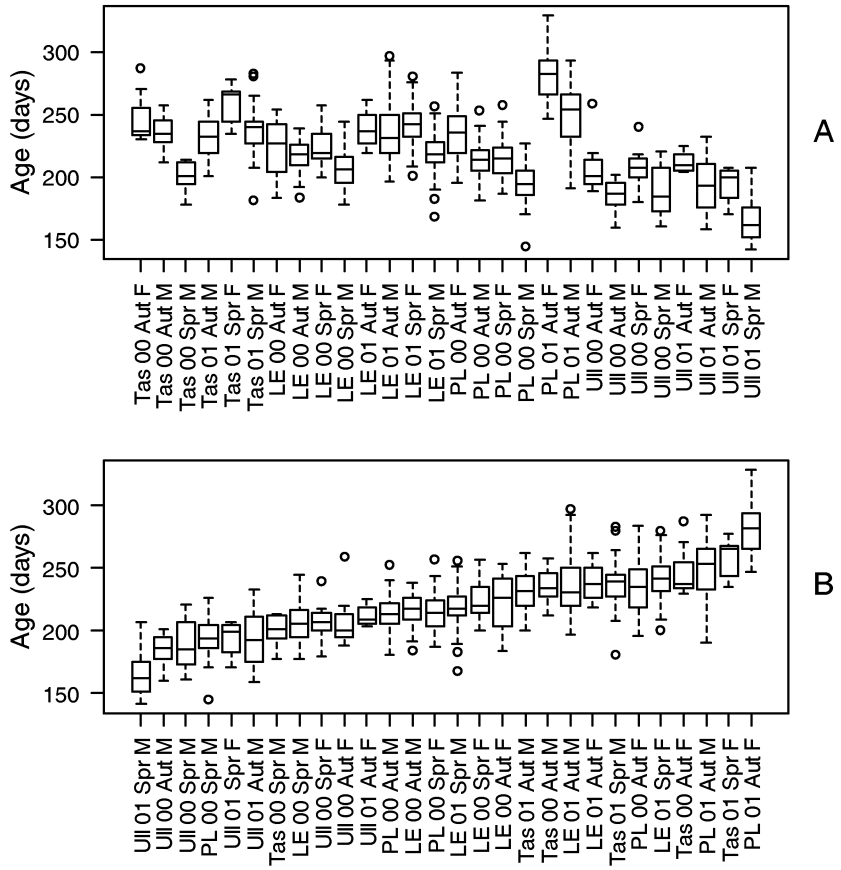


Figure 3.8: The distribution of mature male and female ages according to (A) location and (B) increasing age. F=females, M=males, Ull=Ulladulla, PL= Port Lincoln, LE= Lakes Entrance, Tas=Tasmania, Spr=spring, Aut=autumn, 00= 2000, 01=2001, circles indicate individuals that are outliers.

Average gonad weights of mature individuals

Males

The trend in gonad weights of mature individuals differed between males and females. The three-way full factorial ANOVA of testis weights resulted in a 3-way interaction (location x season x year) (F_{testis}

$_{weight}$ = 24.47, df 2,818, p<0.0001). There were no consistent trends across locations (Fig. 3.9). However, there were two predominant groupings in testis weights; (1) those with lighter testis weights which were indistinguishable from each other (Tasmania 2000 spring, Ulladulla 2000 spring, 2001 spring and autumn, and Port Lincoln 2000 spring and autumn); and (2) those with heavier testis weights also indistinguishable from each other (Port Lincoln autumn 2001, all the Lakes Entrance samples and Tasmania autumn 2000, spring 2001). Ulladulla 2001 spring males had the smallest testis weights and this sample was significantly different from all other samples except for Tasmania spring 2000. Interestingly, Tasmania autumn 2001, had an intermediate testis weight that was significantly different from all other weights except for Tasmania autumn 2000. The difference between the largest median testis weight (7.36 g, Lakes Entrance spring 2001) and the smallest median testis weight (2.25 g, Ulladulla spring 2001) was 227%. The only consistent seasonal trend was that both Tasmania 2000 and Ulladulla 2001 had males with significantly lighter testis weights in spring compared to autumn.

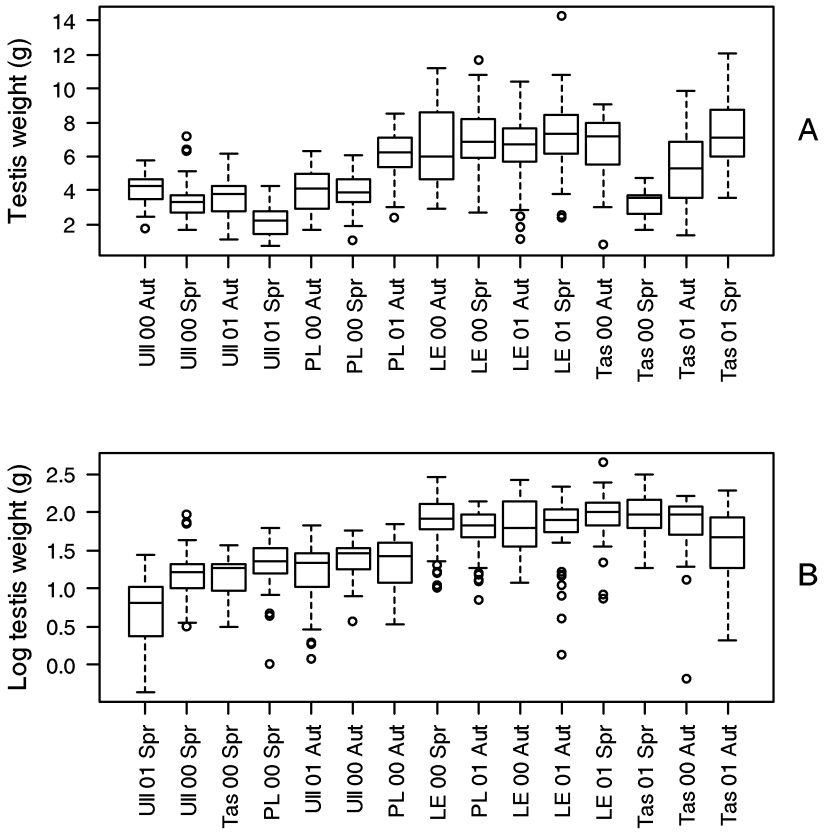


Figure 3.9: The distribution of mature male testis weights according (A) location and the distribution of logged mature male testis weights (B) shown sequentially. Ull=Ulladulla, PL= Port Lincoln, LE= Lakes Entrance, Tas=Tasmania, Spr=spring, Aut=autumn, 00= 2000, 01=2001, circles indicate individuals that are outliers.

Females

Fitting a factorial model to the females only showed evidence of a location x season interaction ($F_{ovary\ weight} = 3.25$, df 3,290, $p<0.05$) which was consistent with the results for body weight. There were no subsets without missing cells to fit a comparative model to. Groupings of the pairwise comparisons were less distinct and revealed predominantly a trend in increasing ovary weight across samples with Ulladulla consistently showing the smallest ovary weights (Fig. 3.10). The only significant seasonal trends in ovary weight were in Port Lincoln 2000 where ovary weight in spring was significantly less than autumn and Lakes Entrance 2000 where the trend was reversed with autumn squid having ovaries that were significantly less than spring squid. The difference between the largest median ovary weight (48.65 g, Lakes Entrance spring 2001) and the smallest median ovary weight (17.53 g, Ulladulla autumn 2000) was 177.5%.

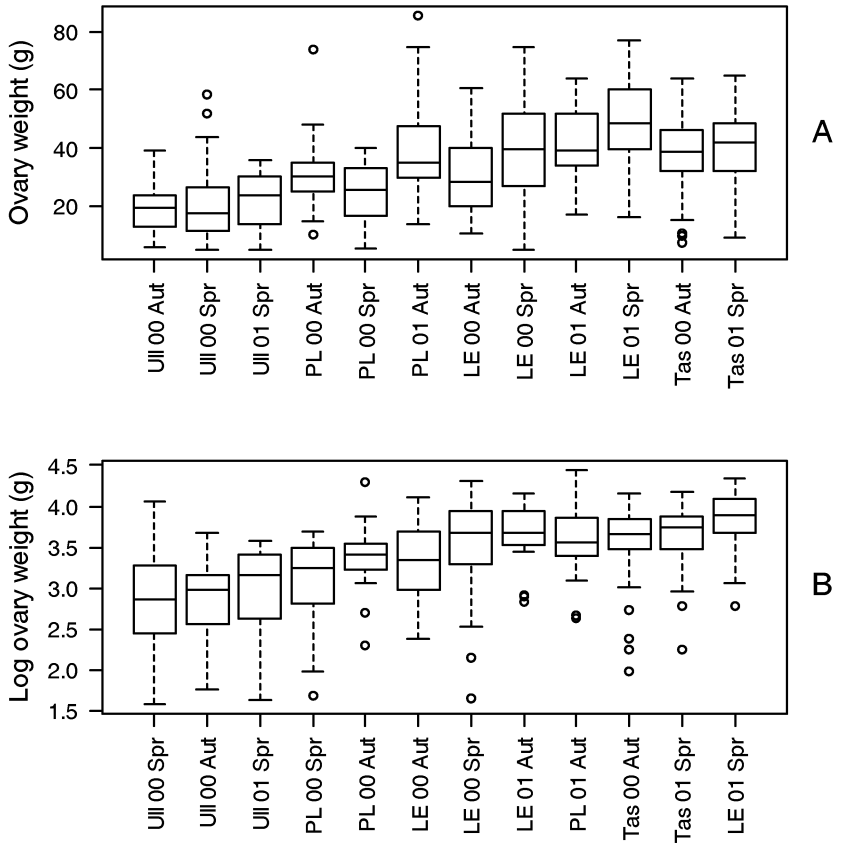


Figure 3.10: The distribution of mature female ovary weights according (A) location and the distribution of logged mature female ovary weights (B) shown sequentially. Ull=Ulladulla, PL= Port Lincoln, LE= Lakes Entrance, Tas=Tasmania, Spr=spring, Aut=autumn, 00= 2000, 01=2001, circles indicate individuals that are outliers.

Trends in maturity stages

Males

There were marked differences in the distribution of maturity stages between sexes and locations (Fig. 3.11). Mature stage 4 and 5 males were generally the most common for Tasmania (except spring 2000 where most were immature), Port Lincoln and Lakes Entrance. While mature males were also predominant in Ulladulla samples, there was generally a greater mix of maturity stages in the Ulladulla samples.

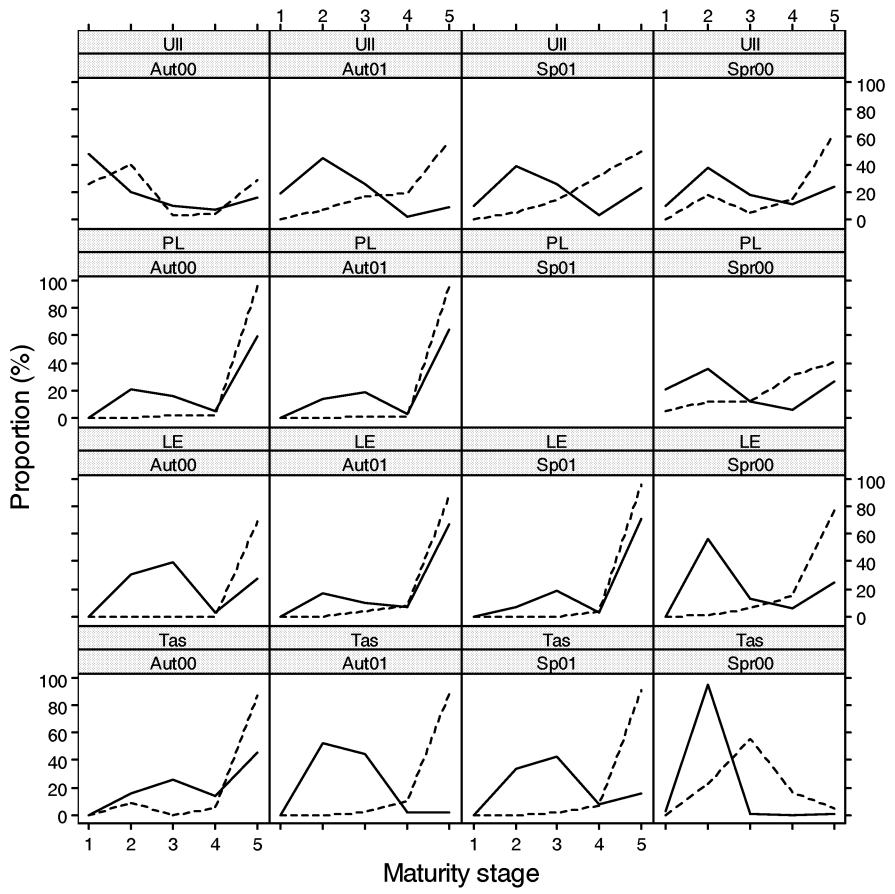


Figure 3.11: The frequency distribution of *Nototodarus gouldi* maturity stages at each of the sites and for each of the seasonal period, females solid line, males dashed line. Ull=Ulladulla, PL= Port Lincoln, LE= Lakes Entrance, Tas=Tasmania, Spr=spring, Aut=autumn, 00= 2000, 01=2001.

Females

The trend in maturity stages was much more variable for females, with a much greater range in maturity stages across sampling sites (Fig. 3.10). Mature females were generally less abundant in all Ulladulla samples and spring 2000 and autumn 2001 in Tasmania. Mature females were more abundant in the autumn samples for Port Lincoln and in both seasons during 2001 for Lakes Entrance.

DISCUSSION

This study revealed just how variable the dynamics of squid populations can be over time and space. We had initially planned a more robust analysis by pooling the seasonal samples over the two years of our study. However, the unexpected difference in the growth dynamics of squid over the two years prevented this and added another layer of complexity to the analysis. What we found was a spectrum of patterns influenced by sites, sexes, seasons and years. While there were some trends, ie., we can state that Ulladulla squid are smaller than Lakes Entrance squid, it is difficult to state what the magnitude of these differences are. There is also a clear lack of consistency. For example, mature Tasmanian male squid are generally large with median weights greater than 400 g, however, in spring 2000 the mature males were considerably smaller (< 300 g) and grouped with the Ulladulla males (Fig. 3.5). The mature males and females from Port Lincoln also show considerable variability in weight depending on the time of capture.

Trends in size

The differences in weight of squid were interesting. Females appear to achieve their larger size by predominantly growing faster than males rather than living longer. Why the marked difference in size for the same species over spatial and temporal scales? Perhaps most dramatic is the small body size of the Ulladulla individuals. These squid of both sexes were consistently the smallest and youngest and generally had the smallest gonads. However, as noted above they seem to sit at the smaller end of the continuum as there were some samples from other locations that overlapped with the Ulladulla squid (eg., low median weights for Port Lincoln and Tasmanian spring 2000 males).

The general trends (small squid in low latitudes and bigger squid in higher latitudes) shown in this study can be best explained in terms of latitudinal differences in temperature. Squid in the cooler water live longer and grow bigger. Such trends have been found in other species sampled over large geographical distances. Pecl (2001) found that individuals of the large Australian loliginid squid *Sepioteuthis australis* were smaller and younger from the low latitude site of Newcastle, New South Wales compared to the higher latitude site of Tasmania. Over even greater distances Jackson & Moltschaniwskyj (2001) found the warm water population of the loliginid *Sepioteuthis lessoniana* in warm tropical Thailand water to be considerably smaller and younger than the sub-tropical population in South Queensland Australia. These two growth strategies were referred to as 'hot' and 'cool' strategies. Interestingly in between these two sample sites, off Townsville North Queensland, Jackson and

Moltschaniwskyj (2002) found that this mid-population alternated between these two growth patterns depending on season, with winter hatched squid displaying the 'cool' growth strategy and summer hatched squid displaying the 'hot' Thailand strategy. Similar differences have also been found between tropical and temperate female populations of the loliginid *Loliolus noctiluca* in Eastern Australia (Jackson & Moltschaniwskyj (2001). While the males of this species also showed dramatic slowing of growth in the cooler location, body size did not increase in the higher latitude population. Similarly, over larger scales, the populations of *Loligo pealeii* of Northeastern North America also show geographical differences in body size although this could be modified depending on season of hatch (Hatfield & Cadrin 2002). Similar trends in cooler bigger slower growing squid vs. warmer smaller faster growing squid has also been observed in *Todarodes sagittatus* populations from the north Atlantic (Rosenberg et al. 1981) compared to tropical Africa (Arkhipkin et al. 1999). Arkhipkin (1996) also found geographical differences in size and growth rates in different populations of *Illex coindetii* in tropical waters off Western Africa. However, it was not possible to relate this to environmental conditions as hatching and juvenile environments were not known.

Age and growth

Trying to establish trends and influences of growth of *N. gouldi* populations in southern Australia was likewise challenging. Some general trends were readily interpretable by known responses of squid to temperature (e.g, Forsythe et al. 2001, Jackson & O'Dor 2001). This is demonstrated by the fact that all squid cohorts hatching out in warmer summer-autumn conditions (at each location) had higher growth rates than cooler winter-spring hatched squid (Table 3.3). However, we were surprised by the consistency for each seasonal cohort for each location that squid hatched in year 1 (1999/2000) often had significantly faster growth rates than the corresponding site/season during year 2 (2000/2001). Furthermore, it was never the other way around, there were never any gender group in year 2 that had faster growth rates than the corresponding group in year 1.

Interestingly, productivity levels as indicated by SSC back-calculated to time of hatching did provide some answers (at least for females). Higher productivity waters at a location, as indicated by the SSC anomaly, resulted in faster winter growth for females at the location, but not summer growth. This may be because temperature is the dominant environmental factor in summer, while at cooler winter temperatures productivity becomes relatively more important. Indeed, the growth anomaly was greater in the waters with highest average SSC (Port Lincoln and Lakes Entrance). The use of such remotely sensed productivity information has immense potential for determining factors of species abundance and recruitment success (Platt et al. 2003).

The productivity analysis was taken from the time of peak squid hatching at each location. It is however, possible that conditions at a time before or after spawning could be more important in determining female growth rates. Alternative critical periods for squid with regard to local productivity and other oceanographic conditions (such as SST) are being investigated in a separate study. These more complex analyses may also help to explain differences in male growth with respect to productivity. Productivity for squid collected at each site was estimated within a 1° box adjacent to that site. The spawning location of the animals is unknown, as is the location of the animals prior to capture. It is possible that the local productivity estimates were not always related to the environment that the squid experienced during their post-hatching/juvenile phase. Seasonal changes in circulation could result in squid populations originating in different regions, or relying on productivity from different regions. Without additional information on the movements of squid, more appropriate areas cannot be selected. It is believed that this species does not move great distances over its life, in contrast to other ommastrephids (e.g., *Illex argentinus*, Waluda et al, 2001, *I. Illecebrosus*, *Todarodes pacificus*, O'Dor 1992). In fact, earlier tagging studies have found *N. gouldi* to not be highly migratory with populations having relatively restricted distributions (Dunning & Förch 1998). Food can have a more important overriding influence than temperature as recently suggested for populations of *Loligo opalescens* off California (Jackson & Domeier 2003) and *Todarodes angolensis* (Villanueva 1992) off Africa, where higher growth rates in squid were attributed to periods of higher productivity rather than higher temperatures.

It is notable that the yearly discrepancies in growth rates were consistent over such a large geographical region and suggests that the squid are possibly responding to broad-scale oceanographic influences. It does appear that the squid are responding as ecosystem indicators as suggested by Jackson & Domeier (2003). The variable growth patterns are probably influenced predominantly by local and seasonal patchiness in the environment as suggested for *Illex illecebrosus* off Nova Scotia (Arkhipkin & Perez 1998).

As typical with other squid populations, the life span of *N. gouldi* in Australian waters appears to not exceed a year. Such short life spans mean that squid can rapidly respond to differing environmental conditions. The growth strategy is also surprisingly similar to the southern Australian teleost blue sprat (*Spratelloides robustus*) that has rapid growth and a short life span (< 300 d) in South Australian waters (Rogers et al. 2003). The age data are similar to what was found for *N. gouldi* and *N. sloanii* in New Zealand waters by Uozumi (1998) although older specimens of *N. gouldi* > 1yr were found in the New Zealand study. *Nototodarus gouldi* appears to live longer than its smaller tropical congener *N. hawaiiensis* that appears to complete its life cycle in < 200 d (Jackson & Wadly 1998).

Our data suggest that *N. gouldi* have protracted spawning with hatching taking place during all seasons of the year. This would contribute to a complex population structure. Year round hatching has also been documented for *N. gouldi* in New Zealand waters (Uozumi 1998).

Small morphs at Ulladulla?

The consistent small size and shorter life spans of the Ulladulla population suggests that environmental conditions are pushing *N. gouldi* to the biological and physiological limits in this region. O'Dor & Lipinski (1998) have suggested that such differences can be caused by either a latitudinal cline in size-at-maturity or genetic isolation leading to distinct growth and maturation patterns regardless of temperature. Jackson & Yeatman (1996) also described a cline in size and age-at maturity in tropical *Photololigo* populations and suggested it might be a combination of environmental, genetic and behavioural factors influencing the cline.

Initial allozyme electrophoresis of the *N. gouldi* population across southern Australia has not found evidence for different species present (Triantafillos et al. submitted). Rather there seems to be considerable genetic mixing across southern Australia. We can't, however, discount genetically distinct regional populations. The fact that Ulladulla-like squid appear other places (eg., Tasmania or Port Lincoln) at certain times suggest that squid populations might be responding rapidly to short term environmental events. Perhaps Ulladulla is self-recruiting and is selecting for 'hot genes' (O'Dor 1998), while Tasmania is maintaining a population of 'cold genes' (or genes selecting for other special characteristics). However, under certain instances, environmental constraints might cause a drastic change in the gene frequencies of the population supporting a population during that season with different gene frequencies than 'normal' for that region. Different morphs have been described for other ommastrephid squid species. Both *Dosidicus gigas* and *Sthenoteuthis oualaniensis* have two size morphs. Anderson & Rodhouse (2001) have suggested an environmental mechanism controlling these morphs, with squid encountering more productive waters growing faster, getting bigger and delaying maturity. We suspect that it is probably a combination of changes in gene frequencies along with environmental influences that shape the structure of *N. gouldi* populations to maintain such marked plasticity in age and growth in southern Australia. Ulladulla squid might be special morphs but flexibility in the population dynamics allow 'Ulladulla-like morphs' to appear at other locations, should the appropriate conditions prevail. The complexity in size and age can be viewed in the light of the Anderson & Rodhouse (2001) hypothesis.

Maturity

Comparing mature individuals across samples provided a means to scale across all locations/times. The weight of mature individuals further emphasised the discrepancy in size among locations. There

were three general trends, (1) large mature individuals at Tasmania and Lakes Entrance, (2) extremely small individuals at Ulladulla and (3) pronounced variability at Port Lincoln. The lack of predictability in size is especially apparent in the weight of mature Tasmanian male squid. Notably, the autumn and spring 2001 samples showed very little variation in median size (around 500 g). However, these contrasted markedly with the 2000 spring sample where individuals were predominantly < 300 g, noticeably younger and grouped with the Ulladulla males. This outlying group goes against general ecological trends of size and age with latitude as discussed above. Our highest latitude squid from Tasmania can alter their life history characteristics to such an extent that they mirror the life history of much lower latitude congeners.

The variation in both weight and age of mature Port Lincoln squid of both sexes is also intriguing. The weight and age of mature individuals at Port Lincoln was variable and inconsistent over time. This suggests that perhaps squid off Port Lincoln may be subject to a more variable environment compared to the other sites.

Conclusion

N. gouldi shows extreme plasticity in growth, body size and reproductive tactics. While there are some general trends apparent, it is the variability in many of the parameters that stand out. *N. gouldi* appear to adapt rapidly to varying environmental conditions and is a successful and abundant species of the southern Australian continental shelf ecosystem. The life style of *N. gouldi* and its success in this ecosystem reflects the adaptability of this species. The extreme phenotypic variability is a prime example of the ultimate law of biology - "whatever works works" (O'Dor 1998). The fact that this species appears to be so adaptable suggests that they probably will act as an ideal ecosystem indicator and productivity integrator as has been shown for *Loligo opalescens* (Jackson & Domeier 2003). Despite the comprehensive site, season and location sampling strategy in this study, a longer time series is probably necessary to interpret the patterns in growth and reproduction in *N. gouldi*. Further analyses incorporating both SST and SSC during different periods of the life cycle may also help to explain growth variation as a response to biological and oceanographic conditions experienced by individuals. More regular samples within a location would also reveal life style changes in this species at a greater resolution.

CHAPTER FOUR

PLASTICITY IN THE REPRODUCTIVE STRATEGIES OF *NOTOTODARUS GOULDI* (CEPHALOPODA: OMMASTREPHIDAE) FROM SOUTHEASTERN AUSTRALIAN WATERS.

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Adapted from: Marine Ecology Progress Series submitted

INTRODUCTION

Recent studies examining the reproductive biology of squid, have largely dismissed the established idea of semelparity and synchronous spawning for all species (Packard 1972, Calow 1987), and instead, have embraced the notion of flexibility or plasticity in patterns of sexual maturation and spawning within the coleoid taxa (Harman et al. 1989, Moltschaniwskyj 1995, Rocha and Guerra 1996, Melo & Sauer 1999). Given the variation evident in the life-styles of different squid species, and the highly variable and unpredictable environments in which they live, a high degree of inter-specific plasticity in reproductive strategies between species is expected (see Rocha et al. 2001). However, individuals from a single species will also encounter highly variable environmental conditions on both spatial and temporal scales. This is because many species occupy broad distributions, and have relatively short life-spans and rapid growth. Therefore, as cohorts rarely experience the same conditions as their parents, or in the case of some small tropical species, their grandparents (e.g. *Idiosepius pygmaeus,* Jackson 1989), the likelihood of all cohorts exhibiting the same reproductive strategy across their entire distribution, is small if the species is to persist (Stearns 1989, Boyle & Boletzky 1996, McNamara & Houston 1996). Thus, it is likely that individuals from the same species will exhibit a high degree of phenotypic variation depending on the environmental conditions encountered.

There are many examples of such intra-specific variation (diversified risk-spreading) in squid populations, particularly in relation to growth patterns among different cohorts. Commonly these variations in growth have been attributed to changes in temperature (Forsythe 1993, Jackson et al. 1997, Hatfield 2000), feeding regime (Forsythe & Van Heukelem 1987, O'Dor 1998, Jackson & Moltschaniwskyj 2001) and light (Mangold 1983). What is less understood is how flexible reproductive strategies are in response to natural environmental fluctuations. Such changes in reproductive

strategies are of great importance when trying to understand population dynamics (Boyle & Boletzky 1996), and the life-history characteristics of a species (Stearns 1989), particularly in relation to how much of an individual's life time is spent depositing eggs (Pecl 2001, Rocha et al. 2001). There is increasing evidence of intra-specific flexibility in reproductive traits relating to variations in size and age-at-maturity (Arkhipkin at al. 2000, Jackson & Moltschaniwskyj 2001, Tafur et al 2001) and resource allocation (Gonzalez & Guerra 1996, Markaida & Sosa-Nishizaki 2001, Pecl 2001, McGrath-Steer & Jackson in press). Furthermore, it is possible that inconsistencies in the literature regarding the type of spawning mode a species adopts, particularly those with broad distributions, are in fact examples of phenotypic variation (e.g. *Illex argentinus*, Rodhouse & Hatfield 1990, Hatfield et al. 1992, Laptikovsky & Nigmatullin 1993).

The ommastrephid squid *Nototodarus gouldi* McCoy 1888, inhabits Australian waters on the east and west coasts from 27°S, and extends across the southern perimeter of the continent. *N. gouldi* is a continental shelf species, and are typically found in depths between 50 and 200 m undertaking diurnal migrations to feed from shallower waters at night. Adult squid range in size up to 400 mm mantle length, and are sexually dimorphic, with females attaining larger sizes than males (Wadley & Dunning 1998). In the southeastern waters off Victoria, females have been found to mature at 30cm ML, whereas in eastern waters off New South Wales, females matured at a smaller size of 22cm (Winstanley et al. 1983), indicating a degree of plasticity in size-at–maturity within the Australian population. Unlike many other commercially exploited ommastrephid squid (e.g. *Illex argentinus* and *Todarodes pacificus*), limited tagging studies have indicated *N. gouldi* does not make large scale migrations (Dunning 1998) and does not seem to be associated with large current systems as are many other ommastrephids (O'Dor 1998). In addition, recent allozyme electrophoresis investigations have shown *N. gouldi* from Australian waters constitutes only the one species of *Nototodarus* (Triantafillos et al. in press), unlike in New Zealand, where both *N. gouldi* and *N. slonii* Grey 1849, are present (Smith et al. 1987). The Australian fishery for *N. gouldi* is currently underexploited, with squid caught incidentally by trawling all year, and intermittently targeted by a jig fishery at specific locations when biomass is sufficient. A recent study by McGrath & Jackson (2002) on the reproductive strategy of *N. gouldi*, caught from its most southern Australian distribution, suggests *N. gouldi* is capable of releasing small batches of eggs over a protracted season, with little evidence of gonad development occurring at the expense of the soma.

The aim of this study was to assess the spatial and temporal variation in the reproductive strategy of *Nototodarus gouldi*. The variability in the reproductive strategies of *N. gouldi* was determined by examining patterns in repro-somatic investment and oocyte storage of squid, across four locations,

over two years and seasons around Australia. Repro-somatic investment, particularly differences in the energy divided between the somatic (mantle and fin) and gonad tissues, relative to maturation and life-time growth rate, were examined to identify trade-offs and variations in resource allocation. In addition, patterns of oocyte storage were examined to help clarify what type of spawning mode was adopted as a function of the variations in energy allocation observed.

MATERIALS AND METHODS

Specimen collection

Individuals were collected from commercial jigging and trawling vessels operating from Ulladulla (35°28S,150°56E), New South Wales; Port Lincoln (33°21S,130°46E), South Australia; Lakes Entrance (35°20S,148°56E), Victoria, and the east coast of Tasmania (43°35S,147°35E), during the austral autumn and spring periods of 2000 and 2001 (Fig 4.1A). These ports were chosen as they represented the broadest distribution of *N. gouldi* from which repetitive samples could be obtained. Mean monthly SST (°C) was also obtained for each of the ports over the study period, using data sourced from the NOAA-CIRES Climate Diagnostic Centre (available at www.cdc.noaa.gov) (Reynolds et al. 2002).

Freshly caught whole specimens were frozen after capture and shipped to Tasmania for processing. After thawing, the total body weight (BW), dorsal mantle length (ML) and maturity stage of each individual was recorded (using Lipinski's Universal scale modified for this species) (Juanicó 1983). The gonad and accessory reproductive organs were weighed, and a repro-somatic index (RSI) was calculated for each individual as:

$$RSI=(\text{Total reproductive weight}/ (\text{total body weight}-\text{total reproductive weight})) \times 100$$

Where total reproductive weight is the combined weight of the ovary + oviducts + oviducal glands + nidamental glands for females, and the combined weight of the testis + spermatophoric complex in males (after Pecl 2001).

Statoliths from all the animals were removed, and a representative sub-sample selected for age estimates from across the size range of individuals. Statoliths were mounted in the thermoplastic cement Crystal Bond on a microscope slide and ground dry on both the anterior and posterior plane to produce a thin section using 30 μm lapping film, and polished using a 5.0 μm lapping film. Total increment counts were taken using a Nikon Eclipse E400 high power microscope (400x) using polarized light. The mean of two counts that varied less than 10% of the mean, was taken as the age estimate in days (after Jackson & Moltschaniwskyj 2001). Generally, however, most counts were very close (within 5%). Mantle and fin weights were also measured and as no spent (stage 6) animals have been recorded for this species previously, special attention was made to thoroughly examine each

animal internally for any sign of deterioration of the muscle or reproductive organs, and externally for body lesions.

Statistical analyses

Initially, the sampling protocol was developed to allow for pooling of the years as replicates, however, as there was such considerable variation in the reproductive parameters measured, unfortunately samples could not be combined. As a result, samples were treated separately for each analysis.

Energy allocation

To assess if any trade-offs occurred between somatic and reproductive processes, length-weight geometric regressions (type II) were performed on log transformed data, for each combination of location and season, for females and males separately (after Green 2001). Regression equations calculated for each location and season combination were: (1) ML – gonad weight and (2) ML – somatic weight (combined weight of the mantle and fin). The residual value gives an indication of how far an individual lies from its predicted weight according to the regression equation. It is assumed in this study that animals with heavier tissues for their ML, and thus higher residual values, have tissues in better condition than animals with lower residuals. The ML - gonad residuals were then correlated with the ML - somatic residuals from the same sample, to identify if gonad development was occurring at a cost to somatic condition (McGrath & Jackson 2002).

Gonad investment and somatic condition

To compare levels of gonad investment and somatic condition of mature animals (stages 4 and 5) among samples, a second set of geometric regressions were calculated for each sex. Differences in the mean residual value among samples, for both males and females were detected using 1-way ANOVA's and Tukey's HSD post hoc test (after Moltschaniwskyj & Semmens 2000). In this way, only the comparisons of interest were examined, for example; (1) temporal variations on a seasonal and annual scale within locations, and (2) spatial variations due to location. This statistical procedure highlighted the comparisons of interest, while allowing each sample to only be analysed twice, thus alleviating the problems of increased type I error rates when performing multiple comparisons.

Mature females and males hold no record of their reproductive past (Moltschaniwskyj & Semmens 2000), therefore the levels of relative gonad investment estimated in this study, are simply an indication of reproductive status at the time of capture. They do not represent the total commitment of each individual, and cannot therefore be used to infer total life-time investment in reproduction.

Life-time growth rate

To determine if life-time growth rate was related to the level of somatic or gonad development in mature animals, a third geometric regression was calculated for the age - BW relationship, again log transforming the data for linearity. As there was not an overlap in sizes of mature animals from varying locations, a regression analysis gave a size-adjusted age for each animal (in the form of a residual), which was then correlated separately against both the ML - somatic and the ML - gonad residuals that were derived for the mature animals. From these correlations we were able to ascertain if animals with positive or negative size-at-age residuals (i.e. faster or slower growing animals) were correlated with an increase or decrease in either somatic condition or gonad investment.

Patterns of oocyte storage

To determine if ovulated oocytes were being held for release in large or small batches, oviduct fullness was calculated for all stage 5 females according to Harman et al (1989), using oviduct weight, not volume (after Pecl 2001). The variation in mean fullness for each sampling period was analysed using a 2-way ANOVA and Tukey's HSD post hoc test. Oviduct fullness was then correlated with female ML for females from each combination of location and season, to determine if larger females had fuller oviducts (after Harman et al 1989). To ascertain if ovarian oocytes were being depleted as ovulated oocytes moved into the oviducts, a ovary to oviduct ratio was calculated, with values > 1.0 indicating a female with an oviduct weight greater than an ovary weight. To assess if females in better somatic condition produced larger batches of ovulated oocytes for spawning, partial correlations were performed using somatic weight and oviduct weight controlling for BW, as both the soma and the oviduct are a function of reproductive status and individual mass (see Moltschaniwskyj & Semmens 2000). Evidence of a previous spawning event in stage 5 females, has been observed in *N. gouldi* (McGrath & Jackson 2002) and *Sepioteuthis australis* (Pecl 2001), due to the presence of stretched empty oviducts, which were distinct from the thin unused oviducts seen in stage 4 animals. Thus, to determine whether females had previously spawned, the number of females with stretched empty oviducts in each sample was also recorded. All figures following the ± symbol refer to standard error.

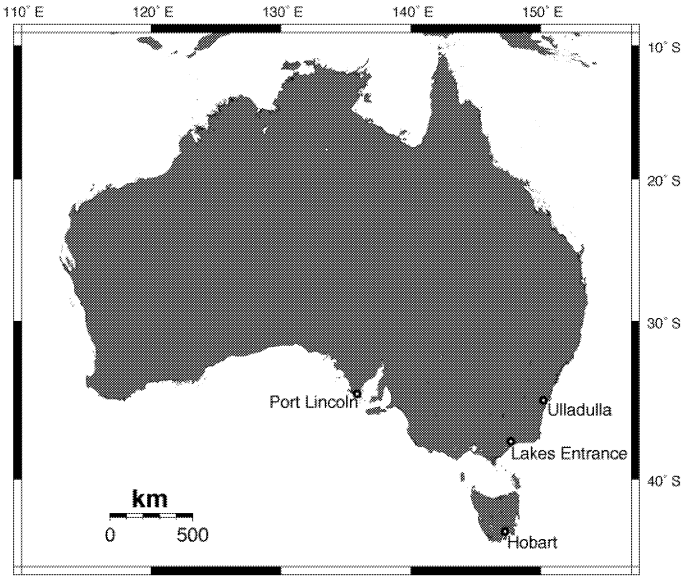
RESULTS

For this study a total of 2064 *Nototodarus gouldi* were collected and dissected (n_{2000} = 1006 and n_{2001} = 1058), comprising 988 females, and 1076 males in total. Unfortunately, a second spring sample was not obtained from Port Lincoln during 2001, because the vessels involved in the fishery moved away from the sampling area into deeper water where few squid were caught. In addition, low numbers of mature females were caught in Tasmanian waters during spring 2000 and autumn 2001. This restricted the comparisons which could be made between groups of individuals, both spatially and temporally. Females ranged from 97 to 393mm ML and from 20 to 1655g in weight, while males varied from 67 to 323mm ML and between 8 and 1057g in weight, indicating that for this species like most

ommastrephid squid, females grow considerably larger than males (Table 4.1). Interestingly, no spent (stage 6) females were recorded. *N. gouldi* from Australian waters were found to have a maximum age of 329 days ($females_{max}$ = 329 days, $males_{max}$ = 297 days) (Table 4.1).

Mean monthly SST (°C) over the period of sampling indicated a decrease in temperature occurred with an increase in latitude (Fig 4.1B). The low latitude sites of Ulladulla and Port Lincoln were influenced by warmer temperatures than Lakes Entrance, which in turn had warmer temperatures than Tasmania. During the austral-summer months both Ulladulla and Port Lincoln had comparable maximum temperatures, however, during the austral-winter months, Ulladulla continually decreased to mean monthly minimums of between 1.5° and 1° below that detected for Port Lincoln, indicating a greater temperature fluctuation at Ulladulla in comparison to Port Lincoln (Fig 4.1B). There was no overlap in SST for either Lakes Entrance or Tasmania (Fig 4.1B).

A



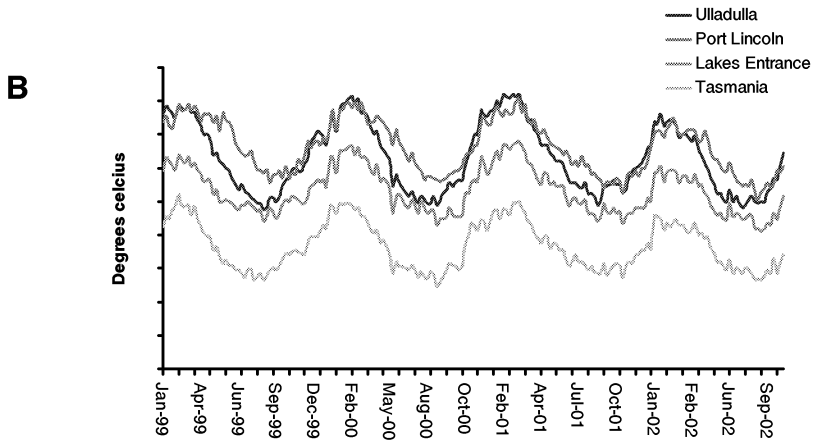


Figure 4.1. (A) Map detailing the sampling sites for *Nototodarus gouldi* and (B) the mean monthly sea surface temperature for each sampling site.

Energy allocation

There was no relationship between ML - gonad and ML - somatic residual pairs, for any females regardless of season or location, suggesting changes in female gonad development was not associated with changes in somatic condition (Table 4.2). The majority of mature males showed significant positive correlations between somatic condition and gonad investment, however, males caught from Tasmania during spring 2000 ($r = -0.051$ $p = 0.841$ $n = 17$) and Lakes Entrance during autumn 2001 ($r = 0.246$ $p = 0.085$ $n = 50$), showed no relationship between the level of somatic condition and gonad investment (Table 4.3). This indicates that in general, male gonad development was associated with an increase in somatic condition.

Table 4.1. *Nototodarus gouldi*. Summary of size and estimated age information for each sex, collected from Australian waters during 2000 and 2001

			Ulladulla				Port Lincoln				Lakes Entrance				Tasmania			
			2000		2001		2000		2001		2000		2001		2000		2001	
Parameter	Sex		Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
ML (mm)	females	mean	170.2	184.3	208.8	183.5	260.7	201.5	293.1	na	275.5	250.4	291.7	308.1	313.9	197.8	289.8	279.1
		±SE	±5.0	±3.4	±5.4	±7.0	±5.1	±4.3	±4.1		±4.2	±4.5	±7.4	±6.9	±3.5	±2.2	±3.4	±3.6
		min	103	97	125	125	186	100	215	na	200	169	226	205	208	161	234	205
		max	262	277	271	271	317	305	393	na	363	367	358	388	355	295	364	360
		n	99	110	41	31	43	86	71	na	58	84	27	39	69	84	70	75
	males	mean	152.8	174.7	205.2	167.8	229.3	186.7	252.1	na	256.7	236.7	257	261	262.8	190.9	267.2	255.1
		±SE	±4.3	±4.0	±4.0	±3.8	±2.8	±2.8	±1.7		±3.2	±2.3	±3.0	±2.5	±7.7	±1.7	±2.7	±2.4
		min	67	104	140	120	170	108	183	na	212	161	206	195	154	151	210	203
		max	237	282	246	203	258	234	282	na	321	308	298	323	289	227	323	292
		n	99	78	41	22	53	84	97	na	68	152	51	85	24	80	75	67
BW (g)	females	mean	161.8	186.6	252.9	187.9	506.1	247.8	725.5	na	589.6	484.8	712	824.5	823.9	204.4	667.1	679.8
		±SE	±13.9	±10.0	±19.0	±23.0	±26.8	±17.9	±32.6		±27.5	±28.2	±55.7	±50.8	±26.6	±8.3	±26.3	±26.4
		min	25	22	56	50	162	20	24	na	207	143	620	190	200	106	343	269
		max	513	613	584	494	851	776	1655	na	1112	1268	1375	1423	1192	686	1250	1279
		n	99	110	41	31	43	86	71	na	58	84	27	39	69	84	70	75
	males	mean	122.6	173	246.9	135.6	369.2	199.5	494.2	na	505.7	440.3	530.4	544	523.2	185.4	578.7	542.9
		±SE	±11.5	±13.7	±15.5	±10.8	±14.7	±9.6	±11.2		±19.6	±13.3	±19.9	±16.7	±38.4	±5.4	±20.3	±16.3
		min	8	23	50	40	136	27	154	na	264	94	219	166	96	89	207	225
		max	372	693	481	269	572	398	730	na	868	803	869	919	728	293	1057	820
		n	99	78	41	22	53	84	97	na	68	152	51	85	24	80	75	67
Age (days)	females	mean	178.5	188.6	193.6	166.7	229.7	196.6	257.3	na	220.6	211.3	227.7	234.9	240.7	199.8	229.6	243.8
		±SE	±4.3	±4.2	±4.1	±3.8	±4.3	±3.6	±5.8		±3.5	±2.8	±3.8	±3.4	±3.0	±2.3	±3.0	±3.4
		min	127	122	137	134	190	151	189	na	179	181	195	198	201	180	193	216
		max	221	217	225	207	284	257	329	na	254	257	262	280	287	228	262	278
		n	44	79	30	31	31	38	35	na	35	34	25	33	34	29	31	34
	males	mean	151.1	174.7	183.3	160.8	213.8	185.8	246.9	na	215.7	203.1	236.5	218.8	233	194.5	230.9	235.6
		±SE	±5.3	±4.5	±4.6	±3.7	±3.0		±5.0		±2.4	±3.1	±4.4	±3.3	±3.9	±2.1	±3.0	±2.9
		min	97	116	148	138	178	135	185	na	184	162	197	168	184	171	201	208
		max	202	221	233	207	253	227	293	na	239	245	297	256	258	218	262	265
		n	32	35	31	22	32	36	31	na	31	152	32	32	19	30	32	28

Gonad investment

Female gonad investment showed little variation in response to date of capture, with only the females caught from Port Lincoln in spring 2000, having a higher level of gonad investment compared with females caught in autumn of 2001 ($df = 2,89$ $F = 1.150$, $p = 0.014$). In comparison, spatial differences in the level of gonad investment were detected between females caught in the same season, particularly between the low and high latitude sites (Fig 4.2). For instance, female squid from both the higher latitude sites of Tasmania and Lakes Entrance showed lower levels of gonad investment, than the lower latitude site of Ulladulla (Fig 4.2). In addition, Tasmanian females also showed less gonad investment than autumn caught females from Port Lincoln during 2000 (autumn 2000 $df = 3,100$ $F = 8.388$, $p < 0.000$; spring 2000 $df = 2,77$ $F = 7.272$, $p = 0.001$; spring 2001 $df = 2,50$ $F = 7.005$, $p = 0.002$) (Fig 4.2). There were no differences detected between females from Lakes Entrance and Port Lincoln, or between females caught from Port Lincoln and Ulladulla.

Average RSI for mature females varied between 8.63% (± 0.56) for Tasmania females and 15.38% (± 1.23) for Lakes Entrance females (Table 4.2). While some females from other locations had an RSI of 25% or above, the highest RSI for any female from Tasmania was only 19.70%, which was substantially lower than females from Ulladulla (max. 25.31%), Port Lincoln (max. 27.19%), or Lakes Entrance (max. 26.91%) (Fig 4.3).

Table 4.2. *Nototodarus gouldi*. Reproductive parameter relationships for mature females collected from Australian waters during 2000 and 2001. * Denotes significance at $\alpha = 0.05$.

Year	Season	Location	ML-gonad residual & ML-somatic residual correlation			Oviduct fullness & ML correlation			Oviduct & ML-somatic partial correlation			Oviduct wt > ovary wt	Stretched oviducts	RSI (%)
			r	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*	%	%	mean
2000	Autumn	Ulladulla	-0.277	0.212	22	0.037	0.895	15	-0.085	0.773	12	5	14	10.48±1.10
		Port Lincoln	0.285	0.176	24	-0.062	0.784	22	-0.573	0.007*	19	14	24	13.56±1.26
		Lakes Entrance	0.092	0.726	17	0.006	0.983	15	0.456	0.101	12	0	7	11.36±1.24
		Tasmania	0.009	0.957	41	0.015	0.935	32	-0.406	0.024*	29	3	18.7	8.63±0.56
	Spring	Ulladulla	-0.276	0.115	34	0.688	0.001*	21	-0.527	0.017*	18	0	8	13.62±1.05
		Port Lincoln	-0.033	0.886	21	0.193	0.475	16	-0.523	0.045*	13	13	23.5	12.25±1.25
		Lakes Entrance	-0.074	0.726	25	0.429	0.059	20	-0.235	0.333	17	15	10	14.46±1.30
		Tasmania	na	na	na	na	na	na	na	na	na	na	na	na
2001	Autumn	Ulladulla	na	na	na	0.873	0.324	3	na	na	na	9	13	11.34±3.27
		Port Lincoln	-0.079	0.597	47	0.396	0.006*	47	-0.106	0.486	43	7	15.2	13.21±0.63
		Lakes Entrance	-0.157	0.521	19	0.581	0.011*	19	-0.517	0.034*	15	17	11	15.38±1.23
		Tasmania	na	na	na	na	na	na	na	na	na	na	na	na
	Spring	Ulladulla	-0.049	0.908	8	0.093	0.843	7	-0.855	0.030*	4	9	6	13.31±1.71
		Port Lincoln	na	na	na	na	na	na	na	na	na	na	na	na
		Lakes Entrance	-0.085	0.675	28	0.502	0.008*	27	-0.165	0.042*	24	11	7	15.00±0.80
		Tasmania	0.116	0.657	17	0.529	0.094	11	-0.609	0.062	8	0	0	8.79±1.04

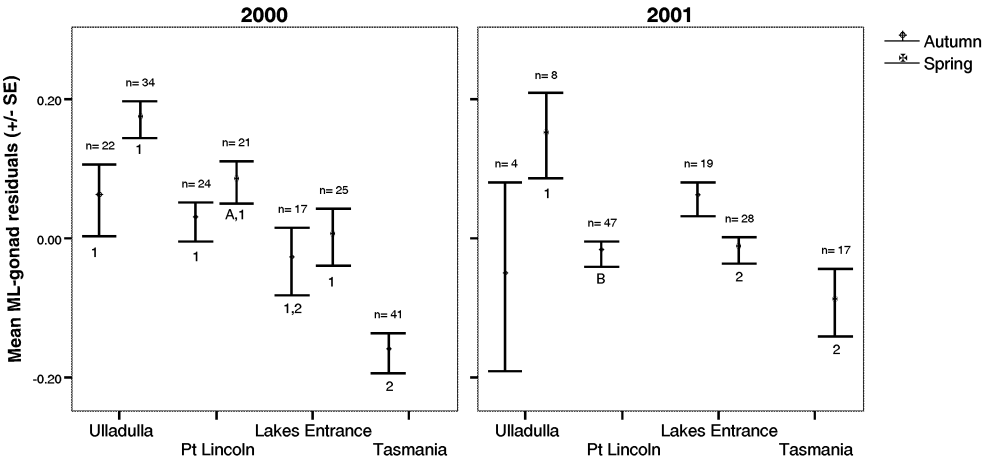


Figure 4.2. *Nototodarus gouldi*. The average residuals from the ML-gonad weight regression for mature females, for each location and season combination. Letters and numbers correspond to significant comparisons of means as identified by Tukey's HSD post hoc test. Letters represent differences in means from seasonal comparisons within location. Numbers represent differences in means from simultaneous comparisons between locations. n= sample size.

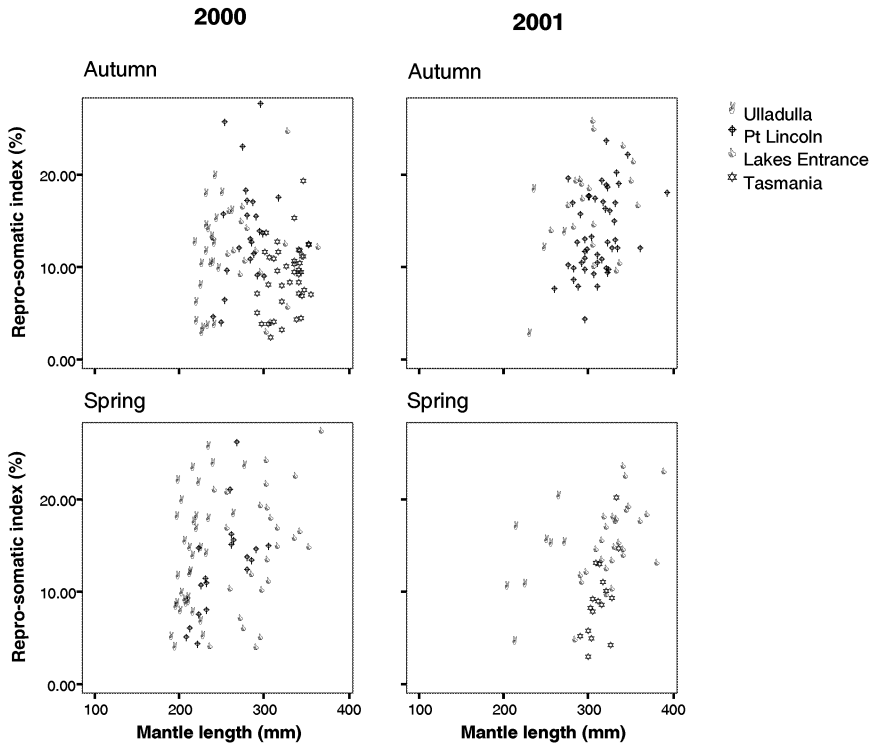


Figure 4.3. *Nototodarus gouldi*. Variation in mature female RSI with ML for spring and autumn 2000 and 2001.

In contrast to the trends observed for females, the levels of male gonad investment varied substantially both temporally and spatially. In all cases where a significant difference was found, spring caught males had heavier gonads for their size when compared with autumn caught males (Ulladulla df = 3,138 F = 14.312 p < 0.001; Port Lincoln df = 2,201 F = 41.422 p < 0.001; Lakes Entrance df = 3,341 F = 39.692 p < 0.001; Tasmania df = 3,174 F = 61.052, p < 0.001) (Fig 4.4). Like the females, males also varied the level of gonad investment spatially, with squid from the higher latitude site of Tasmania having lower levels of gonad investment than males from Ulladulla and Lakes Entrance, for all seasons of capture except spring 2001 (autumn 2000 df = 3,170 F = 19.536 p < 0.001; spring 2000 df = 3,272 F = 5.966 p = 0.001; autumn 2001 df = 3,246 F = 39.701 p < 0.001) (Fig 4.4). Mature male squid had RSI's varying between a maximum of 7.06% for Ulladulla males and a minimum of 0.59% for Tasmanian males (Fig 4.5), with mean RSI's varying between 2.01% (± 0.09) and 3.52 % (± 0.12) for all locations (Table 4.3).

Table 4.3. *Nototodarus gouldi.* Reproductive parameter relationships of mature males caught from Australian waters during 2000 and 2001. * Denotes significance at $\alpha = 0.05$.

Year	Season	Location	ML-somatic residual & ML-gonad residual correlation			RSI (%)
			r	*p*	*n*	mean
2000	Autumn	Ulladulla	0.420	0.019*	31	2.90±0.15
		Port Lincoln	0.664	0.000*	31	2.79±0.10
		Lakes Entrance	0.531	0.000*	68	3.17±0.80
		Tasmania	0.420	0.019*	22	3.17±0.08
	Spring	Ulladulla	0.552	0.000*	60	2.45±0.19
		Port Lincoln	0.491	0.000*	57	3.13±0.11
		Lakes Entrance	0.435	0.000*	142	3.51±0.06
		Tasmania	-0.051	0.841	17	3.42±0.07
2001	Autumn	Ulladulla	0.564	0.001*	32	2.01±0.09
		Port Lincoln	0.476	0.000*	94	3.09±0.07
		Lakes Entrance	0.246	0.085	50	2.96±0.12
		Tasmania	0.499	0.000*	73	2.91±0.12
	Spring	Ulladulla	0.493	0.038*	18	3.19±0.10
		Port Lincoln	na	na	na	na
		Lakes Entrance	0.511	0.000*	85	3.31±0.06
		Tasmania	0.377	0.002*	66	3.31±0.06

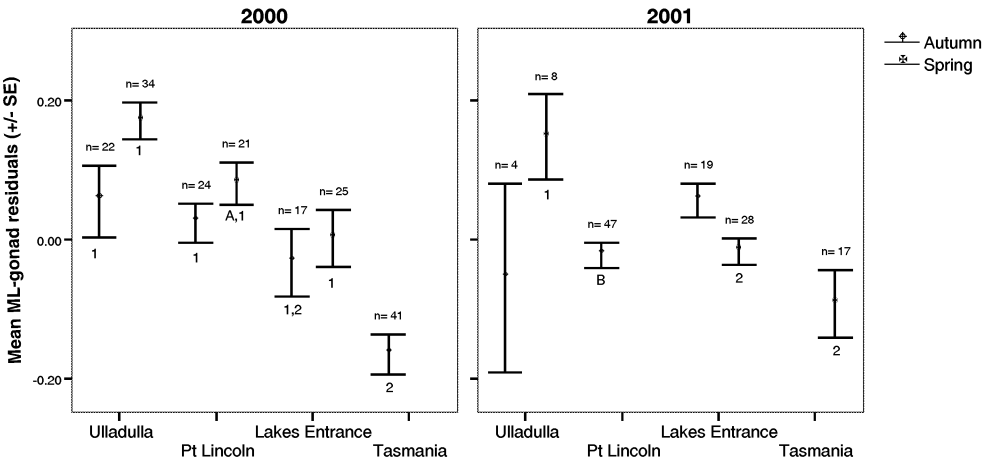


Figure 4.4. *Nototodarus gouldi.* The average residuals from the ML-gonad weight regression for mature males, for each location and season combination. Letters and numbers correspond to significant comparisons of means as identified by Tukey's HSD post hoc test. Letters represent differences in means from seasonal comparisons within each location. Numbers represent differences in means from simultaneous comparisons between locations. n= sample size.

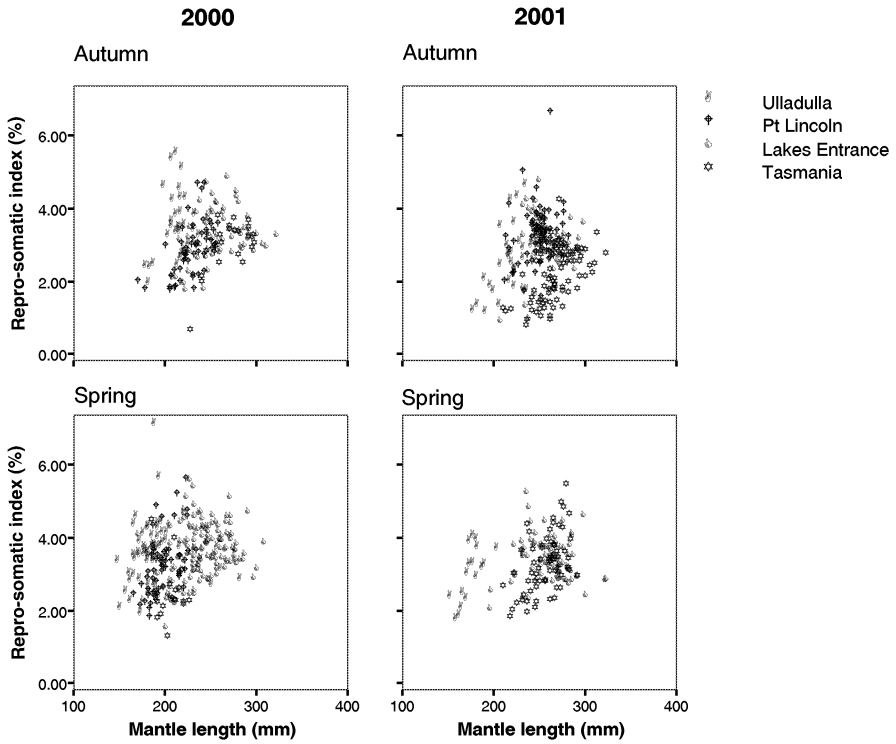


Figure 4.5. *Nototodarus gouldi.* Variation in mature male RSI with ML for spring and autumn 2000 and 2001.

Somatic condition

Temporal variation in the somatic condition of females was only found in squid caught from Port Lincoln, with autumn 2000-caught animals, having better somatic condition than autumn 2001-caught animals ($df = 2,93$ $F = 4.266$ $p = 0.017$) (Fig 4.6A). In contrast, the somatic condition of females differed erratically over geographical scales. Autumn caught females from Tasmania and Ulladulla had a poorer levels of somatic condition than females from Port Lincoln during 2000 ($df = 3,103$ $F = 11.724$ $p < 0.001$); however, females from Tasmania caught in spring 2001, had better somatic condition in comparison to females from both Lakes Entrance and Ulladulla ($df = 2,50$ $F = 12.901$ $p < 0.001$). Likewise, females caught from Lakes Entrance during spring 2000 had better somatic condition than their counterparts from Ulladulla ($df = 2,78$ $F = 4.348$ $p = 0.016$), although during autumn 2001 this trend changed and females from Lakes Entrance had poorer somatic condition than females from both Ulladulla and Port Lincoln ($df = 2,67$ $F = 3.604$ $p = 0.03$) (Fig 4.6A).

Mature male somatic condition showed significant variation in response to both season and location, however, like the females, the variation detected did not produce a clear trend on either a temporal or spatial scale. Seasonal variation in somatic condition was not observed in males from the lower latitude sites of Port Lincoln or Ulladulla, although males from Port Lincoln did show some annual variation with individuals caught during autumn having better somatic condition during 2000 in comparison to 2001 ($df = 2,201$ $F = 25.512$ $p = 0.005$) (Fig 4.6B). On the other hand, males caught from both the higher latitude sites of Lakes Entrance and Tasmania showed marked seasonal variation. Significant increases in somatic condition were observed in spring caught males compared with autumn caught males from (1) Lakes Entrance between spring 2000 and autumn 2001 ($df = 3, 341$ $F = 25.512$ $p < 0.001$) and (2) Tasmania during 2001 ($df = 3,174$ $F = 38.742$ $p < 0.001$). Contrasting with the results obtained from Lakes Entrance between the sampling periods of spring 2000 and autumn 2001, males caught at the same time from Tasmanian waters showed better somatic condition in autumn compared with spring (Fig 4.6B). In terms of geographical differences, again the variation in somatic condition did not follow a set pattern. For both sample periods during 2000, males from Tasmania had significantly poorer somatic condition than all the other sites ($df = 3$, $F = 23.403$, $p < 0.001$) with males from Port Lincoln having the best somatic condition during autumn ($df = 3$, $F = 33.842$, $p < 0.001$). Interestingly, during autumn 2001, again males caught from Port Lincoln appeared to have better somatic condition than all other individuals ($df = 3$, $F = 3.688$, $p = 0.013$), however, during spring of the same year, males caught from Tasmania showed better somatic condition than those from both Port Lincoln and Lakes Entrance ($df = 2$, $F = 22.691$, $p < 0.001$).

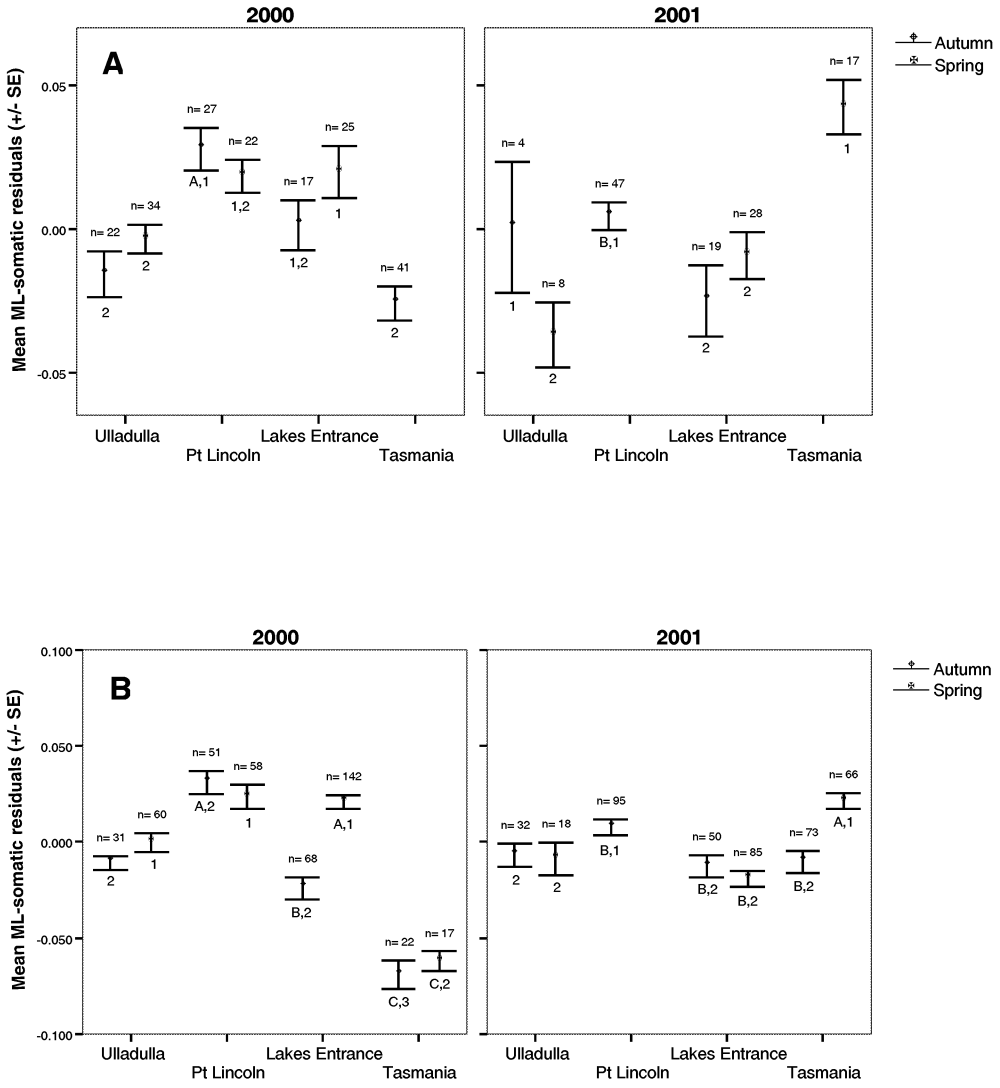


Figure 4.6. *Nototodarus gouldi*. The average residuals from the ML-somatic weight regression for mature (A) females and (B) males, for each location and season combination. Letters and numbers correspond to significant comparisons of means as identified by Tukey's HSD post hoc test. Letters represent differences in means from seasonal comparisons within each location. Numbers represent differences in means from simultaneous comparisons between locations. n= sample size.

Life-time growth rate

Female life-time growth rate was not associated with either increases or decreases in somatic condition or gonad investment (Table 4.4). In comparison, faster life-time growth of mature males showed a positive association with somatic condition in males caught from Port Lincoln (during spring 2000), Lakes Entrance (during autumn 2001), and Tasmania (during autumn 2000 and autumn 2001). An increase in mature male life-time growth rate was associated with an increase in gonad investment for (1) Lakes Entrance during autumn 2000 and (2) Tasmania during autumn 2000. For males caught from Tasmania during autumn 2000 this means that faster life-time growth resulted in both an increase

in somatic and gonad tissue relative to ML. Negative correlations between gonad investment and life-time growth were also observed in males caught from Ulladulla and Lakes Entrance during spring 2000, suggesting for these samples that slower growing males had higher levels of gonad investment.

Patterns of oocyte storage

Females showed little seasonal or spatial variation in oviduct fullness, however, females caught from Lakes Entrance during spring 2001 showed greater oviduct fullness than females from Ulladulla (Fig 4.7). Apart from females caught from Tasmania, overall, mature females did not show a consistent trend in oocyte storage or other reproductive parameters on either a spatial or temporal scale. Tasmanian females consistently showed an insignificant relationship between oviduct fullness and ML (Table 4.2) suggesting at all times, Tasmanian females were not storing ovulated oocytes for long periods of time. Positive relationships between oviduct fullness and ML were found in females caught from Ulladulla (during spring 2000); Port Lincoln (during autumn 2001) and Lakes Entrance (during spring 2000- approaching significance; autumn 2001 and spring 2001), indicating oviduct fullness increased with ML (Table 4.2). A decrease in somatic condition was coupled with an increase in oviduct weight in females caught from Ulladulla (during spring 2000 and spring 2001), Port Lincoln (during autumn 2000 and spring 2000), Lakes Entrance (during autumn 2001 and spring 2001), and Tasmania (during autumn 2000). The percent of females displaying heavier oviducts than ovaries varied between 0% and 17% (Table 4.2) demonstrating only relatively low numbers of females in each sample were possibly depleting ovarian oocytes as mature oocytes moved into the oviducts. The occurrence of stretched empty oviducts in mature females ranged from 6% to 24%, and were found in all samples except Tasmania during spring 2001.

Table 4.4. *Nototodarus gouldi.* Seasonal and annual correlations of residuals derived from the age – BW regression and (1) residuals from the ML – somatic regression and (2) residuals from the ML – gonad regression, to identify the relationship between somatic condition and gonad investment, with life-time growth rate. * Denotes significance at $\alpha = 0.05$.

Sex	Year	Season	Correlation	Ulladulla	Port Lincoln	Lakes Entrance	Tasmania
Females	2000	Autumn	age- somatic	r= 0.076 p=0.813 n=12	r= 0.076 p= 0.813 n= 12	r= 0.076 p= 0.813 n= 12	r= 0.076 p= 0.854 n= 8
			age- gonad	r= 0.220 p= .0492 n= 12	r= -0.386 p= 0.113 n= 18	r= 0.284 p= 0.370 n= 12	r= -0.019 p= 0.946 n= 16
		Spring	age- somatic	r= 0.324 p= 0.259 n= 14	r= 0.324 p= 0.259 n= 14	r= 0.324 p= 0.259 n= 14	na
			age- gonad	r= 0.315 p= 0.272 n= 14	r= 0.172 p= 0.635 n= 10	r= 0.271 p= 0.394 n= 12	na
	2001	Autumn	age- somatic	r= 0.202 p= 0.798 n= 4	r= 0.202 p= 0.798 n= 4	r= 0.202 p= 0.798 n= 4	na
			age- gonad	r= 0.064 p= 0.936 n= 4	r= 0.135 p= 0.592 n= 18	r= -0.402 p= 0.110 n= 17	na
		Spring	age- somatic	r= 0.078 p= 0.854 n= 8	na	r= 0.078 p= 0.854 n= 8	r= 0.078 p= 0.854 n= 8
			age- gonad	r= 0.507 p= 0.199 n= 8	na	r= 0.050 p= 0.826 n= 22	r= -0.395 p= 0.293 n=9
Males	2000	Autumn	age- somatic	r= 0.249 p= 0.459 n= 11	r= 0.206 p= 0.275 n= 30	r= 0.271 p= 0.140 n= 31	r= 0.721 p= 0.001 n= 18
			age- gonad	r= 0.393 p= 0.206 n= 12	r= -0.012 p= 0.951 n= 31	r= 0.408 p= 0.023 n= 31	r= 0.567 p= 0.014 n= 18
		Spring	age- somatic	r= -0.011 p= 0.962 n= 23	r= 0.69 p= 0.000 n= 21	r= -0.271 p= 0.172 n= 27	r= 0.246 p= 0.493 n= 10
			age- gonad	r= -0.475 p= 0.022 n= 23	r= 0.263 p= 0.250 n= 21	r= -0.517 p= 0.006 n= 27	r= -0.554 p= 0.096 n= 10
	2001	Autumn	age- somatic	r= 0.084 p= 0.712 n= 22	r= -0.009 p= 0.961 n= 30	r= 0.641 p= 0.000 n= 31	r= 0.359 p= 0.047 n= 31
			age- gonad	r= -0.057 p= 0.799 n= 22	r= 0.003 p= 0.985 n= 30	r= 0.323 p= 0.076 n= 31	r= 0.314 p= 0.086 n= 31
		Spring	age- somatic	r= 0.196 p= 0.436 n= 18	na	r= 0.346 p= 0.052 n= 32	r= 0.197 p= 0.324 n= 27
			age- gonad	r= 0.286 p= 0.250 n= 18	na	r= -0.102 p= 0.577 n= 32	r= -0.093 p= 0.644 n= 27

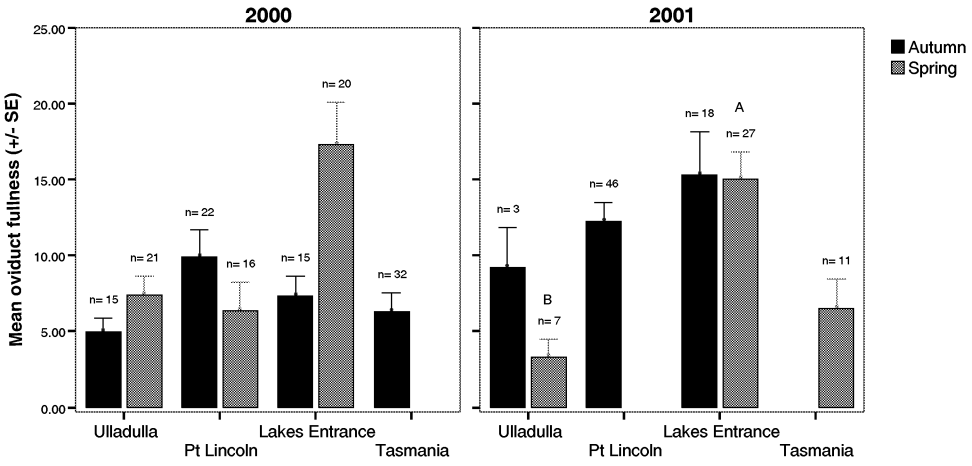


Figure 4.7. *Nototodarus gouldi*. Average oviduct fullness for all mature stage 5 females across all locations and seasons. Letters represent similarity of the means as identified by Tukey's HSD post hoc test. n= sample size

DISCUSSION

This study, along with its companion investigation by Jackson et al. (2003) showed that *Nototodarus gouldi* from Australian waters exhibit highly flexible life-history characteristics, including variations in the patterns of growth and reproductive strategies with ambient environmental conditions. Unfortunately due to the inability to collect mature female squid for three of the sixteen samples, the current examination was somewhat unbalanced, and as a result, some of the variation detected did not produce a clear pattern. However, overall, their appeared to be a basic relationship between changes in SST and the level of gonad investment. These variations appeared associated with seasonal and latitudinal environmental conditions for males, however, for females only latitudinal conditions seemed related to the variations detected.

Energy allocation

Female *N.gouldi* did not seem to compensate somatic condition for gonad development, irrespective of season or location, supporting the previous findings of McGrath & Jackson (2002) for *N. gouldi* from Tasmanian waters, which appeared to have a reproductive strategy more closely resembling *Stenoteuthis oualaniensis* (Harman et al. 1986). In contrast, when monthly samples of *N. gouldi* from southern Australia were analysed, a trade-off was shown to occur between somatic condition and gonad investment during the warmer months (McGrath-Steer & Jackson in press). This suggests that specific environmental conditions can have an influencing role in governing the allocation of energy between competing physiological processes. Furthermore, it is likely that the acquisition of resources of squid, will determine the level of energy available for these processes (Van Noordwijk & De Jong 1986).

Thus during favourable conditions, it may be unlikely that a trade-off occurs, as there are sufficient resources to fuel both reproductive and somatic processes, however, during less favourable conditions, when fewer resources are available a trade-off must take place. This may well indicate that *N. gouldi* will exhibit reproductive characteristics tending towards either end of the spawning continuum (Mangold 1987), with favourable environmental conditions resulting in a spawning strategy like that of *Stenoteuthis oualaniensis* (Harman et al. 1986), while poorer conditions may result in a spawning strategy like that of *Illex illecebrosus* (Hamabe 1963), *I. argentinus* (Laptikhovsky & Nigmatullin 1993), and *Todarodes pacificus* (Ikeda et al. 1993). A trade-off between somatic and gonad development could not be detected for mature male squid, regardless of season or location. Thus, in general males appear to be able to simultaneously allocate energetic resources between competing physiological processes, a trend seen in many squid species (Forsythe & Van Heukelem 1987, Dawe 1988, Harman et al.1989, Jackson & Wadley 1998).

Gonad investment

Although there was one example of seasonal variation in gonad investment, generally, the pattern for mature female *N. gouldi* showed an increase in energy allocation with an increase in latitude. This is in contrast to the mature males, which appeared to alter the level of investment with both seasonal variation and latitudinal variation. It therefore appears, that generally, females will vary their level of gonad investment as a consequence of relatively large changes in environmental conditions such as temperature, or temperature driven processes. Males on the other hand, will vary the level of gonad development in association with smaller changes in ambient conditions, over both seasonal and annual scales. These sex-specific responses appear to support the findings of Jackson & Domeier (2003) and McGrath-Steer & Jackson (in press), who detected greater variation in male life-history characteristics in relation to ambient environmental conditions, in comparison to females.

The reason for the sex specific responses maybe a function of physiological constrains between the two sexes, as female squid must invest considerably more energy into reproductive processes (such as vitellogenesis and the production of secondary sexual organs), compared with males (Sterns 1992). A greater commitment of resources towards sexual reproduction results in less energy available for other metabolic processes that occur concurrently with maturation (Calow 1983). Therefore, if females are to have reproductive success, they inherently have less scope to alter patterns of resource allocation due to the high energetic requirement of reproduction. This suggests the reproductive strategies of female *N. gouldi* may be quite different to that adopted by males. Male squid on the other hand have less energetic requirements for reproduction and so are potentially capable of shifting their patterns of energetic allocation over shorter time-scales. An interesting area for future research would be to investigate what these variations in gonad investment signify for each sex. For instance, for females,

does the variation in gonad investment have any effect on egg size, egg number, egg lipid content, or hatchling condition? Although a little more difficult to assess for the males, the changes in the level of gonad investment could indicate a change in spermatophore size, mating tactic, mating success, or mate competition.

Of interest in this study, was the lack of consistency in detecting patterns of gonad investment through the use of two statistical procedures. Both a residual based analysis which accounts for size, and the calculation of RSI's were used in this investigation, and their application showed the failure of the RSI's to follow the latitudinal patterns detected by the residual-based analysis. These results highlight the long recognized problems associated with ratios and indices (see reviews by Packard & Boardman 1988, Ranta et al. 1994, Green 2001). Of value, however, is the use of gonado-somatic indices to compare levels of energy allocation between species. For instance, the RSI's calculated for mature *N. gouldi* females from the current study, show the variability in reproductive investment with latitude is significant, with Tasmanian females having the lowest RSI's of 8.63%, similar to that calculated for *Stenoteuthis oualaniensis* (Harman et al. 1989), which is suggested to be a multiple spawner. In contrast, maximum values of 25.31%, 27.19% and 26.91% were calculated for mature females from Ulladulla, Port Lincoln and Lakes Entrance respectively. These maximum values are greater than the 20% estimated for *Illex argentinus* (Rodhouse & Hatfield 1990), which is proposed to spawn for only a short period of its life. This further suggests female *N. gouldi* are capable of quite flexible reproductive traits depending on the ambient environmental conditions encountered.

Somatic condition

Generally, the somatic condition of females did not show any trends due to season or location, and only males from the higher latitude sites of Lakes Entrance and Tasmania showed seasonal variation in somatic condition. This is an interesting finding, as it implies that both females and males will retain a specific level of gonad investment according to the environmental conditions encountered, however, somatic condition (although not compromised for either sex), may be more closely associated to other environmental factors, which are temporarily highly variable, such as food availability. A comprehensive study using a near-shore species that is easy to maintain in captivity, and can be held at varying temperature, food, and stocking densities, may provide some insight as to the environmental conditions which govern the allocation of energetic resources between somatic and reproductive processes.

Life-time growth

There were no clear patterns between size-at-age and either (1) somatic condition or (2) gonad investment for either females or males. Females showed little evidence of an association between life-

time growth rate and competing physiological processes. In contrast, an increase in male life-time growth rate in some cases was associated with an increase in either the soma or gonad, and at other times, a decreased life-time growth rate was correlated with an increase in gonad investment without a corresponding decrease in soma. This would suggest, that individual levels of reproductive investment and somatic condition are primarily determined independently of growth rate, similar to *N. hawaiiensis* (Jackson & Wadley 1998), and *Photololigo sp.* (Jackson 1993). These results imply that the relative life-time growth of both female and male *N. gouldi* does not determine the reproductive strategy adopted (as suggested by Mangold 1987, Forsythe & Hanlon 1989). Thus, it may be likely that the rate of growth is secondary to the reproductive needs of squid, in terms of influencing life-history characteristics.

Patterns of oocyte storage

It was not clear from this study how patterns of repro-somatic investment determine spawning mode or patterns of oocyte storage. For instance, it was expected that mature females caught from Ulladulla during autumn 2000 (with high levels of gonad investment) would release their eggs in larger batches in comparison to females caught from Tasmania (with relatively low gonad investment) (following Calow 1979). However, when patterns of oocyte storage were investigated there was little evidence to support this trend. Levels of oviduct fullness were relatively consistent between all females, with the only statistical difference observed between females caught from Ulladulla and Lakes Entrance during spring 2001. However, although not statistically significant, females from Lakes Entrance produced the highest levels of oviduct fullness during spring 2000 and autumn & spring 2001 which corresponded to females which showed positive ML - oviduct fullness relationships (spring 2000 approaching significance; $p=0.059$). This may indicate that females from these three samples were producing larger batches of eggs (higher mean oviduct fullness) by storing them in the oviduct for an extended period of time before release (positive ML-oviduct fullness correlation) (after Harman et al. 1989). In addition, females caught from Lakes Entrance during the autumn 2001, and Ulladulla during spring 2000 also showed a negative oviduct-somatic condition correlation, suggesting there may be a somatic cost for those females in producing larger batches of eggs during that time.

Interestingly, when relationships between various reproductive parameters were being analysed, a small number of mature females showed only a negative relationship between oviduct weight and somatic condition, coupled with the occurrence of stretched empty oviducts, and no other significant correlations. This implies that in a group of spawning females, individuals with low oviduct fullness (or females re-filling) are in better somatic condition than females with higher oviduct fullness. Thus, it appears that each group of females was comprised of individuals at various phases in their spawning cycle, with some individuals that may have only released one or two batches of eggs and were

therefore, in relatively better condition, as well as females which had produced multiple batches, and were in poorer somatic condition. It may therefore be likely, that as a group, these females release their eggs asynchronously through time.

Conclusions

According to life-history theory, females that are typically influenced by highly variable environmental conditions, where adults have better chances of survival than their offspring, will release their gametes in multiple, smaller batches through time (Calow 1979). This is because females in unpredictable environments tend to hedge-their-bets temporally to increase the chance of offspring survival (Hopper 1999), by maintaining control over reproduction and the timing of gamete deposition (Calow 1979). In comparison, organisms which release all their offspring in one or two large batches, are usually associated with predictable environments as they are suggested to have lower rates of survival than their offspring (Calow 1987). Although there is still some conjecture over whether squid are capable of true iteroparity, it is clear that *N. gouldi* are able to release their eggs in multiple batches of various sizes unrelated to gonad investment. Thus it seems female *N. gouldi* may fall somewhere between the extremes of multiple and terminal spawning strategies, depending on their surroundings. By having the flexibility to alter reproductive strategy in response to ambient conditions, *N. gouldi* may be able to provide their offspring with a greater chance of survival. This can be achieved by either allocating large amounts of energy towards gonad development and releasing all their offspring over a short period of time when conditions are favourable for offspring survival, or by hedging-their-bets when conditions are unfavourable, and releasing offspring in smaller batches over a protracted breeding season. As yet the factors affecting intra-specific variations in reproductive strategies in squid are poorly understood. It seems from our study, that temperature or temperature related processes may have some influence on gonad development, however, the factors influencing somatic condition and spawning mode may be more closely associated with other factors such as food availability.

This field-based study supports previous investigations into intra-specific variability of squid reproductive strategies (e.g. *Loligo pealeii*, Maxwell & Hanlon 2000, *Loligo forbesi*, Boyle et al. 1995, *Sepioteuthis*, Pecl 2001, *Illex coindetii,* Arkhipkin et al. 2000 and *N. gouldi* McGrath-Steer & Jackson in press), and provides field observations that confirm the intimate association between squid reproductive characteristics and the environment (Van Heukelem, 1979, Lewis & Choat 1993, Boyle & Boletzky, 1996 and O'Dor 1998). Future work determining the role of the environment in governing the allocation of energy between competing physiological processes is warranted. A more comprehensive examination using a combination of both field and captive based investigations, may be able to identify the environmental triggers for maturation, the rate and use of energy reserves and how this influences reproductive output.

CHAPTER FIVE

TEMPORAL POPULATION DYNAMICS IN ARROW SQUID *NOTOTODARUS GOULDI* IN SOUTHERN AUSTRALIAN WATERS.

Authors: George D. Jackson, Simon Wotherspoon and Belinda McGrath-Steer

INTRODUCTION

Developing techniques for understanding the population dynamics of squids continues to pose challenges. Their short life spans, extended reproductive events, and growth rates that are rapid and closely tied to environmental features produce a complex population structure. Unlike their teleost competitors, squid are designed to live life fast, take advantage of patchy resources and don't have the capacity to store reserves to 'ride out' lean periods (O'Dor & Webber 1986, Jackson & O'Dor 2001). The ability to identify and track cohorts can greatly aid in modelling growth rates and understanding squid population dynamics. The complex sub-structuring of squid populations was suggested to be driven by episodic spawning that produces 'micro-cohorts'. However, alternatively, it is possible that these 'microcohorts' may be more apparent than real and influenced by the sampling interval (Boyle & Boletzky 1996). Furthermore, the life styles of squid mean that their populations are often subject to extreme variations in abundance.

Cephalopod biomass worldwide has been estimated to be up to 500 M t. However, due to the rapid recycling of populations, this high biomass may only occur for a restricted period of time. Biomass of a species may reach a peak as a cohort approaches maturity but subsequently may drop to very low levels. Therefore, while the turnover in biomass of a species might be quite large the biomass of a species for a large proportion of the year might in fact be relatively low (Rodhouse et al. 2001). Understanding the dynamics of squid populations, biomass levels and the rate of population turnover is of profound ecological importance. This is highlighted by the fact that in certain instances there has been a shift in some major fisheries from traditional groundfish to cephalopods, probably as a result of both overfishing finfish along with predator removal (Caddy & Rodhouse 1998). However, it has been suggested that analysing fishing statistics alone may be inflating the degree of ecosystem change that has been hypothesised (Balguerias et al. 2000). Nonetheless, as fisheries around the world continue to be exploited we are going to see increasing pressure on harvesting cephalopod populations and in some regions they may be the only component left to exploit. Thus understanding the population dynamics and their role in the ecosystem will continue to be a high priority.

The Australian arrow squid *Nototodarus gouldi* is an abundant ommastrephid spread across southern Australian and northern New Zealand waters and is common in continental shelf regions and sometimes occurs in shallow and even estuarine waters (Winstanley et al. 1983, Dunning 1998). Recent research has focused on the reproduction and population dynamics of arrow squid in Australian waters. This species appears to be a multiple spawner with eggs being released over time in discrete batches. Reproduction in females occurs at no apparent cost to somatic tissues (McGrath & Jackson 2002). Analysis of the spatial and temporal population dynamics of *N. gouldi* from a number of locations across Southern Australia revealed a complex pattern of growth and reproduction with marked differences in growth rates, body size and maturation (Jackson et al. 2003). It was thus of interest to study temporal population dynamics of this species at a single location to discern how growth, recruitment and maturity changes over time.

For this study we chose continental shelf waters off Portland, Australia to follow the trends in the population of *N. gouldi*. This location served our research purposes well as it is an established fishing port and squid can be obtained from commercial vessels throughout the year. It is also of oceanographic interest as this maritime region of Australia is subject to regular and predictable upwelling events (Bonney Upwelling area) which greatly enhance the productivity of these waters (Butler et al. 2002).

METHODS

Samples of *Nototodarus gouldi* (≥100) were obtained from commercial fishing vessels trawling in waters off the coast of Portland (Victoria), Australia. During 2001, monthly samples were obtained from February to September, and November. Squid were frozen within 12 hrs of capture and subsequently shipped to and dissected at the Institute of Antarctic and Southern Ocean Studies laboratory. Data recorded for each specimen included sex, dorsal mantle length (ML, mm) and total body weight (TBW, g). Additionally, each squid was also assigned a maturity stage after Lipinski (1979). Statoliths were removed, rinsed with water and stored dry at room temperature.

A sub-sample of statoliths was chosen for age estimates representing the entire size range of individuals for each month. In preparation for ageing, statoliths were mounted in the thermoplastic cement Crystal Bond on a microscope slide. Statoliths were then ground dry on the anterior and posterior plane to create a thin section using 30 μm lapping film and polished using a 5.0 μm lapping film.

A Nikon Eclipse E400 high power microscope (400x) under polarized light was used in estimating the total number of increment counts. The mean of two counts that varied less than 10% of the mean,

was taken as the age estimate in days. The majority of counts were very close to each other (within 5%).

An experiment for validating the periodicity of statolith increments was carried out during January 1991 using squid captured in waters off Tasmania. Squid were captured at night using a commercial jigging vessel and were maintained in holding tanks on board the vessel. They were then transferred to a large circular 20,000 litre tank with flow-through seawater at the University of Tasmania, Tasmanian Aquaculture and Fisheries Institute. Squid were fed ad-libitum with locally captured fish. Before release into the maintenance tank, squid were injected in the region of the base of arm I with a saturated solution of tetracycline-seawater. Statoliths were later examined under a fluorescent microscope and photographed. These photographs were then used as a guide for identifying the region of the tetracycline mark under light microscopy.

Statistical analysis

For growth analysis, individuals were grouped according to hatch season; Winter 2000, Spring 2000, Summer 2000/01 and Autumn 2001. The winter 2000 sample also included 14 individuals that had hatched in the last 12 days of autumn. Growth (weight-at-age) was compared across sexes and hatch seasons using factorial ANOVA. In each case, season was treated as a fixed factor in the analysis. Pairwise comparisons amongst groups were computed using the logical constraints method of Westfall (1997), except for the comparisons of age, where strong heterogeneity of variance was detected. In this case pairwise comparisons across months using Bonferroni adjusted t-tests assuming un-equal variance were used.

Growth was analysed by fitting separate lines regression to log transformed weight at age data to determine the effects of season and sex on body weight. The model was fitted with both log age (i.e. assuming an underlying power law growth model) and age (assuming an underlying exponential growth model) as covariate. As growth data are more closely influenced by conditions experienced post-hatching individuals were grouped according to season of hatch.

To model the cohort structure of the sample a finite Normal mixture model was fitted to the totality of hatch dates. That is, the hatch dates within each cohort were assumed to be Normally distributed, with density

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

and mean μ and variance σ^2. The overall distribution of hatch dates is then a mixture of Normal components,

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

one component for each cohort, the multipliers λ_i representing the proportion that each cohort makes up of the entire distribution.

The mixture model was fitted using the mclust library (Fraley and Raftery 2002) in Splus. This library fits Normal mixture models by the EM algorithm, using the Schwarz Bayesian information criterion (BIC) (Schwarz 1978) for model selection.

RESULTS

RESULTS

Validation experiment

Eight specimens were successfully maintained in captivity for up to 10 d post capture. In all specimens an obvious tetracycline mark was apparent under fluorescent microscopy. Increments could not be easily discerned in the region post staining. As an alternative measure the width of the statolith region that had grown post staining was measured for each individual. The total width in the post-staining region appeared to be linear with time, suggesting regular growth (Fig. 5.1). However, the spread of the data suggested that error variance increases with width. We therefore fitted a model in which error variance was proportional to the fitted values by generalized least squares. While there was no evidence of a nonlinear trend, there was insufficient data to rule this out entirely. The average daily increment width was 0.75 microns, with a 95% confidence interval of 0.60 to 0.90 microns.

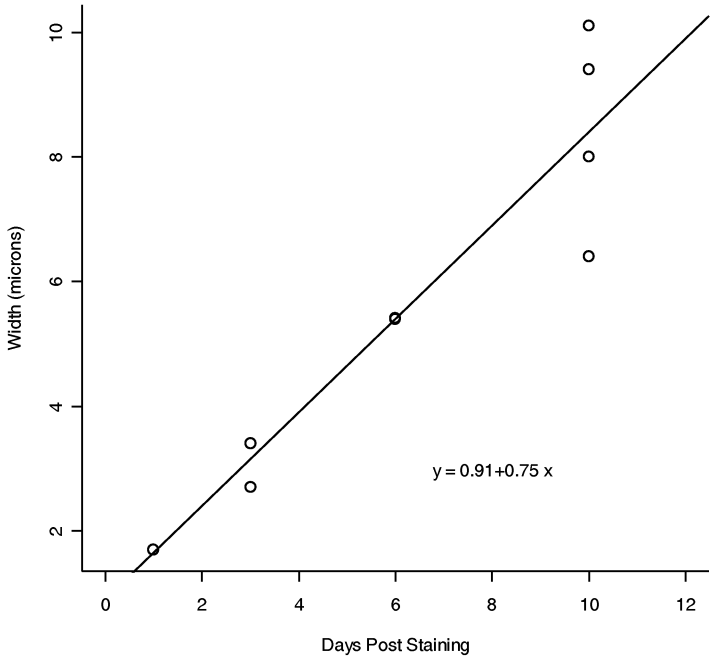


Figure 5.1: The relationship between the number of days post-staining and the width of the statolith during the period of maintenance.

Portland study

Overall we collected and processed 856 individuals (n_{males} = 503, $n_{females}$ = 353) with an average of 40 females and 55 males sampled per month. Of these a total of 602 (70%) were aged (n_{males} = 309, $n_{females}$ = 209), with an average of 34 males and 33 females aged each month. The size of squid captured were greater than 90 mm length (ML) and the majority of squid caught were > 200 mm ML. This would have been due to the selectivity of the net along with the fact that the fishers probably did not collect smaller squid. The length frequency distribution over the study period did not reveal any obvious progression in the modes (Fig. 5.2). This suggested that multiple cohorts were being sampled. However, during some consecutive months there was the suggestion that we may have sampled the same cohort (e.g., April-June, females; April-May, males, Fig. 5.2). Females had a greater mantle length range than males with individuals ≥ 400 mm while males did not exceed 350 mm.

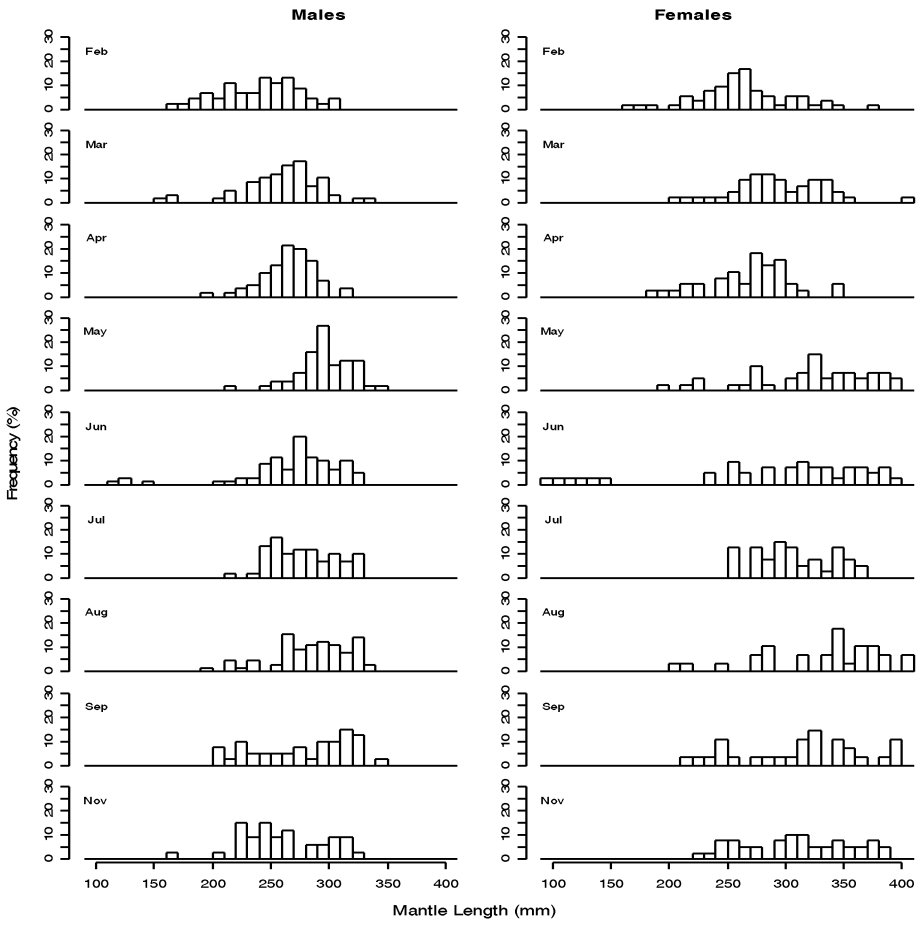


Figure 5.2: The length frequency distribution of all males and females in this study captured off Portland, Victoria.

The majority of males captured in all months were mature (Fig. 5.3). This is especially apparent after June where there were very few immature males caught in the second half of the year. The highest number of immature males was caught during February where immature males were 23.9%. Alternatively, females showed considerable variability in the proportion of individuals at each maturity stage (Fig. 5.3). Mature females were only in the majority for the months of May, August, September and November with the greatest percent maturity of 76.9% in November. In contrast, 95% of females were immature in April and there was a majority of immature females for February (70.3%), March (61.9%), June (60.5%) and July (67.5%).

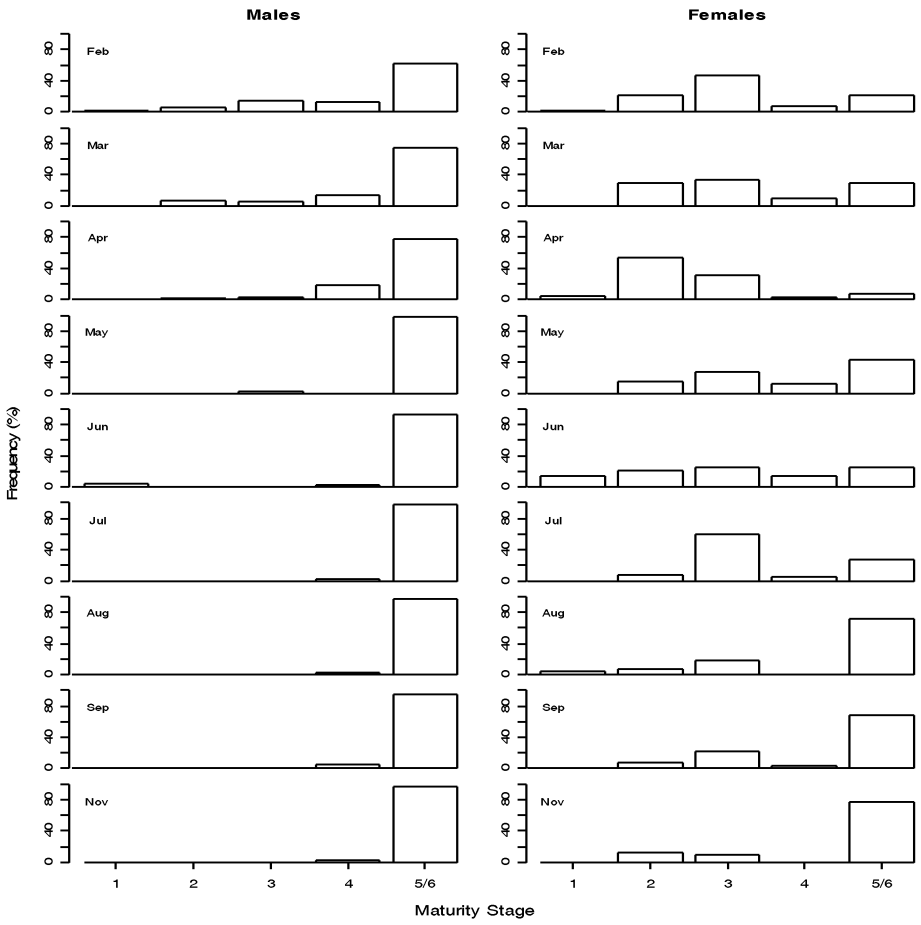


Figure 5.3: The distribution in maturity stages for *Nototodarus gouldi* males and females for each month of the study off Portland, Victoria.

The observed age range was 150 to 325 days for males, and 153 to 360 days for females. The mean age distribution for each sex across all months of capture did not show a great level of variability (Fig. 5.4). Our data showed a strong degree of heterogeneity of variance among months, we therefore compared individual months using a Bonferroni adjusted pairwise t-tests assuming unequal variances. The mean age for males in March was significantly less than all later months but not significantly different to February. There was however, no clear pattern in the distribution of mean ages of females. The high degree of variance in some months (eg., June, Fig. 5.4) suggests that during some sampling months there were a mixture of cohorts.

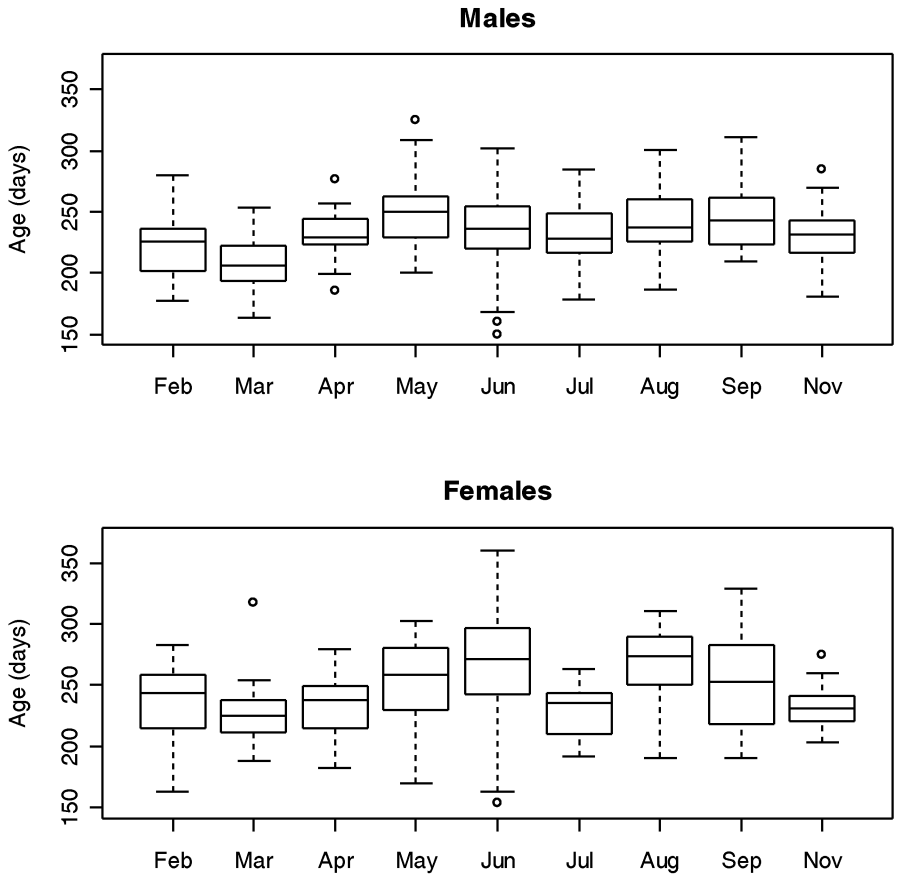


Figure 5.4: The distribution of ages of *Nototodarus gouldi*, for males (top) and females (bottom) for each month of capture.

In order to identify how many cohorts were present, we examined hatch date by capture month for both sexes combined (Fig. 5.5). There appear to be at least four discernible cohorts, the first disappearing after February, the second lasting from March through June, the third from May through to at least September, and the final cohort appearing in September and clearly evident in November. The broader distribution of hatch dates in February and May could be attributed to longer survival times during these months.

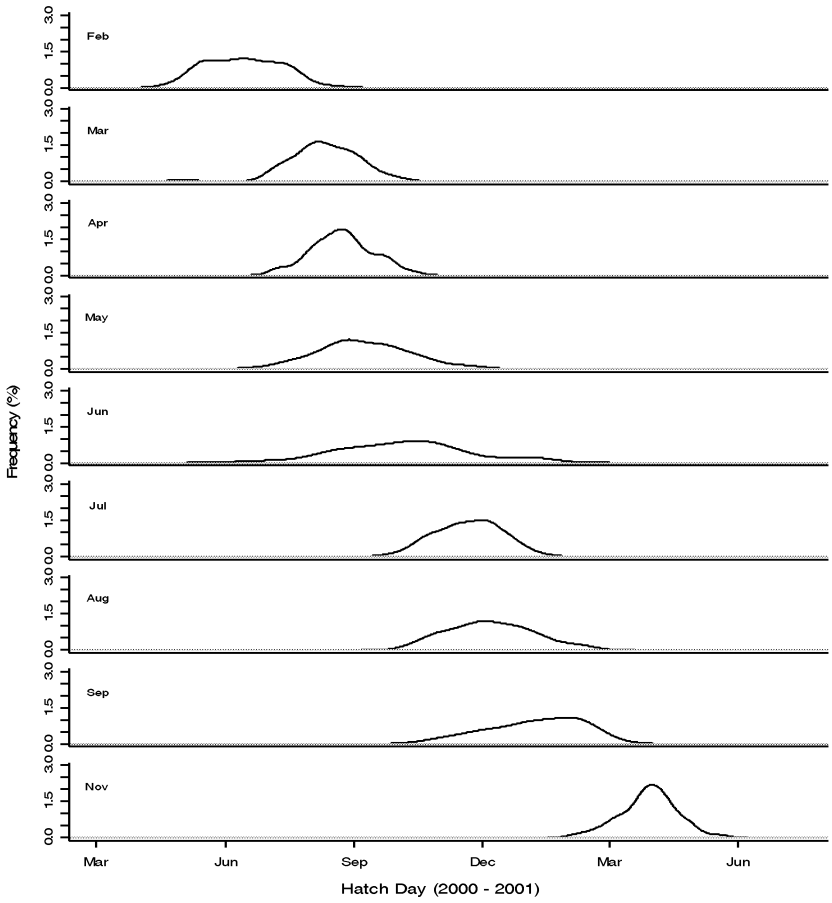


Figure 5.5: The age frequency of *Nototodarus gouldi* for each month of capture of the study period.

Proceeding on the assumption that there are a number of discrete cohorts, we fitted a finite Normal mixture to the totality of hatch dates to model the cohort structure. Based on BIC, the best fitting model had four components (Fig. 5.6), each with a common variance. This suggestion of multiple cohorts was also consistent with the complex monthly length frequencies (Fig. 5.2).

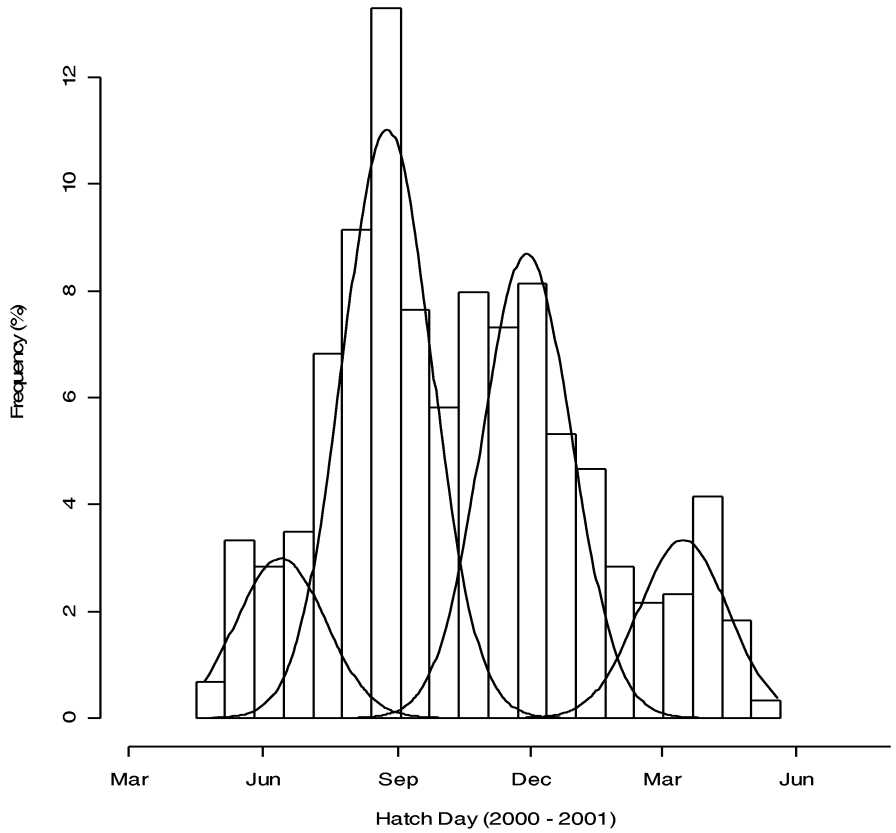


Figure 5.6: The Normal mixture model fitted to the totality of all hatch dates for *Nototodarus gouldi*. The x axis is the number of days from January 1, 2000.

Growth –Tests based on grouping squid according to hatch month showed few significant differences, possibly due to the smaller number of observations per degree of freedom. Therefore growth in weight was analysed according to season of hatch (Fig. 5.7). There was also little discernible difference in adequacy of fit for the power law and exponential models, as over the age range considered, log age is very nearly linear. The differences in these two models would be more apparent if we had a fuller compliment of younger individuals. As both models yield ostensibly the same conclusions, we present only the results of the exponential model to allow comparison with future work.

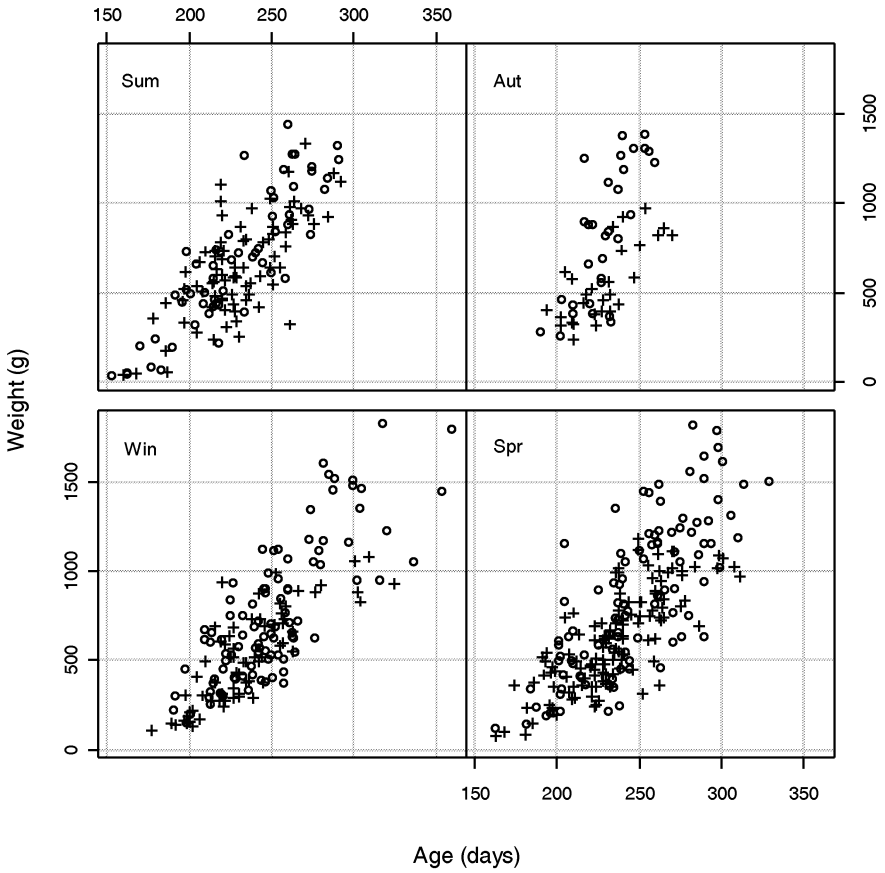


Figure 5.7: The relationship between age and total body weight for *Nototodarus gouldi* grouped according to hatch season.

Separate lines regression models were fitted to age vs. log weight (exponential growth model) allowing both slopes and intercepts to vary with hatch season and gender. The model showed strong evidence ($p=0.009$, $F=3.8$, $df=3,577$) of a sex × age × hatch season interaction, and to simplify further analysis, the two sexes were considered separately.

Multiple comparisons of slopes for the males revealed no evidence of seasonal differences in growth rates (Fig. 5.8a). In contrast, multiple comparisons of slopes for the females revealed a different pattern, with strong evidence for a hatch season by age interaction ($P<0.001$, $F=9.80$, $df=3,282$). At the 0.05 level of significance, the summer-hatched sample had a significantly faster growth rate compared to both winter and spring. The growth rate for the autumn sample appeared to be most similar to that of the summer sample. However, presumably due to the lower sample size, the autumn sample could not be shown to be different to the winter and spring samples at the 0.05 level, but was found to be different to the winter sample at the 0.1 level (Fig. 5.8b).

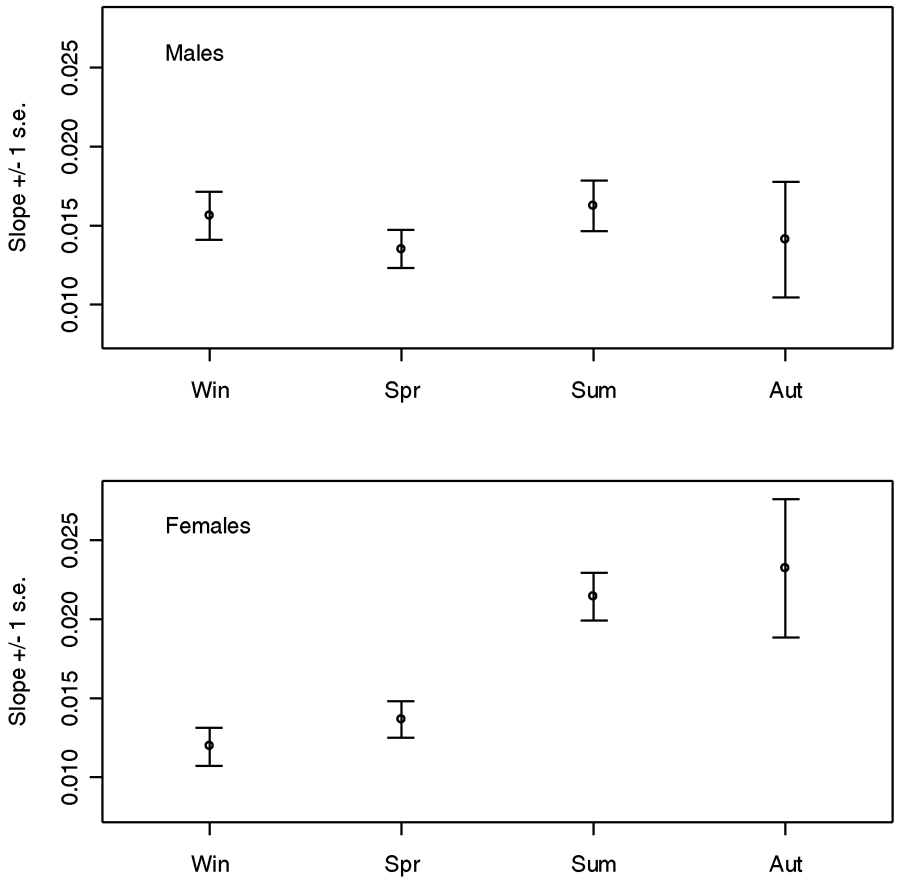


Figure 5.8: The mean slope of the separate lines regression model fitted to log transformed weight at age data for each seasonal hatch group of *Nototodarus gouldi*.

DISCUSSION

A number of techniques were used to discern important temporal patterns in age, growth, maturity and cohort structure off Portland. The combination of sequential sampling along with statolith ageing has proven to be an effective means to obtain important parameters in the population dynamics of *N. gouldi*. While we know that this species has a short life span and rapid growth, this study extends the use of statolith-derived age data to identify temporal patterns in cohort structure. Such an analysis holds promise for similar studies on other key squid species. This study also compliments earlier work that focused on broad-scale spatial and temporal dynamics of growth and reproduction in *N. gouldi* across a number of sites in southern Australia (Jackson et al. 2003). Ages of the Portland squid were somewhat higher than in the previous study. The total age of females of 360 d and males 325 d contrasts with the previous study where maximum age obtained was 329 d. These data further suggest an annual life cycle for *N. gouldi*.

We continue to assume daily periodicity in increment formation in *N. gouldi*. While statolith-based ageing studies have been carried out on ommastrephids (review Jackson & O'Dor 2001) there have been no validation studies on ommastrephids for about a decade (review Jackson 1994). However, earlier successful studies based on direct chemical marking and captive maintenance of *Illex illecebrosus* and *Todarodes pacificus*, along with indirect field-based methods of *Nototodarus sloani* and *Illex argentinus* (review Jackson 1994) give some confidence on the daily nature of statolith increments in ommastrephids. While our attempts at validation of *N. gouldi* were disappointing, the assumption of one increment per day is not unreasonable. Our experiments do highlight the difficulties with capturing and maintaining ommastrephid squid in good condition. The mean increment width of 0.75 microns per day seems to be narrow based on work on other ommastrephids where increment widths range between 1.5- 5.0 microns (eg., Morris & Aldrich 1985, Hurley et al. 1985, Arkhipkin 1993, Uozumi & O'Hara 1993). This suggests that the individuals of *N. gouldi* were probably growing at a considerably slower rate due to the captive maintenance conditions. This would also be a factor in the poor increment resolution in the period of growth during maintenance.

Previous large-scale spatial and temporal analysis of size, age and maturity of *N. gouldi* also found a complexity in growth dynamics (Jackson et al. 2003). This species appears to show considerable flexibility in reproduction and growth dynamics. The differing patterns in seasonal growth rates between males and females suggests that males and females are responding to the environment differently. It is interesting that we could not discern any seasonal changes in growth of the males compared to the females, which showed faster growth in the summer cohort. Jackson et al. (2003) found that sea surface colour (SSC) helped explain differences in growth rates of females captured during winter but not summer. Similarly SSC failed to explain variability in male growth rates for any season. Females thus seem to be more sensitive to environmental change compared to the males.

A companion study to this research, (McGrath Steer & Jackson in press) also found sex differences in reproductive investment during the same sampling months off Portland. Both females and males showed lower gonad investment during winter and greater investment during summer, with males showing a drop in gonad investment several months prior to females. An increase in gonad investment in females resulted in a drop in somatic investment. This suggested that gonad investment in females occurred at the expense of somatic growth. However, males showed a different pattern. An increase in gonad investment in males in the warmer months was generally associated with a concomitant increase in somatic investment. Therefore, during some periods, males appear to be able to allocate energy to both gonads and somatic tissue.

Given the gender differences in both the relationship between growth rate and productivity (Jackson et al. 2003) and in gonad investment (McGrath Steer & Jackson in press) it is not surprising that we found differences between males and females in seasonal growth rates. However, the mechanisms driving these differences remain unclear. The lack of seasonal growth in males was unexpected and

suggests a certain level of consistency in growth rates despite seasonal changes. However, this may be simply due to low power due to insufficient sample size and deserves further research. The oceanography off Portland is complex and influenced by regular upwelling events that occur in this region between November/December and March/April as part of the Bonney Upwelling Area. This is the most prominent and regular upwelling region in southeast Australia and results in a highly productive marine environment. This is driven predominantly by alongshore winds that produce classical upwelling plumes (Butler et al. 2002). Currently, the timing of periods of high productivity during our study is unknown. This is the focus of future research on the relationship between both SSC and SST on squid growth rate.

The high growth rates displayed by the summer-hatched females are consistent with our understanding on the influence of warm temperature accelerating squid growth (Forsythe 1993, Forsythe et al. 2001, Jackson et al. 1997). However, the Portland situation is complicated by the lack of any variation in growth rates detected in males. *Nototodarus gouldi* is clearly capable of inhabiting a wide range of habitats (Dunning 1998, Jackson et al. 2003) with flexible growth patterns according to local conditions experienced. If we assume that food is not limited off Portland, then perhaps other factors are controlling the difference in growth responses observed between males and females. Jackson & Domeier (2003) found that male individuals of *Loligo opalescens* off California tracked the environment more closely than females. This was also suggested to be an important factor in the differences in gonad and somatic investment observed between males and females for these squid off Portland (McGrath Steer & Jackson in press). However, for arrow squid off Portland, it seems that the growth rates of females are responding to seasonal changes rather than males. This is also partially supported by the fact that only female growth rate could be correlated with SSC during winter (Jackson et al. 2003) as mentioned above. The sex differences in *N. gouldi* contrasts with work by Brodziak & Macy (1996) for *Loligo* pealei and Hatfield (2000) for *Loligo gahi*, in which both studies found seasonally induced differences in growth rates were reflected in both males and females. Further oceanographic analysis in relation to squid growth may help to elucidate the role the environment is playing in influencing squid growth rate, especially for females.

Identifying squid cohorts within squid populations can be extremely difficult due to problems of uncoupling of squid size and age. This study identifies the usefulness of the combination of age data and sequential samples for identifying the numbers of cohorts present over time. Without age data it can be virtually impossible to identify cohorts. Arkhipkin (1993) employed statolith ageing techniques to identify 'waves' of individuals of *Illex argentinus* passing through fishing grounds in the South Atlantic. Thus, ageing identified that the fishery was not harvesting squid from a stationary population but rather, the situation was much more dynamic with successive cohorts passing through the fishing region. Similarly, Jackson & Pecl (2003) also found marked dynamics in spawning groups of the near-shore Australian loliginid *Sepioteuthis australis* in Tasmanian waters. Samples of this species taken

approximately weekly found no difference in either the size or age structure of squid sampled sequentially. While discrete cohorts were not identified by Jackson & Pecl (2003), their work displayed that there was a continuation of new recruits moving through the spawning region at least on a weekly basis.

The mixture model we used assumes that we have fully sampled each cohort. This is unlikely for the two end cohorts in our study off Portland. It seems more reasonable to assume that our sampling has missed the early-hatched squid in the first cohort, and the later hatched squid in the last cohort. This will lead to bias in estimates of the cohort means. Most likely the first cohort occurs somewhat earlier than we have estimated, and the last cohort somewhat later. This problem could be better addressed by a longer time series of collections off Portland. Furthermore, a longer time series would also help to identify the number of cohorts that may move through an area that is subject to fishing pressure. We now know that the fishery is targeting a transient population off Portland with at least four cohorts occurring on nearly a seasonal basis. Although these cohorts appear to be distinct, this may be a by-product of our sampling scheme. It may be that there is a single extended cohort that appears as a sequence of distinct cohorts due to the nature of our sampling scheme, as has been alluded to by Boyle & Boletzky (1996).

There is a substantial proportion of the population that we do not sample. For example, very few immature males are captured. This can influence when we detect a new cohort. While we can detect a new cohort when the animals are large enough (usually older than 150 days) that cohort is likely to be present earlier although we will not detect it with our sampling regime. Continued work will help provide a clearer insight into the important dynamics of the stock structure in this region.

Conclusions

This work demonstrates for the first time that at the fishing grounds off Portland there are waves of cohorts moving through the fishing grounds during the year. This has important implications for understanding and managing the arrow squid fishery. Rather than fishers targeting a stationary population there is a trend of new squid moving on to the fishing grounds over the course of the year. This is not unexpected due to the large degree of genetic mixing as highlighted in the genetics study (Chapter 1). These waves of squid consisted of at least 4 cohorts spread throughout the year. However, our results were somewhat limited by our number of samples. Undertaking sampling for a longer period of time or even taking samples at greater intervals may provide us with a clearer picture of population dynamics of arrow squid off Portland. Being able to sample the smaller, younger segment of the population would also help to identify sequential cohorts more clearly.

CHAPTER SIX

TEMPORAL SHIFTS IN THE ALLOCATION OF ENERGY IN THE ARROW SQUID, *NOTOTODARUS GOULDI*; SEX SPECIFIC RESPONSES

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Adapted from: *Marine Biology* 2004 (in press)

INTRODUCTION

Plasticity in reproductive traits has recently been recognized as a major characteristic of squid biology (e.g. Boyle et al. 1995; Pecl 2001; Tafur et al. 2001) and has greatly enhanced our understanding of the complexity in squid life histories. Recent evidence suggests that the timing of squid maturation is strongly influenced by a range of biotic and abiotic factors including temperature (Jackson 1993), nutritive stress (Rowe and Mangold 1975; O'Dor et al. 1977), and day length (O'Dor et al. 1977, Arkhipkin et al. 2000). These factors fluctuate in the natural environment and can lead to significant variations in size-at-maturity (Mangold 1987; Boyle 1990; Collins et al. 1995), and age-at-maturity (Jackson et al. 1997; Arkhipkin et al. 2000). In addition, environmental conditions may have a significant effect on (1) batch size (Lewis and Choat 1993) however, this is not typical for all squids (see Maxwell and Hanlon 2000) and (2) batch quality (Lewis and Choat 1993; Steer et al. 2003c), suggesting that ambient conditions may influence the rate and timing of energetic allocation, maternal investment and potentially offspring survival.

Intra-specific differences in female repro-somatic investment have been found for inshore squid species, with the allocation of energetic resources apparently depending on the time of hatch or the environmental regimes encountered during the early life-history phase (e.g. *Photololigo* sp., *Idiosepius pygmaeus* Jackson 1993; *Sepioteuthis australis* Pecl 2001; *Sepioteuthis lessoniana* Jackson & Moltschaniwskyj 2002). For instance, summer caught mature females of both *Sepioteuthis australis* and *Sepioteuthis lessoniana* have twice the gonado-somatic index of winter-caught squids (Pecl 2001; Jackson and Moltschaniwskyj 2002). In addition, *S. australis* also showed significant variation in batch size with summer-caught females producing larger clutches than winter-caught females (Pecl 2001). This suggests a strong seasonal component to the level of repro-somatic investment, and indicates that considerable flexibility within reproductive strategies (as defined by Rochet 2000) of loliginid squids, is

possible over relatively small time-scales. In comparison, relatively little work has focused on the intra-specific variations in reproductive traits of ommastrephid squid. Nevertheless, as ommastrephid squid are highly mobile and broadly distributed throughout varying oceanic environments, they may have considerably different energetic needs and thus energetic limitations in comparison to loliginid squids. Like the loliginids, however, ommastrephid squid are influenced by considerable seasonal variation in ambient conditions, and thus patterns of energy allocation as a function of varying environments should perhaps be more prevalent.

The ommastrephid squid *Nototodarus gouldi* is caught incidentally by trawling all year round, and is also targeted by a jig fishery during the austral winter in waters off Portland (Victoria) on the southeast coast of Australia. These squid are easily obtained from the fishery, enabling samples to be taken over short time-intervals. Although movement patterns have not been ascertained for this species in Australian waters, it has been shown to have very little population structuring, with high levels of gene-flow throughout its southern Australian distribution (Triantafillos et al. submitted). McGrath and Jackson (2002) have detailed the general reproductive biology of female *N. gouldi* from Tasmanian waters and suggested that maturation occurs concurrently with somatic development producing gonado-somatic indexes comparable to *Stenoteuthis oualaniensis* (Harman et al. 1989). In addition, *N. gouldi* probably produced multiple batches of eggs over a protracted spawning season. Due to its relative ease of access, availability and potential to spawn multiple batches, *N. gouldi* is an ideal species to use for investigating short-term temporal changes in ommastrephid reproductive traits.

The aim of this study was to investigate temporal variation within the reproductive strategy of *N. gouldi*. This was achieved by assessing changes in energy allocation and levels of reproductive investment and somatic condition on a monthly basis, in conjunction with other morphological measures and biological information. In addition, patterns of oocyte storage were examined, to help clarify what type of spawning mode was adopted as a function of the variations in energy allocation observed.

METHODS AND MATERIALS

Collection and processing methods

Monthly samples ($n \geq 100$) of *N. gouldi* were collected from the trawl fishery that operates in waters off the coast of Portland (Victoria), Australia during all months of 2001 except for January, October and December. Squid were collected fresh and then frozen within 12 hrs of capture and sent to Tasmania for processing. Squid were defrosted and the total body weight (BW) and dorsal mantle length (ML) were measured for each specimen. All animals were dissected and assigned a maturity stage (using

Lipinski's Universal scale modified for this species) (Juanicó 1983). Weights were taken for the gonad, accessory reproductive structures, mantle and fin. As no spent females have been previously observed for *N. gouldi*, each female was examined for any signs of tissue or gonad degeneration, as well as for internal and external lesions. Evidence of spermatophore deposition around the buccal membrane was also noted to establish at what maturity level mating occurred.

To aid in a comparison of *N. gouldi* with other squid species, the proportion of total weight allocated towards reproductive processes was calculated as a repro-somatic index (after Pecl 2001) for each mature individual (stage 4 and 5) as:

(Total reproductive weight/ (total body weight- total reproductive weight)) x 100

Where total reproductive weight is the combined weight of the ovary + oviducts + oviducal glands + nidamental glands for females, and the combined weight of the testis + spermatophoric complex in males.

Calculation of instantaneous growth

A representative sub-sample of statoliths from across the size distribution of individuals was selected for age estimates. After removal, statoliths were rinsed with tap water and mounted on a microscope slide in the thermoplastic cement Crystal Bond. Statoliths were ground dry on both the anterior and posterior planes to produce a thin section using 30 μm lapping film and polished using 5.0 μm lapping film. Total increment counts were taken from the dorsal dome using a Nikon Eclipse E400 high power microscope (400x) with polarized light. The mean of two counts that varied less than 10% of the mean, were taken as the age estimate in days (after Jackson and Moltschaniwskyj 2001), however, most counts were within 5%. Instantaneous growth rate (G) was estimated for each animal using the equation:

$$G = (\ln W_2 - \ln W_1 / T_2 - T_1) * 100$$

Where W = total BW (g) and T = time (days) giving a % increase in BW day-1 (after Forsythe and Van Heukelem 1987).

This analysis assumed a hatching weight of 0.15mg based on *Illex illecebrosus* hatchling weights (O'Dor et al. 1986), an ommastrephid squid which has very similar oviduct egg sizes and reaches comparable weights and lengths as *N. gouldi* when an adult.

Body condition during maturation

Relative body condition can be determined by the mantle length-weight relationship, because individuals can be described as being either heavier or lighter than average for their length according to their weight (Moltschaniwskyj 1995). Thus, to identify changes in body condition of animals through maturation, geometric mean (type II) regressions were calculated for each sex separately using log transformed data (after Green 2001). Residual values from this analysis allows animals to be compared

irrespective of body size, as those individuals sitting above the regression line with positive residual values are heavier for their length (as predicted by the model) than individuals below the regression line with negative residual values. Variations in female condition with reproductive stage were determined for each month by comparing means using a 1-way ANOVA and Tukey's HSD post hoc test.

Patterns of oocyte storage

To determine if females were releasing their eggs in large or small batches, oviduct fullness was calculated according to Harman et al. (1989), using oviduct weight, not volume (after Pecl 2001), and correlated against ML (Harman et al. 1989). There is evidence to suggest that oviduct fullness is not a trigger for spawning, and that females will maintain the frequency of spawning events, regardless of body size or oviduct fullness. (Lewis and Choat 1993; Maxwell and Hanlon 2000). Thus, it is likely that a group of mature females will release all their ovulated eggs at the same time, producing a concurrent spawning event. If this occurs, then oviduct re-filling should also occur concurrently, in preparation for the next spawning event. Moreover, since larger females would have relatively larger gonads, they would be expected to accumulate slightly more eggs in their oviducts than smaller females over time (as larger females are more fecund). Thus, when sampling a number of females that spawn concurrently, a positive correlation between increasing female ML and increasing oviduct weight would occur. However, this trend may also arise because a positive ML - oviduct weight correlation may also be found in females storing oocytes in their oviducts to produce larger batches, for release when the oviduct is full. Thus, to ascertain whether females were either spawning at the same time, or holding their ovulated eggs for release as large batches, the ML - oviduct weight relationship was assessed in conjunction with the ML - oviduct fullness relationship. This would then determine if oocyte storage is function of spawning time or batch size (see Fig. 6.1 for graphic representation).

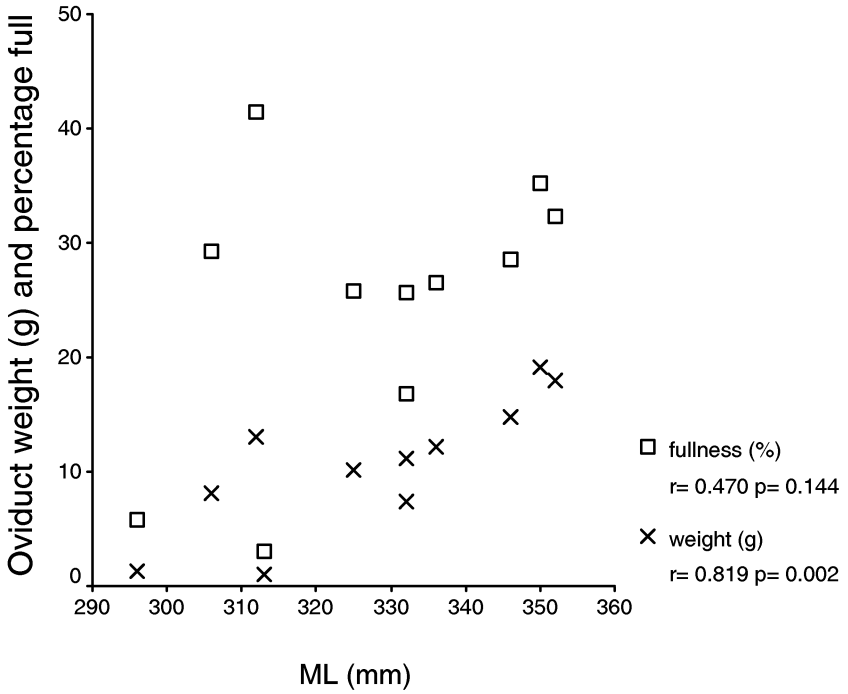


Figure 6.1. *Nototodarus gouldi.* A representative graph using data derived from the same individuals showing a positive relationship between ML & oviduct weight and an insignificant relationship between ML & oviduct fullness. In this situation, it is proposed that females of all sizes are concurrently filling (or re-filling) their oviducts and thus a positive ML – oviduct weight relationship is found. However, as smaller females will have greater oviduct fullness at lower oviduct weights (by virtue of their size), a poor relationship between ML & oviduct fullness is also observed. This pattern is expected if all females spawn and re- fill their oviducts simultaneously and spawning events are not triggered by oviduct fullness.

Allocation of energy

To investigate differences in levels of gonad investment and somatic condition in mature animals among months, two geometric regressions were performed on animals from each sex separately, using (1) ML - gonad weight and (2) ML - somatic weight (mantle + fin weight) after log transformation. The standardized residuals obtained from these two calculations provide a size-independent estimate of the relative weight of the gonad or somatic tissue, and the monthly means of these residuals can be compared using 1-way ANOVA and Tukey's HSD post hoc test. To determine if the allocation of energy between somatic and reproductive processes was a function of an energetic trade-off, the standardized residuals derived from the ML - gonad weight and the ML - somatic weight relationships were compared using correlation analysis. As the division of energetic resources between somatic and reproductive development may also affect growth rate, so growth averaged over the life-time (instantaneous growth) was correlated with gonad investment and somatic condition. This would determine if individuals with an increased growth rate (% BW day^{-1}) were preferentially allocating energy towards either the gonad or the soma. We were also interested in examining the effect of gonad investment and somatic condition on egg protection, with the premise that females in better

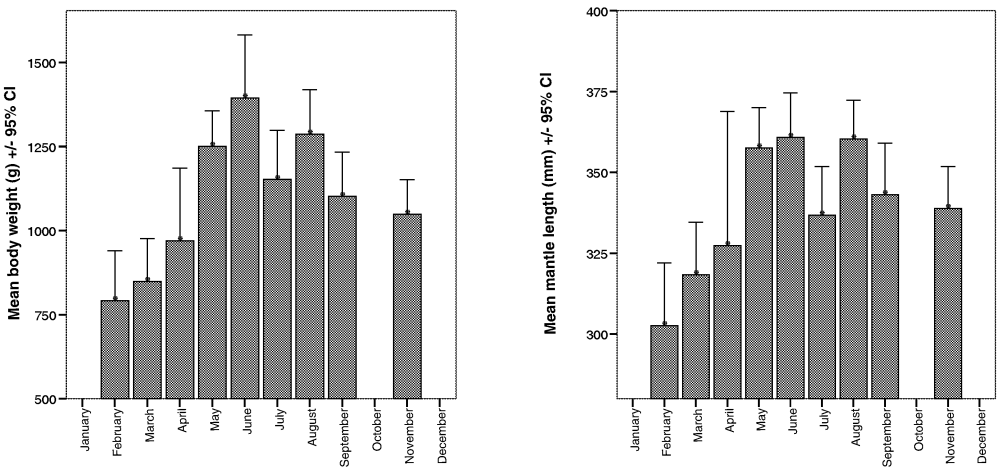
somatic condition may provide more protective egg coating than those females in poorer somatic condition. Therefore, geometric regressions were also calculated for the ML - nidamental gland weight (NG) relationship and the residuals (ML - NG) were correlated against (1) ML - gonad residuals, (2) ML - somatic residuals, and (3) oviduct fullness.

RESULTS

Size

Mature females had lower ML and BW during the warmer months in comparison to cooler months, with animals caught during February and March consistently smaller than animals caught during May, June and August. In addition, females caught in February had smaller ML than individuals caught during September, and females caught in April and November had lower BW than June females (Fig. 6.2). Mature male BW was significantly different among months, with males caught in May having the greatest BW, followed by males from the cooler months of July and August, whereas males caught during summer (February) had the lowest BW of all months. Like BW, the ML of mature males was also greatest in May, followed by August-caught individuals. Again, males caught during February showed the smallest ML of all months (Fig. 6.2).

A



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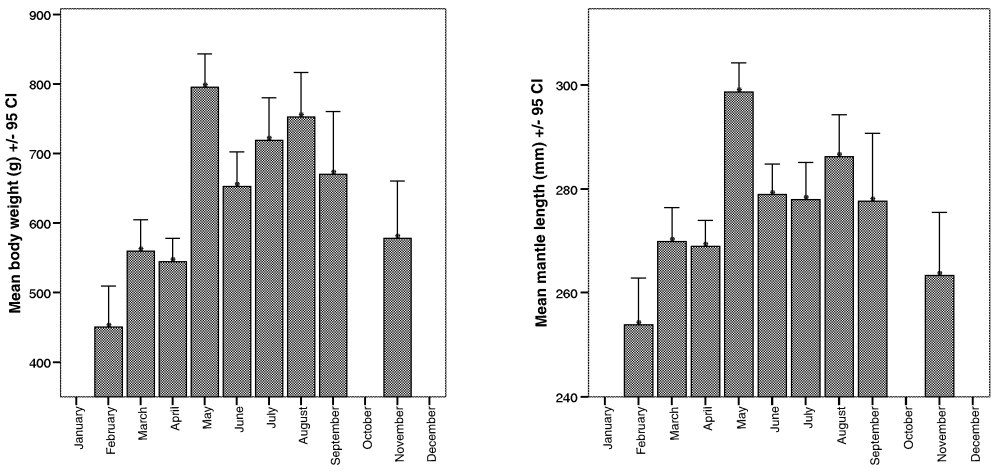

Figure 6.2. *Nototodarus gouldi*. Variations in mature a) females and b) males BW and ML from all months sampled.

Energy allocation, trade-offs and instantaneous growth

Body condition of female squid was poorer in mature squid than in immature or preparatory squid during March (df = 3,37 F = 5.169, p = 0.004), August (df = 3,24 F = 5.427 p= 0.005), September (df = 3,22 F = 8.661, p = 0.001), and November (df = 2,30 F = 8.572, p = 0.001). In comparison, mature females caught during June were in better condition than females from all other reproductive stages (df = 4,37 F = 5.268, p = 0.002). Mature female gonad investment (as described by the ML – gonad relationship) was found to vary significantly among months. Females caught during May and June had lower levels of gonad investment than all other months except July-caught females, which also had the same amount of investment as females from May (Fig. 6.3a). In contrast, levels of somatic condition were higher in females caught from May to July in comparison to females from other months. For instance, females caught during May had better somatic condition than those caught in November; while June caught females were in better somatic condition than September and November-caught females; and July caught females were found in better condition than those caught from February to April, August, September and November (Fig. 6.3a). Across all months a correlation between ML - ovary weight and ML - somatic weight standardized residuals showed a significant negative relationship, indicating that females with higher gonad investment also had poorer somatic condition (Table 6.1). Gonad investment of mature males, like the females, was greatest in the warmer months, with males from February showing the highest level of gonad investment followed by August and November. On the other hand, males caught from March to June had the lowest levels of gonad investment (Fig. 6.3b). Mature male somatic condition did not vary greatly between months, with July-caught males in better somatic condition than males from all other months (Fig. 6.3b). Unlike the

females however, mature males with heavier gonads for their size also had heavier somatic tissue (Table 6.1) indicating males with greater relative gonad mass were in better condition. Mature female RSI varied between 2.54 and 23.92% with monthly means ranging between 7.22% during July and 15.07% in September. Mature male RSI ranged between 0.66% and 5.8 % with monthly means showing little variation (maximum of 3.1% and a minimum of 2.2%).

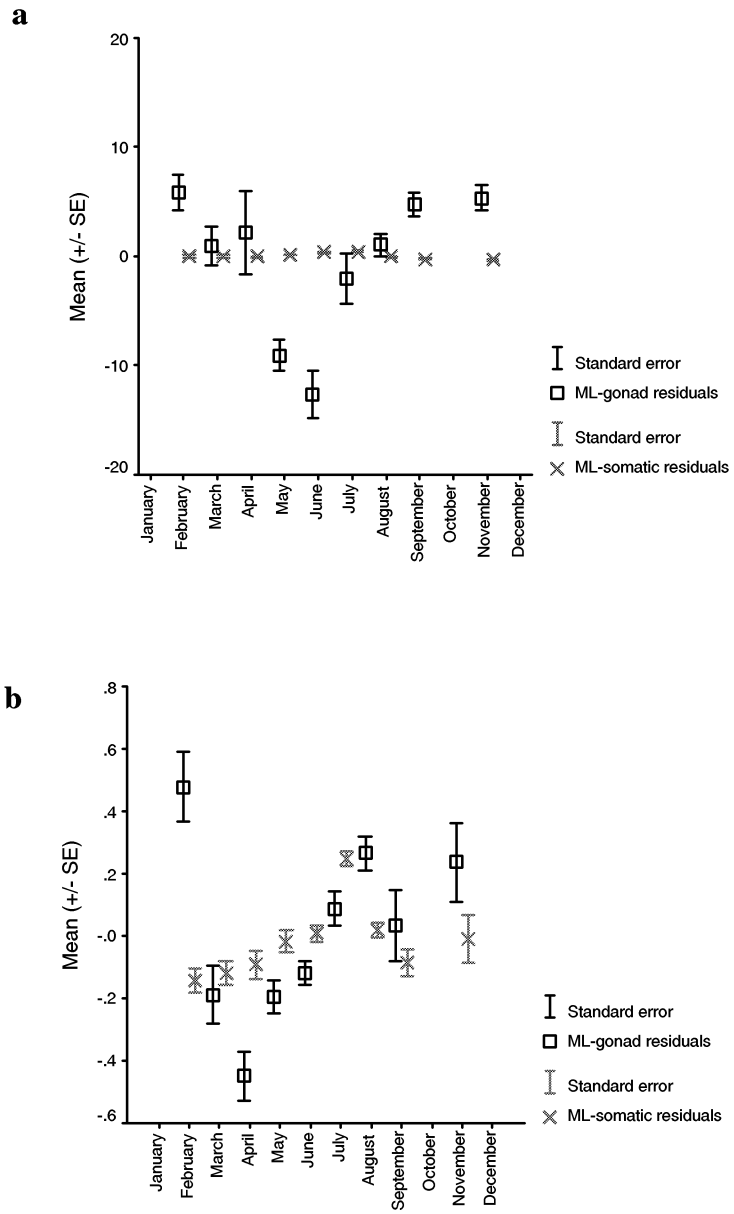

Figure 6.3. *Nototodarus gouldi.* Average gonad investment (ML – gonad residuals) and somatic condition (ML – somatic residuals) of mature **a)** females and **b)** males, from all months sampled.

A significant positive correlation was detected between instantaneous growth and gonad investment in mature females, however, as no correlation was detected between relative growth and somatic condition. This suggests that females with larger ovaries for their length also had faster life-time

growth rates (Table 6.1). Correlations between instantaneous growth and (1) ML - gonad weight and (2) ML - somatic weight indicated that mature males with higher life-time growth rates also had heavier testis and somatic weight for their size (Table 6.1).

Table 6.1. *Nototodarus gouldi*. Correlations to identify trade-offs and the relationship between gonad and somatic structures and life-time growth. All residuals are standardized and * denotes significance at $\alpha = 0.05$

Correlation	ML-gonad wt & ML-somatic wt			ML – gonad wt & G			ML – somatic wt & G		
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*
Females	145	-0.229*	0.007	122	0.332*	<0.001	123	0.001	0.995
Males	470	0.260*	<0.001	279	0.121*	0.044	279	0.178*	0.003

Patterns of oocyte storage and egg protection in females

A significant positive relationship between oviduct fullness and ML was detected only in mature females caught in June. In addition, all females except those from May showed a significant positive oviduct weight – ML relationship (Table 6.2). During all months except August and September, mating was observed to start when females reached a maturity stage of 3. The percentage of females that had mated increased with maturation, with 100% of stage 5 females having some spermatophores deposited around the buccal membrane (Fig. 6.4). Across all months, ML-NG residuals were positively correlated with both gonad investment ($r = 0.756$, $p < 0.001$, $n = 122$), and oviduct fullness ($r = 0.568$, $p < 0.001$, $n = 123$) and negatively correlated with somatic condition ($r = -0.229$, $p = 0.011$, $n = 123$).

Table 6.2. *Nototodarus gouldi*. Correlations of reproductive parameters of mature (stage 5) females for each month. * Denotes significance at $\alpha = 0.05$.

Correlation	Oviduct & ML			Oviduct fullness & ML		
	r	*p*	*n*	*r*	*p*	*n*
February	0.617*	0.003	12	0.210	0.512	12
March	0.927*	<0.001	12	0.537	0.072	12
April	0.980*	0.016	3	0.946	0.211	3
May	0.034	0.898	17	-0.194	0.455	17
June	0.707*	0.022	10	0.673*	0.033	10
July	0.700*	0.036	9	0.569	0.141	8
August	0.490*	0.028	20	0.099	0.679	20
September	0.498*	0.035	18	0.040	0.871	19
November	0.443*	0.014	30	0.213	0.297	26

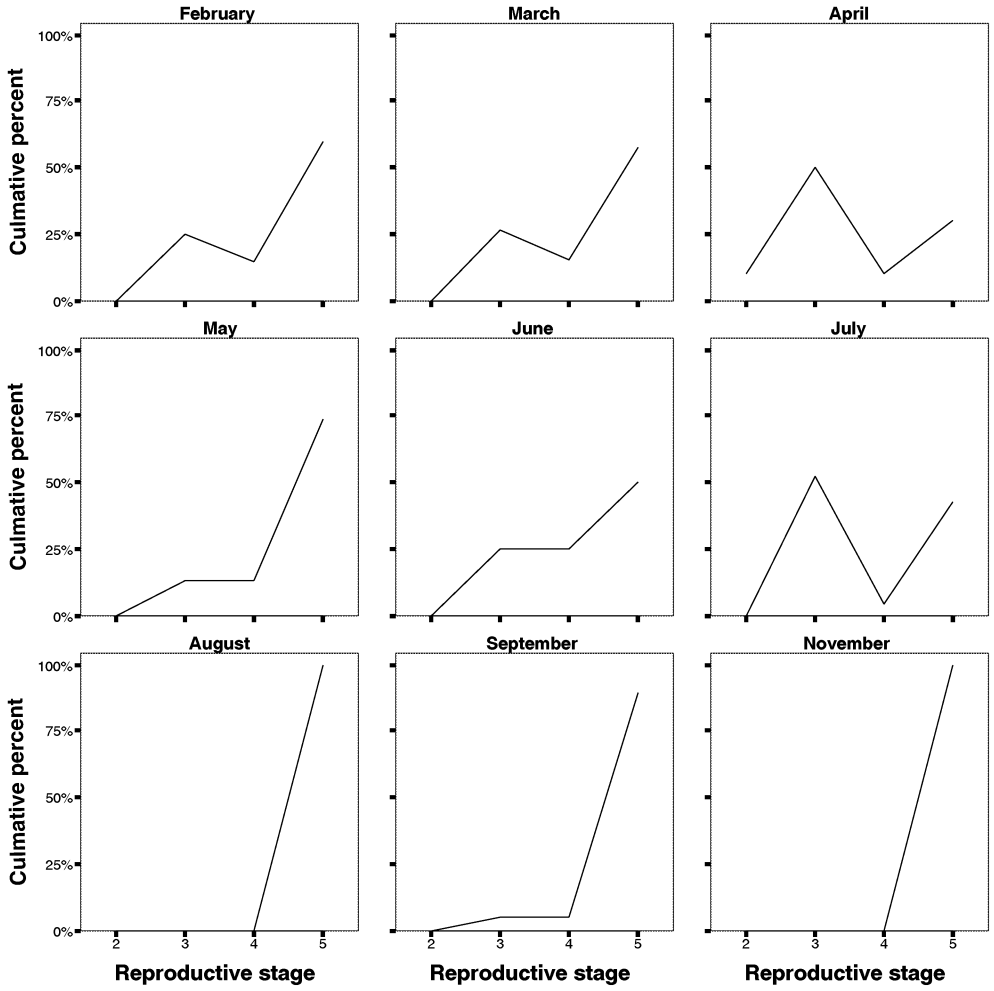

Figure 6.4. Nototodarus gouldi. Percentage of mated females found in each maturation stage across all months.

DISCUSSION

This study provides new information relating to the understanding of energy allocation in squid, particularly the sex specific responses to seasonal change. Intra-specific variation in the reproductive traits of squid has received little attention in the literature although the subsequent implications on life-histories are significant (see Boyle et al. 1995; Pecl 2001 as notable exceptions). This is because variations in life-history traits probably provide the mechanism for squid to successfully compete in a resource-limited environment (Boyle and Boletzky 1996).

Overall both mature males and females showed a significant decline in gonad investment during the autumn/winter months, however, there were considerable differences in the timing of the decline and the patterns of energy allocation between the sexes. Males showed the lowest levels of gonad investment from March to June; whereas females showed a considerable decline from May to July, suggesting that females may be delayed in their response to temporal variation in the environment, in comparison to males (supporting Jackson and Domeier 2003). This is likely to be a function of the relative energetic needs of reproduction in females compared with males, as males require relatively fewer energetic resources directed towards gonad investment for successful reproduction (Stearns 1992). This possibly means that males have less constraint on patterns of energy allocation and are therefore able to adjust resource allocation to suit their ambient environment more responsively. The tight coupling of gonad investment with somatic condition in mature males (established by the positive ML – gonad & ML – somatic residual correlation) highlights this pattern of change, for all months except February. Consequently a decline in either the relative gonad or somatic weight, results in the decline of the other. In contrast, mature females require higher commitments of energy towards reproduction (Stearns 1992) and potentially have less scope to alter patterns of investment rapidly, in response to the environment. The higher energetic requirements of female maturation and the subsequent effect this has on energy partitioning in response to environmental pressures, are confirmed by the negative correlation between gonad investment and somatic condition. This indicates that females, which increase resource allocation towards gonad development, subsequently decrease somatic condition. This associated cost or trade-off between competing physiological processes suggests females, unlike males, have large energetic constraints, which may explain why the decline in female gonad investment occurs slightly after the males.

The comparable drop in female gonad investment during autumn/winter in contrast to males was considerable. Again, this is probably a reflection of relative energetic investment between the sexes. For instance, as males invest less into reproduction and have a tighter coupling between gonad and somatic investment, so the absolute fluctuations in male resources will be less. Females on the other hand invest considerably larger amounts of energy into gonad tissue and thus their responses will be on a larger scale. Interestingly however, is the observation that mature females in contrast to the males, appear to stabilize levels of somatic condition among months (with only small relative increases occurring during autumn/winter) in comparison to the quite distinctive declines in gonad investment at the same time. This is likely to occur if a specific level of somatic condition must be reached before energy is directed towards gonad development. Maintaining somatic condition explains the poor relationship between gonad weight and size, which also occurs for *Photololigo* spp. (Semmens and Moltschaniwskyj 2000) and *Loligo forbesi* (using total egg number, Boyle et al. 1995). The premise that females sustain a certain level of somatic condition and then channel additional available resources into

gonad investment, is further supported by a correlation between life-time growth rates and gonad investment, which is not observed with somatic condition. This suggests females with faster life-time growth rates, preferentially increase gonad weight relative to their ML, but not somatic weight. Interestingly, mature females from the winter months of June and July also exhibit the highest somatic condition, further suggesting that slower-growing, winter-caught females with higher ML and BW, and lower gonad investment, preferentially maintain somatic condition over gonad investment. This is a very different trend to that observed for males, because increases in life-time growth rate resulted in both an increase in gonad and somatic weight relative to ML, implying that for males, if resource acquisition increases this additional energy is directed without preference.

Energy partitioning between gonad and somatic tissues, appears to have implications for oocyte storage and release, as mature females caught during the warmer months possibly released their eggs concurrently with other females regardless of size. This is suggested to occur in all months from; February - April; July - September; and during November, as females with higher gonad investment had a positive relationship between oviduct weight & ML, but not oviduct fullness & ML. On the other hand, females with the lowest level of gonad investment that were caught during June, also had a positive oviduct fullness & ML relationship, suggesting that these females release their eggs in larger batches (Harman et al. 1989; Moltschaniwskyj 1995; Gonzales and Guerra 1996). Although these results seem to disagree with the results of Pecl (2001) which found that smaller, summer caught female *Sepioteuthis australis* with high gonado-somatic indexes release their eggs in large batches, this may not necessarily be the case. This is because egg release in *N. gouldi* during the cool winter months, was more likely to be a function of oocyte maturation time, rather than batch size. For instance, holding eggs in the oviduct for long periods may simply be a necessity due to longer gamete development time and not a consequence of batch size. This longer development time may potentially be due to minimal acquisition of resources to fuel gamete development (as suggested by Lewis and Choat 1993), which would also result in the low levels of gonad investment observed in June-caught squid. This premise is supported by the observation that mature females caught during May, which have slightly lower gonad investment than those caught in June, release their eggs in smaller batches, not at the same time as other females. Thus, resource acquisition was possibly not limiting gamete development in May-caught females as it may have in June-caught females.

Although variation in size-at-maturity and age-at-maturity have been documented for a number of squid species both inter-specifically (Rocha et al. 2001) and intra-specifically (Boyle et al. 1995; Arkhipkin et al. 2000), this investigation supports a growing number of studies, showing reproductive output and gonad investment is not necessarily determined by size or age (Maxwell and Hanlon 2000; Pecl 2001). This has substantial implications for life histories since it cannot be assumed that larger squid necessarily have higher gonad investment than smaller squid. Moreover, patterns of egg release

are likely to be a function of the rate and timing of energy use (Calow 1979; Moltschaniwskyj and Semmens 2000). For instance, to put the flexibility in reproductive traits observed in this study (on one species from one location) in context, monthly RSI means of mature females varied from a minimum of 7.22% similar to the average obtained for the multiple spawning ommastrephid *Stenoteuthis oualaniensis* (Harman et al. 1989) and *N. gouldi* at its highest latitudinal range (McGrath and Jackson 2002) to a maximum of 15.07% comparable to *Sepioteuthis australis* (Newcastle-caught), which is further towards the terminal end of the spawning continuum (Pecl 2001). In some cases mature female *N. gouldi* displayed an RSI of up to 23.92%, higher than the mean obtained for *Illex argentinus* (20%, Rodhouse and Hatfield 1990) which is proposed to spawn for only a short period of its life.

Possible implications for squid life history

Since males have fewer energetic demands for gonad maturation and gamete release in comparison to females (Stearns 1992), and female *N. gouldi* are able to store sperm from a preparatory reproductive stage, it is possible that intra-specific differences in *N. gouldi* female reproductive traits, have considerable influence on offspring survival. So what are the possible ecological consequences of variations in intra-specific plasticity? If females have very different reproductive traits, what are the relative levels of female contribution to the population? Although somewhat speculative, there is limited evidence from this study to suggest that female *N. gouldi* do not compromise egg protection when in poor somatic condition. This may suggest that females will preferentially maintain oocyte viability irrespective of batch size or the reproductive strategy adopted. The notion that *N. gouldi* possibly retains oocyte viability, supports the conclusions of Lewis and Choat (1993) and Steer et al. (2003c), who found that regardless of maternal ration, egg size and quality was always maintained. This may indicate that females produce a similar quality of offspring temporally, however, they may alter their spawning strategy to maximize offspring survival. For instance, females preferentially allocating large amounts of energy into gonad development, rather than somatic condition (caught during the warmer months), may have released their eggs over a shorter time period in comparison to females with low gonad investment (Calow 1987; Roff 1992), because environmental conditions were more favorable to offspring survival (Roff 1992). Moreover, by releasing eggs concurrently during warm conditions, this could provide greater dispersion of offspring (Chai 1974) and the hatching of paralarve would occur at warmer temperatures, potentially speeding the development of young squid through the early vulnerable life-stages (Calow 1987). This type of strategy is compatible with the oceanographic environment of the Portland region during March/April and November/December (austral autumn and summer) as the Bonney upwelling produces a temporally predictable abundance of phytoplankton and krill (Butler et al 2002). Thus, an optimum time for gamete release by squid may correspond to the Bonney upwelling events, which would provide high levels of resource availability

for undeveloped offspring. On the other hand, females with low gonad investment caught during the resource limited coolest months, may release their clutches over a longer period of time to hedge-their-bets or increase the likelihood of some offspring encountering a favourable environment (Philippi and Seger 1989; Hopper 1999). This also supports life history theory, which suggests that females encountering fluctuating ambient conditions should release smaller batches of eggs to minimize competition between offspring for limited resources (Roff 1992). In addition, the embryonic development of eggs hatched in cooler conditions typically takes more time and produces larger hatchlings for many marine invertebrates, including *Littorina* spp. (Hughes and Roberts 1980) and *Sepioteuthis australis* (Steer et al. 2003a). As a consequence, offspring emerging during cooler conditions may in fact have better survival due to their larger size (Yampolsky and Scheiner 1996; Steer et al. 2003c).

Conclusions

Further studies incorporating investigations in egg size, number and a measure of egg quality via proximal analysis, could provide valuable insights into the early life-history consequences of variation in female reproductive traits. In order to clarify these ecological consequences, future research focusing on the survival of offspring from females that were held under varying conditions, could yield promising results. The considerable flexibility in reproductive traits based on temporal variation in the environment, highlights the very intimate relationship between ambient conditions and energy allocation in squids. We propose from the results obtained in this study that environmental variability, in particular the acquisition of resources, is the primary driving mechanism for much of the phenotypic plasticity within squid reproductive strategies. Furthermore, it is the ability of squid to quickly adjust and respond physiologically to this resource variability that may account for their success in a competitive pelagic environment (Boyle and Boletzky 1996; Rocha et al. 2001).

CHAPTER SEVEN

IS THERE A COST ASSOCIATED WITH MATURATION? MUSCLE TISSUE DYNAMICS OF THE ARROW SQUID *NOTOTODARUS GOULDI*.

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INTRODUCTION

Recent investigations suggest that mantle muscle may be the only potential source of stored energy to fuel physiological processes such as reproduction in squid (Semmens 1998; Moltschaniwskyj and Semmens 2000; Rocha et al. 2001). Previous studies using histological techniques to examine changes in mantle muscle structure with maturation have identified varying levels of muscle use associated with reproductive development (e.g. Hatfield et al. 1992, Jackson and Mladenov 1994, Moltschaniwskyj 1995). For instance, the use of mantle muscle as a fuel for reproductive processes has been shown for the terminal spawning deep-sea onychoteuthid squid, *Moroteuthis ingens,* which confirmed an irreversible breakdown of the muscle structure with the onset of maturity (Jackson and Mladenov 1994). In contrast, histological examination of the mantle muscle structure of the inshore multiple spawning loliginid species *Photololigo* sp. identified that only small changes to mantle-muscle fibres were associated with maturation (Moltschaniwskyj 1995), and instead the needs of reproduction in this species was probably being met by food intake (Moltschaniwskyj and Semmens 2000). Thus, whether an individual uses stored energy reserves to fuel maturation may largely depend on reproductive strategy. Therefore, this research aimed to investigate changes in the mantle muscle structure with maturation, in an attempt to quantify the somatic cost of reproduction at the cellular level.

At the whole animals level, the timing and rate of reproductive processes in cephalopods is primarily controlled by a hormone (gonadotrophin) produced in the optic gland (O'Dor and Wells 1978; Mangold 1987). Although maturation in cephalopods is influenced by both biotic and abiotic factors, none of the ambient conditions assessed to-date has had as great an affect on sexual reproduction as stimulating the optic gland by the surgical removal of the subpedunculate lobe (O'Dor and Webber 1986). This suggests a complex endocrine system principally regulates vitellogenesis and the use of the mantle muscle as an energy store. Although the role of the hormone (or hormones) responsible for maturation and the associated changes in somatic tissue are not fully understood, it has been hypothesized that varying levels of the gonadotrophic hormone will influence the reproductive strategy adopted. For instance, it may be that high levels of secretion inhibit feeding and promote gonad growth at the expense of the soma, resulting in an irreversible process of emaciation; or conversely at

lower hormone levels, feeding and growth continues and mantle muscle structure is maintained, resulting in protracted spawning (Mangold 1987).

Life-history theory also predicts that if an individual is unable to obtain sufficient resources or nutrients to sustain both somatic and reproductive growth, a trade-off between these competing processes is likely to occur (Roff 1992). This may result in the reduction of somatic growth by either (1) the preferential allocation of resources towards reproduction and thus the intrinsic rate of increase in somatic tissue will decline or (2) somatic tissue may be used to fuel maturation and thus reproduction occurs at the expense of the soma. The change or breakdown in mantle muscle structure with sexual maturation may therefore be primarily a function of reproductive strategy, with the rate of mantle breakdown possibly dependent on the level of resource acquisition (Van Noordwijk and De Jong 1986).

Given the relative importance of the squid mantle muscle to various physiological processes such as locomotion and respiration, relatively few studies have examined the impacts of maturation on somatic structures at the cellular level (see Moltschaniwskjy 1995 as a notable exception). Squid mantle muscle increases in mass by both hyperplasia (formation of new muscle fibres), and hypertrophy (increase in muscle fibre number) throughout the lifespan (Moltschaniwskyj 1995, Pecl and Moltschaniwskyj 1997). Therefore structural changes as a result of maturation may be due to either fibre utilisation, changes in the relative importance of fibre generation and/or growth, or a combination of both.

Mantle tissue is principally composed of obliquely striated circular muscle fibres (~ 80%) partitioned into rectangular bands by thin strips of radial fibres (Ward and Wainwright 1972; Moon and Hulbert 1975). These radial fibres insert into both the inner and outer tunics (Bone et al. 1981) and contribute to less of the mantle tissue than the circular fibres. The circular muscle fibres are typically separated into two morphologically and biochemically distinct types; large, mitochondrial-rich fibres and smaller mitochondrial-poor fibres. The mitochondria-rich circular fibres are oxidative and usually found in a thin layer on the inner and outer regions of the mantle, and are responsible for slower, steady-state swimming (Mommsen et al. 1981). In contrast, the mitochondria-poor fibres are found within the central zone of the mantle tissue and possess Na^+ channels in squid, which allow an all-or-nothing response for use during feeding or escape (Mommsen et al. 1981; Rogers et al. 1997). In addition, a network of inelastic collagen fibres produces an intramuscular network that provides support and a means for potential energy to be stored and released within the mantle structure (Bone et al. 1981, MacGillivray et al. 1999). This mantle muscular arrangement provides structural and morphological regions within the mantle tissue, possibly identifying specific functional areas. Thus the quantification

of changes in mantle structure with reproductive processes may yield interesting results in terms of energy transfer and the associated physiological consequences for needs such as predation, predator avoidance and migration. The focus of this study was to therefore quantify changes in mantle muscle structure and fibre organisation with sexual maturation in the female multiple spawning arrow squid *Nototodarus gouldi*. Changes in somatic condition with sexual maturation have not been found in previous investigations of this species at the whole animal level; however, as *N. gouldi* is a highly mobile oceanic predator, it is possible that changes in mantle structure with maturation do occur at lower levels of organisation. A further positive development from this research is the initial description of muscle fibre organisation in an ommastrephid squid.

MATERIALS AND METHODS

Specimen collection

Female *N. gouldi* (n = 41) were collected from commercial vessels trawling and jigging in the waters off Portland, Victoria, Australia (38.35°S, 141.62°E). All squid were obtained within the same 12 hour period during July of 2001, and processed fresh in Portland within 18 hours of capture. Each animal was weighed (BW) and measured (ML) before being assigned a maturity stage according to Lipinski's universal scale (Juanicó 1983) modified for this species. The reproductive organs and the mantle were dissected out and weighed separately for each individual, and then a small sample of mantle tissue was taken for histological analysis. Statoliths were also removed for age analysis (according to Jackson et al. in review).

Mantle muscle-tissue analysis

A sample of mantle tissue was taken from three positions along the mantle from each individual (excluding juveniles); anteriorly (AM), obtained adjacent to the mantle locking mechanism; mid-mantle (MM), taken half way along the mantle and; posteriorly (PM), adjacent to the fin. Mantle tissue was fixed in a formalin acetic-acid calcium-chloride solution (FAACC:10ml of 37% formaldehyde, 5ml glacial acetic acid, 13g calcium chloride-dehydrate and 100ml water) for two weeks then transferred to 70% ethanol for storage prior to processing. Tissue was dehydrated in a graded ethanol series, cleared in toluene, and infiltrated with paraffin wax. After embedding, blocks were sectioned at 6 μm and stained with Hematoxylin and Eosin. A sub-sample of sections taken from animals (n = 7) ranging in maturity was stained with the trichrome Mallory-Heidenhain to identify collagen and elastin fibres. Sections of mantle muscle-tissue were examined and measured using a Leica DC 300F camera and Leica IM 50 software. The widths of 5 muscle blocks (not including radial muscle partitions), and the largest diameter of 20 mitochondria-rich fibres and 50 mitochondria-poor fibres were randomly measured from each position along the mantle for all individuals. The width of the inner and outer

mitochondria-rich zones was also determined for each muscle block measured. Muscle fibre size and mean muscle block width were then compared between maturity stages. In addition, the width of the mitochondria-rich zones as a proportion of total mantle width was compared between maturity stages.

Statistical analysis

To identify changes in mantle condition with sexual maturation, a geometric mean (type II) regression was performed on ML and mantle weight after log transformation (after Green 2001). This enabled a size-independent measure of mantle condition to be obtained for each female (as a residual value), which could then be compared using a 1-way ANOVA (after Moltschaniwskyj and Semmens 2000). Females below the regression line, with smaller residual values, have lighter mantle weights for their length than the model predicts, and therefore are considered to have poorer mantle condition. In contrast, females with heavier mantle weights have higher residual values and are thus were considered in better mantle condition.

Ovary weight is a useful proxy for maturation status, although, it is also a function of animal size. Therefore, partial correlations, controlling for BW, were used to determine the relationship between ovary weight and the average muscle block width of each individual (after Moltschaniwskyj and Semmens 2000).

As meaningful transformations of the non-independent data would not produce normality or homogeneity of variance, it was not clear how to adequately summarise changes to the proportion of the mitochondria-rich zones at the inner and outer mantle regions with maturation. Therefore it was decided that analysis of the mitochondria-rich zones would primarily be achieved through graphical observation of the means for each animal, with some statistical support to confirm the visual observations. As an initial inspection of the data revealed a highly variable distribution of zone widths both among positions along the mantle, and among maturity stages, a logistic regression was used to examine the relationship between zone width and maturity stage. Zones of mitochondria-rich fibres were described as either wide or narrow, depending on whether they occurred above or below a reference value that divided the data at $y=0$ when a binomial distribution was present. Values of 25% and 7% were chosen as the reference value for dividing wide and narrow mitochondria-rich widths for the inner and outer zones respectively. (see Fig 7.3 and 7.4). As the order in which terms are fitted is important for logistic regression, both reproductive stage and ML terms were fitted in both orders to judge their relative importance in explaining the variation in the data. However, as statistical analysis of the mitochondria-rich regions was only used to provide a descriptive analysis to help support graphical patterns and not to test hypotheses, statistical outcomes are not presented; nevertheless,

graphical displays do adequately show changes in the width of mitochondria-rich zones with maturation.

Changes in the diameters of mitochondria-rich and poor fibres with maturation were analysed using logistic regression, which examined variation in the proportion of large and small fibres with maturity stage. Fibres were classified as being either large or small depending on whether they were larger or smaller than the median value.

RESULTS

Mantle condition

The ML-mantle weight relationship was relatively strong ($r^2 = 0.99$, n = 41) with little variation around the model. In this study, there was no evidence of maturation occurring at a cost to mantle condition (n = 41, df = 4,36 p = 0.745)(Fig. 7.1) at the whole animal level, as females from all maturity stages were found both above and below the regression line.

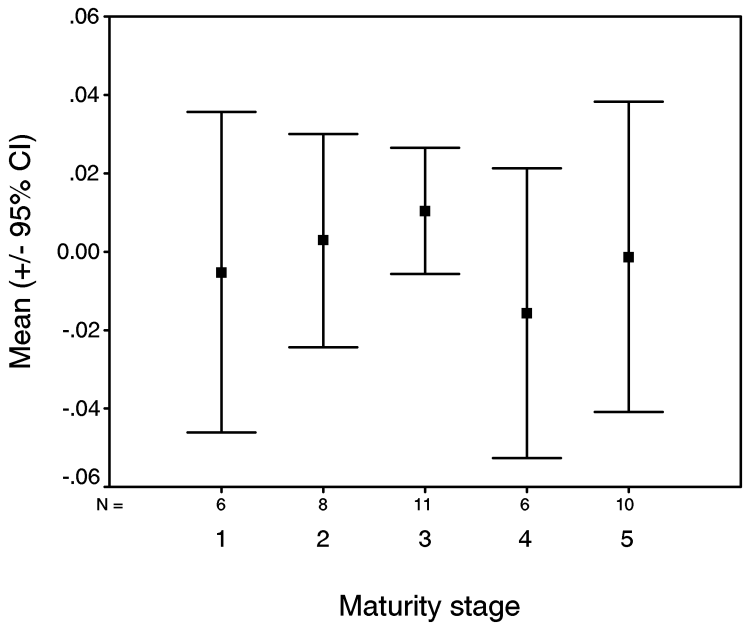

Figure 7.1. *Nototodarus gouldi.* Average ML-mantle weight residuals with maturation.

Mantle muscle organisation

In *N. gouldi,* muscle fibres ran longitudinally bordering the circular muscle fibres at both the PM and AM positions (Fig. 7.2b and 7.2d). This longitudinal muscle was positioned just internally to the outer tunic and was thicker at the posterior end of the mantle; where adjacent to the fin it comprised

approximately half of the total mantle thickness. In addition, there was not the usual region of outer mitochondria-rich fibres bordering the longitudinal muscle, indicating only an inner region of mitochondria-rich fibres occurred posteriorly.

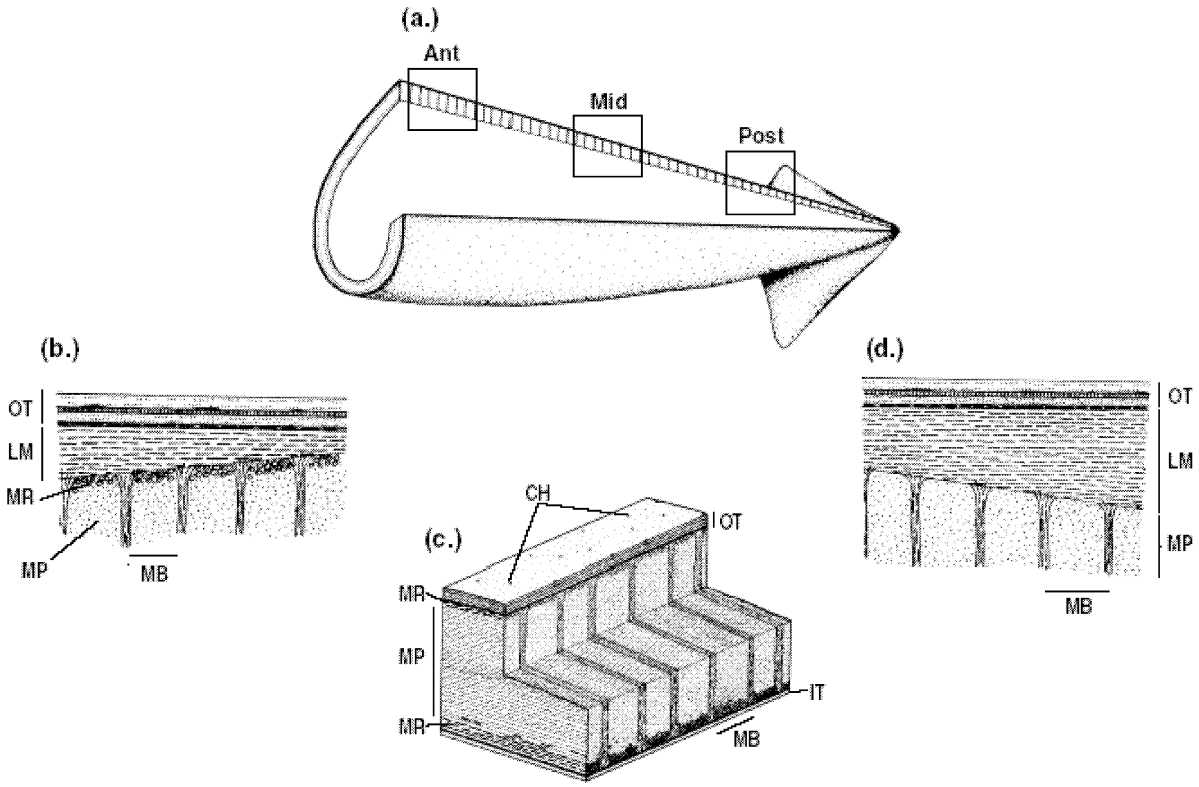

Figure 7.2. Schematic diagram of the arrangement of muscle fibres in *Nototodarus gouldi* mantle muscle (not to scale). Samples were taken from the anterior (b), mid (c) and posterior (d) positions along the mantle. Mantle muscle consisted of an outer tunic (OT) containing chromatophores (CH), a region of inner and outer mitochondria-rich circular muscle fibres (MR), a central region of mitochondria-poor circular muscle fibres (MP) and an inner tunic (IT). Circular muscle fibres were organised into muscle blocks (MB) by radial fibres. A region of longitudinal muscle fibres (LM) were also present adjacent to the outer tunic at both the anterior and posterior positions.

Muscle block width

The average muscle block width was 235.124 (± 4.379) with blocks ranging between 67.04 and 630.66. Correlations between ovary weight and muscle block width at each mantle position were relatively strong at the AM position only ($r = -0.431$, $n = 29$, $p = 0.026$). In contrast, at the MM and PM

positions the muscle block width did not appear to alter with maturation (MM: r = 0.286, n = 30, p = 0.140; PM: r = -0.252, n = 28, p = 0.224).

Mitochondria-rich zone

Inner

Although a clear pattern could not be found, taken as a whole, the proportion of the inner mitochondria-rich zone decreased with maturation (Fig 7.3). The proportion of mitochondria-rich fibres at the AM position, ranged from 10.02 - 57.24% at the immature stage, and declined to between 3.06 and 17.58% when females were mature (Fig 7.3a), whereas at the MM position, the zone declined from a range of 7.56 - 30.68% to 3.08 - 16.58% with maturity (Fig 7.3b). The PM showed the greatest decline with maturation, with the proportion of the mitochondria-rich zone reducing from a range of 28.98 – 82.20% when females were immature to a range of 6.57 – 16.71% when mature (Fig 7.3c). Logistic regression supported these observations, identifying a reduction in the proportional width of the mitochondria-rich zone with both reproductive development and ML at all three positions. However, at each position, the logistic regression analysis identified different factors as the driving mechanism for the decline. At the AM position, maturation or ML could equally explain the reduction in the proportion of the mitochondria-rich zone, whereas at the MM position, reproductive development explained more of the variability than the ML, and therefore was more influential in the reduction of the mitochondria-rich zone at the MM position. Posteriorly, both maturation and ML explained some of the decline in the proportion of mitochondria-rich fibres, with maturation explaining some of the reduction not explained by ML. In addition, when the inner mitochondria-rich zone was viewed as a width of total mantle width and not as a proportion, the width of the inner mitochondria-rich zone decreased with maturity (Table 7.1). This suggests that mitochondria-rich fibres comprised less of the total mantle width, and therefore these fibres either declined in number, size, or were recruited as small fibres that did not undergo hyperplasia, during maturation.

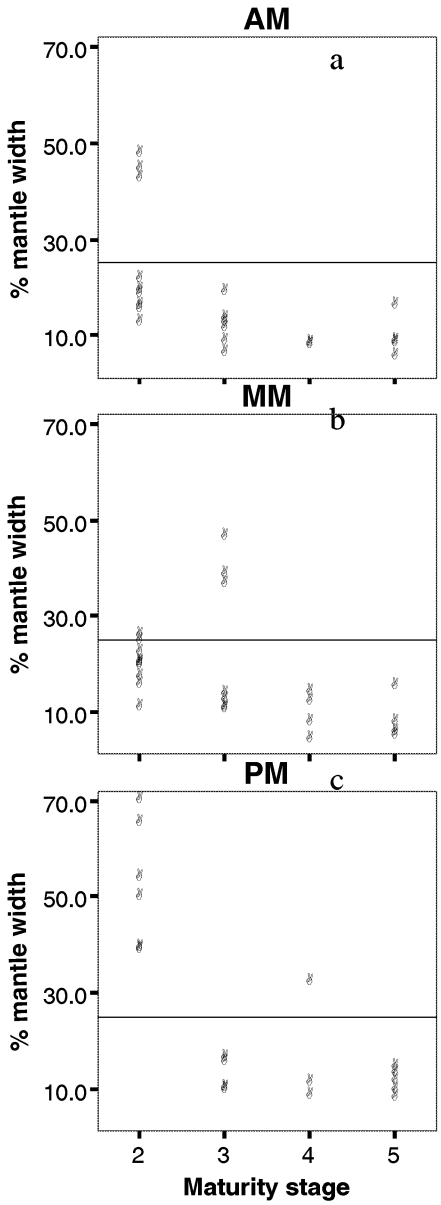

Figure 7.3. *Nototodarus gouldi*. Changes in the proportion of the mitochondria rich zone with maturation and position along the mantle (a-c) at the inner region. A 25% reference line is included to delineate between wide (>25%) and narrow (<25%) values of mitochondria-rich zones as a function of mantle width. For sample sizes see fig 1.

Table 7.1. *Nototodarus gouldi.* Width of the inner mitochondria-rich zone among positions along the mantle and maturity stages. Overall, at both positions, the total width of mitochondria-rich fibres at the inner zone decreases with maturation

Position	maturity	n	mean ± SE	minimum	maximum
Anterior	2	43	266.09 ± 21.53	93.2	612.4
	3	35	135.03 ± 7.06	75	213.7
	4	18	124.75 ± 9.44	55.1	224.8
	5	25	145.37 ± 11.85	47.1	254.9
Mid	2	44	249.53 ± 8.84	105.9	362.0
	3	29	222.75 ± 32.57	0.0	694.8
	4	20	124.49 ± 10.45	41.5	184.8
	5	20	136.50 ± 14.95	54.2	271.8
Posterior	2	30	216.55 ± 11.57	113.0	381.2
	3	20	68.72 ± 4.18	43.7	103.1
	4	15	80.33 ± 14.14	27.8	178.8
	5	27	62.02 ± 3.32	36.2	103.7

Outer

As there were no mitochondria-rich fibres present at the PM position (see mantle muscle organisation above), the summary and analysis of mitochondria-rich fibres within muscle blocks for each maturity stage only included the AM and MM positions. At the AM position, the proportion of the mitochondria-rich zone declined with maturity, from a range of 3.18 – 10.75% when females were immature, to a range of 1.44 – 7.14% when mature (Fig 7.4a). Similarly, at the MM position, the proportion of the zone decreased from a range of 3.42 – 16.46% to 1.89 – 4.18% as females matured (Fig 7.4b). These observations were supported by a logistic regression analysis, which identified maturation as the primary influence on the reduction in the mitochondria-rich zone both anteriorly and mid mantle. Again, when the zone of mitochondria-rich fibres was observed as a width of the total mantle, the zone of mitochondria-rich fibres declined in width with maturation (Table 7.2). This indicates, that like the inner zone, the outer mitochondria-rich zone not only encompassed less of the mantle width, but also decreased in number, size, or were recruited as small fibres without undergoing subsequent hyperplasia, with maturity.

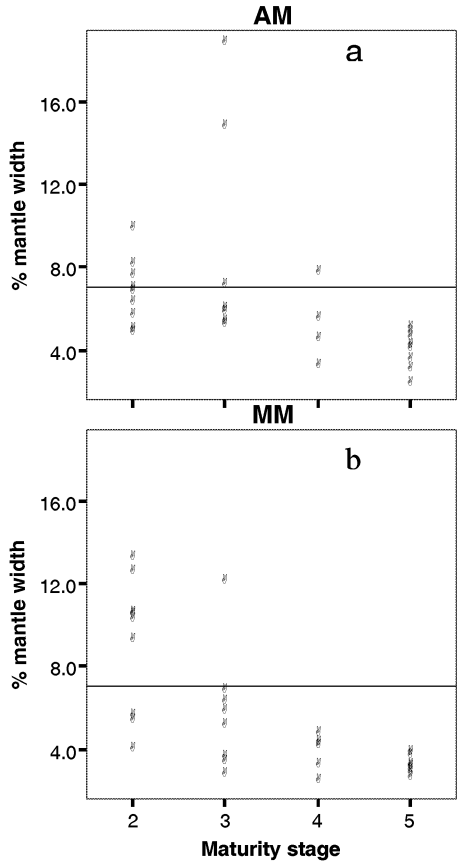

Figure 7.4. *Nototodarus gouldi.* Changes in the proportion of the mitochondria-rich zone with maturation and position along the mantle (a-b) at the outer region. A 7% reference line is included to delineate between wide (>7%) and narrow (<7%) values of mitochondria-rich zones as a function of mantle width. For sample sizes see fig. 1.

Table 7.2. *Nototodarus gouldi.* Width of the outer mitochondria-rich zone among positions along the mantle and maturity stages. Overall, at both positions, the total width of mitochondria-rich fibres at the outer zone decreases with maturation.

Position	maturity	n	mean ± SE	minimum	maximum
Anterior	2	43	68.26 ± 2.412	37.22	104.23
	3	35	107.55 ± 9.05	47.96	228.27
	4	18	68.95 ± 4.87	29.89	117.71
	5	49	63.12 ± 2.28	26.02	90.54
Posterior	2	44	116.97 ± 7.69	39.28	255.15
	3	33	69.44 ± 5.63	27.86	219.05
	4	30	49.15 ± 2.80	23.38	83.11
	5	45	51.69 ± 1.37	32.91	68.15

Muscle fibres

Mitochondria-rich muscle fibres

Mitochondria-rich muscle fibre diameter varied in size from 0.24 to 16.85 μm with a median of 6.2 μm. The proportion of large and small muscle fibres within a muscle block altered with maturity stage at the AM ($df = 3, 557$, $p < 0.001$) and MM ($df = 3, 566$, $p < 0.001$) positions, with no variation being detected at the PM position ($df = 3, 469$, $P = 0.88$). At the AM position a clear pattern was evident, with a decrease in the proportion of large fibres associated with reproductive development (Fig. 7.5a). In contrast, no clear pattern in the proportion of muscle fibre size could be discerned at the MM position, and it appeared that the proportions of the two different fibres sizes alternated with maturity stage (Fig. 7.5b).

Mitochondria-poor muscle fibres

Mitochondria-poor muscle fibres ranged in diameter from 0.28 to 9.54 μm with a median value of 2.77 μm. The proportion of large and small muscle fibres varied with maturity at all positions along the mantle (AM: $df = 3, 1428$, $p = 0.02$; MM: $df = 3, 1450$, $P = 0.02$; PM: $df = 3, 1252$, $P < 0.001$). Like the mitochondria-rich muscle fibres, at the AM position the proportion of large mitochondria-poor fibres declined with maturity stage (Fig. 7.6a). In general, at both the PM and MM positions, a greater proportion of large muscle fibres were associated with an increase in maturity stage (Fig. 7.6b and 7.6c).

Collagen matrix

Apart from the muscle structure described above, in a small number of animals ($n = 4$) an area of the inner mantle appeared to have a small region where no muscle fibres were present and only an intra-muscular collagen network remained (Fig. 7.7b). The matrix stained blue with the trichrome Mallory-Heidenhain suggesting its collagen content. The location of the collagen matrix along the mantle wall was not consistent with size or maturity stage (Table 7.3). The smallest, least mature individual (stage 3) only had the collagen matrix in the MM position, whereas larger, maturing (stage 4) animals had the collagen matrix in both the MM and PM, with a higher percent found in the PM than the MM. The largest and only mature (stage 5) female that appeared to have the collagen matrix, showed a lack of muscle fibres in all three mantle positions (AM, MM and PM) with the MM showing the greatest amount.

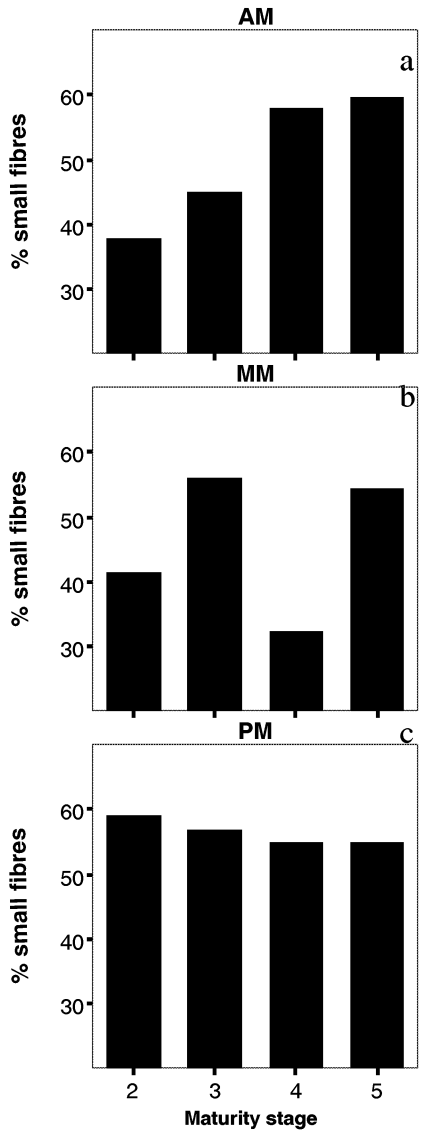

Figure 7.5. *Nototodarus gouldi*. Differences in the proportion of small mitochondria-rich muscle fibres with maturation and position along the mantle (a-c). Fibres were classified as either large or small depending on whether they were larger or smaller than the median value of 6.2 μm.

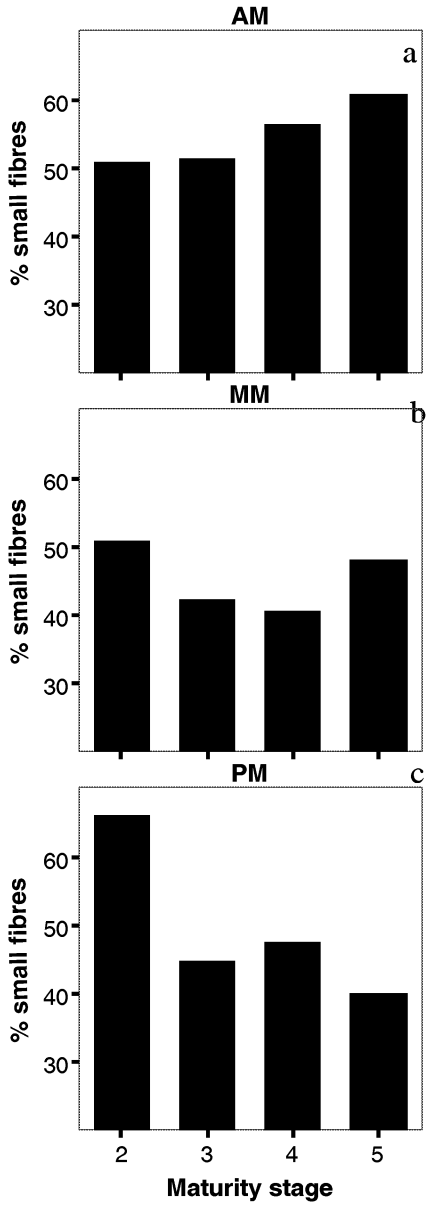

Figure 7.6. *Nototodarus gouldi.* Differences in the proportion of small mitochondria-poor muscle fibres with maturation and position along the mantle (a-c). Fibres were classified as either large or small depending on whether they were larger or smaller than the median value of 2.7 μm.

Table 7.3. *Nototodarus gouldi.* Females with inner collagen matrix present. Regions of mantle affected are anterior mantle (AM), mid-mantle (MM) and posterior mantle (PM). Calculations are taken from the total width of circular fibres only, and therefore do not include the width of longitudinal muscle found in the MA and MP.

Maturity	ML (mm)	BW (g)	Age (days)	% circular fibre (± SE) AM	MM	PM
3	313	763	271		42.90±3.43	
4	355	1154	294		14.71±1.11	41.74±0.91
4	370	1507	300		41.05±0.83	90.11±2.67
5	379	1782	297	29.83±2.17	50.61±2.00	27.45±1.30

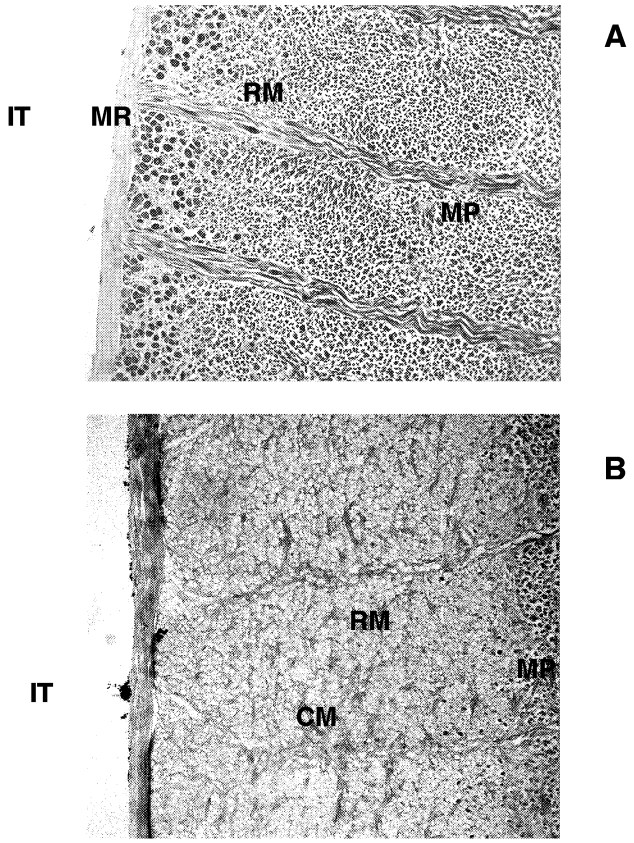

Figure 7.7. *Nototodarus gouldi.* Formation of the collagenous intra-musculature matrix (CM) identified in some individuals. Illustration shows the inner region of the mantle muscle fibre arrangement, with the inner tunic (IT), mitochondria-rich fibres (MR), mitochondria-poor (MP), and the radial muscle fibres (RM), in a section of normal tissue (a), and a section without any circular fibres present with a collagenous matrix remaining (b).

DISCUSSION

Generally, the structure of *N. gouldi* mantle-muscle is like that described for other squid species (see Packard and Trueman 1974; Moon and Hulbert 1975; Bone et al. 1981), with the exception of the longitudinal fibres that lie internally to the outer mantle tunic at the AM and PM positions. The occurrence of longitudinal fibres, external to the circular fibre arrangement, suggests that *N. gouldi* mantle-muscle more closely resembles that described for *Sepia officinalis* (Bone et al. 1981) in comparison to loliginids (Ward and Wainwright 1972; Bone et al. 1981). Although the function of longitudinal muscle-fibres within the mantle structure is not understood, it is possible that it provides some control over longitudinal mantle extension as seems to occur in the fin of *Sepia officinalis* (Kier 1989). In addition, as these fibres are far more abundant in the posterior position of the mantle, it may also be likely that they provide an important part of the hydrostatic skeletal support necessary for active fin movements. There are few studies on ommastrephid mantle-muscle structure and

organisation (see Moon and Hulbert 1975 as an exception) thus, a comparison of within-family muscle-fibre organisation is limited.

Changes in mantle-muscle organisation with maturation, suggests *N. gouldi* undergoes some muscle re-organisation, possibly including muscle-fibre utilisation, as a result of maturation, unlike that observed for *Illex argentinus* (Hatfield et al. 1992). A reduction in muscle block width and size of both mitochondria-rich and poor muscle fibres with maturation at the anterior position, and the decline in the proportion of mitochondria-rich muscle fibres at all positions, indicates a change in either the growth mechanism of muscle fibres, or the re-absorption of protein from the mantle at the cellular level. In addition, as the growth of *N. gouldi* is continuous (Jackson et al. 2003), and the formation of muscle blocks may occur more rapidly anteriorly as it does in other species (using mantle fibre analysis see Moltschaniwskyj 1994; Pecl and Moltschaniwskyj 1997; Martinez and Moltschaniwskyj 1999; for gladius growth analysis see Perez et al. 1996), the occurrence of smaller sized muscle-blocks and a greater proportion of small muscle-fibres at the anterior end of the mantle, suggests the AM may be a major site of muscle growth by new fibre and muscle block generation, during the later stages of maturation. Thus, it is probable maturation effects the generation of mitochondria-rich and poor muscle fibres and the formation of new muscle blocks, but not existing fibres or blocks (as also suggested by Moltschaniwskyj 1994).

The width of the mitochondria-rich zone within the mantle muscle declined with maturation; therefore, either of two possible processes influenced the amount of mitochondria-rich fibres within the mantle. Either mitochondria-rich fibres were being absorbed, or alternatively, the content of mitochondria within the fibre decreased. Pecl and Moltschaniwskyj (1997) found that the circular muscle-fibres of small *Idiosepius pygmaeus* had larger cores of mitochondria than large individuals, suggesting a change in fibre structure with size. If the reduction in mitochondria is considerable, this could possibly result in a change from one fibre type to the other, although this process has not been documented in any squid species. This in turn, would have functional significance, as mitochondria-rich and mitochondria-poor muscle fibres in squid, are analogues of red and white muscle fibres of vertebrates (Mommsen et al. 1981). Alternatively, and perhaps more credible, is the absorption or utilisation of mitochondria-rich fibres during reproductive development. Unfortunately, the mechanisms of how mitochondria-rich fibres are produced are still not known, thus the mechanism driving the decline cannot be clarified.

As the relative proportion and total width of both mitochondria-rich zones declined noticeably with maturation in *N. gouldi*, it is likely that the production of new fibres in mature females was

predominately of the mitochondria-poor type, similar to *Photololigo* sp. (Moltschaniwskyj 1994), although unlike the smaller *Idiosepius pygmaeus* (Pecl and Moltschaniwskyj 1997). This may have occurred through either the recruitment of new mitochondria-poor fibres or the growth of existing fibres, or more probable, a combination of both. At the AM position, smaller mitochondria-rich and poor fibres were in greater proportion. Therefore, it is likely that a decrease in the width of the mitochondria-rich zone was due either to the formation of new smaller mitochondria-poor fibres, which did not then undergo hyperplasia, or the partial-absorption of existing fibres. In addition, as squid have continuous growth, it is likely that a decrease in the proportion of large mitochondria-poor fibres at the AM position, could be a result of partial-absorption of larger fibres, and continued production of smaller fibres. As squid grow, possibly through the addition of muscle blocks and muscle fibres anteriorly (e.g. Moltschaniwskyj 1994) it seems squid compensate for a reduction in the energy available for growth due to maturation, by utilising or partially absorbing mitochondria-rich fibres and producing smaller mitochondria-rich fibres at the anterior position.

Interestingly, at the MM position, the proportional width of the mitochondria-rich zone decreased with maturation, however this was not associated with a clear decline in the proportion of smaller mitochondria-rich fibres. However, correlated with this decline, is the subsequent increase in the proportion of large mitochondria-poor muscle fibres, which probably occurred through hyperplasia, as it would seem unlikely that new muscle fibres could be recruited without forming as small muscle fibres first, that undergo subsequent increases in size. Thus it appears at the MM position, an economical use of energy during maturation may be to preferentially utilise whole mitochondria-rich fibres. It appears from these results that the relative importance of hypertrophy, hyperplasia and absorption at the MM position requires more research before firm conclusions can be drawn.

As there was no change detected in the proportion of either small or large mitochondria-rich fibres with maturation at the PM position although the total proportion of mitochondria-rich fibres decreased, this would suggest both large and small fibres were being absorbed in equal proportion, maintaining the relative amounts of each size, although, resulting in an overall decrease in mitochondria-rich fibre number. This probable decline in mitochondria-rich fibre number was associated with an increase in the proportion of large mitochondria-poor fibres, which as with the MM position, is possibly due to hyperplasia. Overall, it would appear that *N. gouldi* utilizes a number of mechanisms to compensate for the energetic constraints on mantle muscle development with maturation, and use of these depends on maturity stage and position along the mantle.

The need to retain or increase the proportion of mitochondria-poor fibres within muscle blocks of pelagic squids, indicates that these fibres are potentially of greater importance to the function of the mantle-muscle, or, that they are needed in greater proportion than mitochondria-rich fibres. This is supported by the observation that the proportion of large mitochondria-poor fibres increased with maturation at the MM and PM positions, whereas the proportion of mitochondria-rich fibres sizes altered inconsistently at the MM position and remained constant at the PM position. This reduction in the proportion of mitochondria-rich fibres with maturity may have been due to the relative ease of absorbing muscle fibres where capillary networks are greatest. Capillary plexuses are denser in the inner and outer surfaces of the mantle in contrast to the central mantle region (Bone et al. 1981). Capillaries appear to be spaced only ~5-8 muscle fibres apart in the inner and outer surface regions (Bone et al. 1981), probably due to the need to transport oxygen to the aerobic mitochondria-rich fibres found there. In contrast, capillaries may be spaced up to 20 or more fibres apart in the central region (as in *Alloteuthis* Bone et al. 1981). Therefore, the same mechanism that supplies oxygen to the mitochondria-rich fibres may also be responsible for their preferential absorption

A clear decrease in the proportion of large mitochondria-rich fibres was observed at the AM, whereas at the PM they were found in similar proportions. In contrast to these two patterns, there was an inconsistent change in the proportion of mitochondria-rich fibre size with maturation at the MM. The lack of a definitive pattern at the MM position may have been a consequence of exactly where along the mantle the tissue samples were obtained, and while care was taken to standardize the sampling protocol, a slight alteration in sampling of the MM position either anteriorly or posteriorly could have yielded different results. In addition, as squid were collected from a wild fishery, it is probable that individuals experienced different ambient environmental conditions depending on month of hatching, resulting in differences in maturation and growth patterns. An experiment in which cultured animals were kept under controlled conditions at known temperature and dietary regimes, and sacrificially sampled throughout maturation, may provide valuable insight into how energy is utilised for mantle growth during maturation.

The importance of mitochondria-poor muscle fibres within the mantle of pelagic squids may be a function of their lifestyle. Ommastrephids are primarily oceanic, and exhibit broader movement patterns than most near-shore species. Therefore, their demands for movement may arguably be higher than many loliginid squids, which have been the subject of the majority of mantle muscle structure studies (e.g. Bone et al. 1981, Mommsen et al. 1981,.Gosline et al. 1983, Moltschaniwskyj 1994) As such, mitochondria-poor muscle fibres in pelagic species such as *N. gouldi* would appear to be needed in a greater proportion, as these are the primary muscle fibres used for both intermediate

and rapid movement (Bartol et al. 2001), and for use during predation and escape (Mommsen et al. 1981; Gosline et al. 1983). Although historically, the muscle contraction needed for respiration and steady-state swimming has been attributed to the use of the non-fatiguing mitochondria-rich muscle fibres (Bone et al. 1981), recent examinations into the structure and mechanisms of squid mantle muscle have emphasised the role of the intra-muscular collagen network in providing a store for potential energy (MacGillvray et al. 1999; Bairati et al. 2003). This collagen network may play a pivotal role in providing much of the recoil mechanism for respiration and non-escape locomotion, possibly minimising the need for mitochondria-rich fibres, which were traditionally thought to carry out this function in loliginids (Gosline et al. 1983). Therefore, as maturation in *N. gouldi* results in a decline in the proportion of mitochondria-rich fibres within the mantle, mature animals may rely primarily on the use of the intra-muscular collagen network, radial muscle fibres, and mitochondria-poor circular fibres for much of their mantle-muscle contractions.

The observation of a small number of squid with a possible collagen intra-muscular matrix without any circular fibres is an interesting result. A structure of this type has only been described once before on previously frozen then fixed tissue of *Moroteuthis ingens* (Jackson and Mladenov 1994) and is distinct from the nodes of disorganised circular muscle fibres described by Moltschaniwskyj (1997). It is unlikely the matrix described in the present study is an artefact due to histological examination, as a sample of the original fixed tissue was observed under a dissecting microscope and clearly showed a white band separating the normal circular fibres from the inner tunic where the matrix occurred. In addition, as all squid were fresh and not frozen and as the order of processing occurred as follows; mature, immature, preparatory, then maturing, the presence of the matrix is probably not a result of handling or contamination. Similarly, if the matrix was due to problems with fixation, then the occurrence would be expected in the central mitochondrial-poor region of the mantle where fixative may not have penetrated (Moltschaniwskyj 1997). Unfortunately there were not enough individuals with this condition to perform a statistical analysis; however, as the matrix failed to appear in immature animals and an increase in the extent of the matrix was coupled with an increase in size and/or maturation, the matrix phenomenon is probably genuine. It is therefore likely that the collagen matrix is either the only structure remaining after total absorption of the inner mitochondria-rich zone, or it has grown in place of the mitochondria-rich fibres. As the results of this study show a marked decline in the width of the inner mitochondria-rich zone with maturation, it is perhaps possible that the collagen matrix is what is left following the total absorption of fibres along the inner region of mantle.

Conclusions

Throughout maturation, the structure and organisation of *Nototodarus gouldi* mantle muscle changed. These changes could not be detected at the whole animal level suggesting that either a re-organisation

of fibre type within the mantle tissue occurs with maturation, or the cellular changes are too subtle to detect using whole tissue weights. Alternatively, changes in mantle structure may have been masked at higher levels of organisation, potentially due to the uptake of water as a replacement for protein resources. However, such correlations between maturation and the proportion of water in the mantle have not been found in other squid species, where variations due to reproductive status have been examined via proximal analysis (e.g. Moltschaniwskyj and Semmens 2000, Semmens and Moltschaniwskyj 2000). It therefore appears that the mantle tissue of *N. gouldi* like *Photololigo* (Moltschaniwskyj 1994, 1995) is affected by reproduction at the cellular level, although the changes do not correspond to declines in mantle condition at higher levels of organisation. Further examinations of changes in mantle muscle structure using spent individuals would allow us to understand the absolute consequences of maturation on mantle integrity. Moreover, studies comparing changes in individuals obtained from varying ambient conditions could provide insight into the relationship between patterns of repro-somatic investment and mantle muscle integrity.

BENEFITS AND ADOPTION

This research project has provided needed information for the developing management policies for the arrow squid fishery. Ongoing information has been presented to both squidMAC and squidFAG meetings during the course of the research. This report provides a comprehensive presentation of the results obtained.

These results will directly benefit AFMA as it continues to work to develop appropriate management tools for enabling the sustainability of the arrow squid fishery. The primary beneficiaries will be the commercial fishers who are engaged under the commonwealth arrow squid fishery. However, the state fishery in Tasmania and the Great Australian Bight fishery will also benefit (both of which catch arrow squid).

Since the arrow squid fishery is still in the early phase of exploitation and so little was known on the biology of arrow squid, we had much to find out. Thus it is difficult to quantify direct flow of benefits as much if the data obtained provides basic biological information necessary for understanding the dynamics of this species.

The project has also contributed generally to the scientific community by enhancing our understanding of squid biology in a broader context. This data will be of interest to scientists around the world who are working in the fields of fishery management and ecosystem management generally. The significance of the results are highlighted by the publishing of some of the work already in international journals such as *Marine Biology*, *Marine Ecology Progress Series* and *ICES Journal of Marine Science*.

FURTHER DEVELOPMENT

While this research project has resulted in a much greater understanding of arrow squid biology, there are a number of research areas that need to be pursued to further answer critical questions. Some of these further areas of research include:

More comprehensive genetic studies to determine stock structure of arrow squid.

Research into movement and migration patterns.

Biological differences between jigged and trawl-caught squid as future TAC's may be needed to be applied separately to each of these fishing sectors.

Comprehensive diet analysis to better understand how arrow squid fit into the broader ecosystem.

Further analysis of how environmental variables (such as sea surface temperature and sea surface colour) can help to explain variations in squid growth. This technique also has the exciting potential to develop a predictive model where environmental parameters may help to predict times of successful squid growth and recruitment.

PLANNED OUTCOMES

Not applicable – (at the time of the application, planned outcomes were not a requested part of the application)

CONCLUSIONS

Objective 1: undertake extensive statolith age studies to determine validated age, growth rates and life spans throughout the fishing region both spatially and temporally.

Use of statolith ageing for understanding the spatial and temporal aspects of arrow squid growth as an integral part of this project. This was used extensively over two years of spatial/seasonal sampling at the four regions (Ulladulla, Port Lincoln, Lakes Entrance, Tasmania) and over the temporal study over the 12 month study at Portland. Overall 2,969 squid were collected and processed in the lab. For these squid detailed dissections were undertaken which provided much of the data for the project. Of these collected squid, 1,586 were aged using statolith ageing techniques. This provided us with an extensive data set for the study. This data was thus used to obtain a comprehensive understanding of the spatial and temporal differences in growth rates and life spans across many regions of the fishery. Our research showed that squid completed their life cycle in < 1 year and appear to hatch throughout the year. Trends in size, growth and maturity varied considerably between sites, seasons and years. Squids hatched in summer-autumn grew consistently faster than squid that hatched in winter-spring, presumably due to the influence of temperature on growth. Squid in 1999/2000 also grew faster than squid in 2000/2001. Growth of female squid in winter correlated with sea surface colour (SSC) during peak hatch periods but the SSC relationship did not exist for males. Ulladulla squid were generally smaller, younger and had smaller gonads than most other squid and were possibly a smaller morph of the species. Tasmania and Lakes Entrance tended to have larger older individuals with larger gonads while Port Lincoln was variable and intermediate. However, during spring 2001 both Tasmania and Port Lincoln had individuals that were much smaller than the other seasons for these sites and were more like Ulladulla. Trends in age of mature individuals showed considerable variability (over 100 d from youngest to oldest) and there appeared to be a cline across all sites/seasons. Arrow squid appear to reveal marked plasticity in age, growth and maturity parameters but currently the extent to what environment or genetics control plasticity is unclear.

The temporal patterns in growth off Portland revealed considerable variability in growth rates between males and females with no clear seasonal trends in growth rates evident. The ageing work off Portland was especially valuable in revealing the presence of at least four cohorts present in this region over the course of the year.

Unfortunately, the results of the validation experiment were inconclusive. However, the assumption of daily periodicity in statolith increment production is not unreasonable. The experiments carried out highlighted the difficulties with collecting and maintaining oceanic squids successfully.

Objective 2: Assess rates and timing of maturity, and the effect that the maturation process has on muscle growth and body condition of arrow squid.

The underlying reproductive strategy of female *N. gouldi* was determined, with the relative weight of the mantle, fin and digestive gland remaining unchanged during ovarian development, suggesting energy was not being diverted away from somatic growth during sexual development, and consequently neither muscle nor digestive gland was being utilised as an energy store. Mean GSI was low, which is characteristic of a multiple spawning strategy and it is likely that the cost of maturation is largely being met by food intake. The presence of stretched empty oviducts is further evidence that egg production in N. gouldi is slow and steady, with ova being released in discrete batches over a period of time.

Changes in reproductive strategies of *N. gouldi* were sex specific and varied over both broad spatial (4 locations) and temporal scales (bi-annual) and also over finer, monthly temporal scales. Over broad geographic scales the division of energetic resources showed little evidence of gonad development occurring at the expense of the soma regardless of season, sex, location or life-time growth rate. The only variation in female strategies found on a broad-scale was between high and low latitude sites, with female squid caught from lower latitude sites showing higher levels of gonad investment in comparison to their higher latitude counterparts. In contrast, when females were caught on a monthly basis, females caught during the cool months were larger, grew slower and had lower gonad investment and better somatic condition than females caught during the warmer months, suggesting a trade-off between gonad investment and somatic condition. Males on the other hand showed both broad-scale spatial and temporal changes as well as small-scale temporal changes in reproductive traits, with spring caught males having greater levels of investment than in autumn; in addition males from low latitude had higher gonad investment than those from higher latitudes. Patterns of repro-somatic investment had implications for spawning strategies as females with higher gonad investment apparently released eggs simultaneously, whereas females with low gonad investment possibly spawned eggs independently of one another.

Muscle fibre dynamics of *N. gouldi* were investigated relative to reproductive development to quantify the cellular cost of reproduction in a highly mobile species. Changes in the proportion of large and small muscle fibres, a decline in the proportional zones of mitochondria-rich fibres throughout the mantle, and a decrease in the width of muscle blocks at the anterior end, suggests a decline in energy available for muscle growth occurs with maturation. As these cellular changes could not be identified at the whole animal level, the cost of reproduction to mantle tissue is likely to be small.

Objective 3: Identify squid stocks using genetic tools to determine if there is a single or multiple stocks and whether the Australian stock is separated from the New Zealand stock.

Allozyme electrophoresis was used to examine species boundaries and stock structure among arrow squid populations across southern Australia. Samples collected from six localities around southern Australia, separated by distances of between 700 and 4300 km, were examined for allozyme variation at 48 loci. The data revealed no evidence of more than a single species among the 203 squid examined. Nine polymorphic loci were detected, although only three were sufficiently variable to provide real insight into the population structure of arrow squid. There were no significant deviations from Hardy-Weinberg expectations for any locus, population or for the metapopulation. Pairwise comparisons of allele frequencies revealed minor evidence of stock structure, with the Iluka, north New South Wales sample set displaying significant allelic differences from the Tasmanian sample set at *Acyc* and from the Ulladulla, south New South Wales sample set at *Sordh*. F-statistics also provided weak support that the Australian metapopulation is not panmictic. Further studies are needed to delineate the degree of stock segregation within the Australian/New Zealand region in order to successfully manage the arrow squid fishery in these waters.

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APPENDIX 1: Intellectual property

Not applicable

APPENDIX 2: Staff involved

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