

G. D. Jackson · J. M. Semmens · K. L. Phillips
C. H. Jackson

Reproduction in the deepwater squid *Moroteuthis ingens*, what does it cost?

Received: 28 July 2003 / Accepted: 30 March 2004 / Published online: 7 May 2004
© Springer-Verlag 2004

Abstract The cost of reproduction for the terminal spawning onychoteuthid squid, *Moroteuthis ingens*, was analysed using measures of condition and tissue biochemistry. Both males and females showed a dramatic drop in the weight of the gonad in stage 6 (spent) individuals. The mantle weight and nidamental gland weight of females also decreased during the maturation process. Males, however, had a marked increase in both the penis and spermatophoric complex weight in spent individuals, while female oviducal gland weight and nidamental gland length also increased in stage 6 individuals. Residual analysis indicated that testis growth was not developing at the expense of mantle growth, although there was a suggestion of cost to the fins. Females showed that the development of the ovary occurred at a cost to both the mantle and fins. Overall body condition also declined with maturity stage for both males and females, with stage 6 individuals of both sexes in poor condition. Very few females had eggs in the oviducts, suggesting that the oviducts are used as ducts instead of storage organs. Proximal analysis revealed a loss of constituents within the mantle during maturation, with an associated increase in water, indicating the remobilization of energy from the mantle to fuel reproduction. This study suggests that the digestive gland is not used as an energy store in this species.

Introduction

Reproductive strategies and the associated costs of maturation in cephalopods are currently a topical issue. Additionally, there continue to be challenges with classifying the reproductive mode, due in part to the general paucity of information for many species (Rocha et al. 2001). Historically, squids have been viewed as semelparous with a single spawning event followed by death. This has been supported for some species (e.g., *Moroteuthis ingens*, Jackson and Mladenov 1994; *Galiteuthis glacialis*, Nesis et al. 1998; *Gonatus onyx*, Seibel et al. 2000; *Gonatus fabricii*, Bjorke et al. 1997). Furthermore, *Gonatus* spp. provide egg care and brooding that is more akin to an octopod strategy, where the female stays with the eggs to maintain and protect them. Conversely, research is continuing to reveal that repeated spawning events, along with continued feeding and growth, are more common in many squid species (Harman et al. 1989; Lewis and Choat 1993; Moltschaniwskyj 1995; Melo and Sauer 1999; Maxwell and Hanlon 2000; Pecl 2001; McGrath and Jackson 2002).

The reproductive strategy exhibited by a species is inextricably linked to the source of reproductive energy (i.e., feeding or storage), which in turn impacts on the relationship between reproductive growth and somatic condition. Life history theory predicts that semelparous species invest more in their single spawning event compared to multiple spawning species (Calow 1973). This in turn can result in energy being sequestered from somatic tissues to fuel the large reproductive event. Substantial loss of somatic tissue to fuel reproduction has been documented in both octopus, *Octopus vulgaris* (O'Dor and Wells 1978), and *Loligo opalescens* (Fields 1965). In contrast, an investigation of the mode and cost of reproduction in females of the Australian ommastrephid squid, *Nototodarus gouldi*, found that this species produced multiple, small batches of eggs with no discernible cost to somatic tissues (McGrath and Jackson 2002). Thus, feeding during the maturation phase fuelled

Communicated by M.S. Johnson, Crawley

G. D. Jackson (✉) · K. L. Phillips · C. H. Jackson
Institute of Antarctic and Southern Ocean Studies,
University of Tasmania, Private Bag 77, 7001 Hobart,
Tasmania, Australia
E-mail: george.jackson@utas.edu.au
Tel.: +61-3-62262975
Fax: +61-3-62262973

J. M. Semmens
Marine Research Laboratories,
Tasmanian Aquaculture and Fisheries Institute,
University of Tasmania, PO Box 252–49,
7053, Tasmania, Australia

reproduction. Such a strategy has also been documented for other ommastrephids, namely *Sthenoteuthis oualensis* (Harman et al. 1989) and *Illex argentinus* (Rodhouse and Hatfield 1992; Hatfield et al. 1992). It has been suggested that there is likely to be a continuum of reproductive strategies among cephalopods generally, (Jackson and Mladenov 1994), from the 'big bang' single spawning squid (such as *M. ingens*, *L. opalescens* and octopus) to the multiple spawning species as described above. Furthermore, there appears to be flexibility in the mode of spawning within a species or species complex (e.g., *Sepioteuthis australis*, Pecl 2001).

Moroteuthis ingens is one of the more important sub-Antarctic deepwater squid that has a circumpolar distribution between the sub-tropical and polar fronts. Studies on the distribution of *M. ingens* from both the Patagonian Shelf (Jackson et al. 1998a) and southern New Zealand waters (Jackson et al. 2000a) have shown this species to be widespread in the demersal habitat. Throughout the Southern Ocean there seems to be little genetic difference among populations of *M. ingens* (Sands et al. 2003). *M. ingens* is also an important predator of mesopelagic fish (Jackson et al. 1998b; Phillips et al. 2001, 2002) and is significant in the diet of a number of marine mammals and birds (Jackson et al. 1998a; Cherel and Weimerskirch 1999). Stage 6 (spent) onychoteuthids are generally thought to float to the ocean surface and subsequently become available to a number of seabird species (Cherel and Weimerskirch 1999). Thus, obtaining a greater understanding of the reproductive process in this squid has significance to food chain and ecosystem issues in the Southern Ocean.

In this study, we investigated the cost of reproduction to the somatic condition of a single spawning species, *M. ingens*. This extends previous work by Jackson and Mladenov (1994) and Jackson (2001) that established *M. ingens* as a terminal spawner. *Moroteuthis ingens* is sexually dimorphic with females obtaining a much larger size than males. Maturation in females produces a huge ovary that can in fact exceed the total body weight of males (Jackson and Mladenov 1994). As the ovary develops there is a concomitant and dramatic tissue breakdown in the females. This is particularly evident in the mantle and tentacles. The mantle becomes thinned and floppy, virtually losing all its muscle fibre until only a collagen matrix remains, while the tentacles change from thick and muscular to thin, ribbon-like structures. Males also exhibit similar breakdown in the muscle of the tentacles, but this is not so apparent in the mantle.

We know that *M. ingens* is an important species in the sub-Antarctic ecosystem and that the mode of reproduction appears to be a means of transferring energy from the deep ocean to surface predators. This process is driven by the gelatinisation that transforms an individual from a muscular squid to a gelatinous 'balloon'. However, the process by which this takes place at a lower organisational level is unknown.

Therefore, the aim of this study was to document and expand our understanding of the morphological and biochemical constituent changes affiliated with this dramatic maturation and process of tissue disintegration.

Materials and methods

Collection of specimens for morphometric analysis

Individuals ($n=398$; males=208, females=190) were collected in bottom trawls from a number of cruises undertaken by the New Zealand National Institute of Water and Atmospheric Research Limited (NIWA) on the RV "Tangaroa" or the "san Waitaki". Sampling locations were the Chatham Rise region during June/July 1997, ($n=86$, Jackson 2001), July 2000 ($n=11$) and October/November 2000 ($n=24$); and in waters south of New Zealand and extending down to the Campbell and Auckland Islands region ($n=262$). Additionally, squids were acquired ($n=15$) from four cruises during 2000/2001 without location or specific date information. All the morphometric analyses were undertaken on these New Zealand specimens. However, due to damage in some individuals and weight of the oviducts not taken in some samples, sample sizes for the ovary weight /oviduct analysis ($n=14$) and GSI analysis ($n=5$) were increased by using specimens caught in the commercial trawl fishery from waters south of Tasmania, Australia during January 2002 and February/March 2003.

The majority of specimens were frozen then transported to the laboratory prior to dissection. A number of measurements were taken (all wet weights on defrosted or in some instances freshly caught individuals) including, total weight (W) dorsal mantle length (ML), mantle weight and fin weight. Male measurements included testis weight and weight of all other reproductive structures (penis + spermatophoric complex, hereafter referred to as the PSC), while female measurements included weights of the ovary, oviducts, oviducal glands and nidamental glands. Hereafter, oviduct, oviducal and nidamental gland weights all refer to the combined weights of these paired organs. A GSI was calculated for 13 mature females as total reproductive weight / total body weight $\times 100$. Total reproductive weight for females was taken as the combined weights of the ovary + oviducts + oviducal glands + nidamental glands. For seven of the females, oviduct weight was not available so a mean (9 g) was used based on oviduct weight from the other mature individuals.

Collection of specimens for biochemical analysis

Fifty-four individuals of *M. ingens* were collected by the RV *Tangaroa* in southern, sub-Antarctic New Zealand waters, between 46°S and 54°S and between 166°W and

175°W during October–December 2000, using bottom trawls. The digestive gland, mantle muscle and gonad (ovary of females and testis of males) were weighed for most specimens. A sample was taken from each of the digestive gland, muscle and gonad, and frozen for water, lipid, protein and carbohydrate analysis. Only one mature female was collected during this cruise; however, three more mature females (dissected fresh) were collected from commercial fishing operations in waters south of Tasmania during January 2002. Additionally, three fresh frozen individuals were also included from the Chatham Rise, New Zealand, collected during July 2002.

Biochemical analysis

Frozen digestive gland, mantle muscle and gonad were weighed before and after freeze-drying in order to calculate water content. For all analyses, except for lipid in the digestive gland, the proximal composition of tissue was quantified colorimetrically using a spectrophotometer at the appropriate wavelength, with absorbency recorded and converted to μg of protein, carbohydrate or lipid via a standard curve. Protein concentration was determined using a modification of the Lowry method (Lowry et al. 1951), with the standard curve constructed using bovine serum albumin. Carbohydrate concentration was quantified using a modification of the methodology of Mann and Gallagher (1985), via a standard curve constructed using D-glucose. Lipid concentration was determined by a chloroform/methanol extraction (Bligh and Dyer, 1959) and for muscle and gonad tissue converted to μg of lipid via a standard curve constructed using tripalmitin. The concentration of lipid in the digestive gland was determined by weighing the extracted sample, as the high lipid content of the digestive gland made the colorimetric method unreliable. For all analyses, duplicates were run for each individual. The concentrations of the compositional elements were expressed as a percentage of the total mantle muscle, digestive gland and gonad dry weights. All these analyses were undertaken on the 60 individuals used in the biochemical study.

Maturity stages

Individuals were staged according to the Lipinski Universal scale (Lipinski 1979; Sauer and Lipinski 1990). However, for the purpose of this study stage 5 and 6 were modified. Very rarely are eggs ever found within the oviducts of *M. ingens* females. Therefore, we based mature specimens on the presence of mature eggs within the ovary as in other studies (Jackson and Mladenov 1994; Jackson 2001). These eggs were obvious by their size and often fell free during dissections.

Previously, males were classified as stage 6 if no spermatophores were present in the penis or spermatophoric sac (e.g. Jackson 2001). However, it became apparent that often specimens with a thin ribbon-like regressed testis (Jackson and Mladenov 1994); but a large PSC; might also have some or numerous spermatophores. Thus, we chose to designate stage 6 males on the basis of testis weight rather than the presence of spermatophores. If individuals had a testis weight < 3.77 g we considered them stage 6 even if some spermatophores were present. Males with a testis weight > 3.77 g (all of these had spermatophores present) were considered mature (stage 5). The classification of stage 5 and 6 males in this study therefore differs from earlier work by Jackson (2001). The numbers collected for each maturity stage for the morphometric analyses, for males and females respectively were (stage 1: 0,1; stage 2: 0,48; stage 3: 1,109; stage 4: 20,4; stage 5: 109,14; stage 6: 78,14).

Statistical analysis

To investigate the relative investment between somatic and gonad growth we calculated geometric mean regression (Model II) equations (after Green 2001 and McGrath and Jackson 2002) for ML-MW, ML-fin weight, ML-gonad weight for males and females separately. Based on these equations, standardised residuals were calculated for each individual. By regressing each of these tissue weights against ML, the residual value of each individual provides a size-independent method to compare the relative condition of each tissue. Residuals of individuals that were relatively heavier for their length were above the regression line (and were considered in better condition), whereas individuals with lighter residuals were below the regression line (and were considered in poorer condition).

For males and females separately, the ML-gonad residuals were correlated with both ML-fin weight residuals and ML-MW residuals. Similarly, ML-fin weight residuals were also correlated with ML-MW residuals. This type of analysis can highlight any trade-offs occurring between somatic and reproductive growth (McGrath and Jackson 2002). We used ML-MW as a measure of somatic condition. Then, a mean ML-MW condition residual was calculated for each of the maturity stages, for males and females, to determine how somatic condition changed with maturity.

Pearson correlation coefficients were used to determine the association between the individual proximal elements (lipid, protein, carbohydrate and water) of muscle tissue, gonad and the digestive gland and between these proximal elements and the weights of each of these organs (mantle muscle, gonad and digestive gland). Probability values were used as a guide due to the higher probability of type I error rates when conducting a number of correlations on the same data (Moltschanivskyj and Semmens 2000).

Results

Growth with maturity

Males

Mantle length, total weight, mantle weight, fin weight and PSC weights increased with maturity stage (Fig. 1A–E). However, there was a marked drop (89.5%) in mean testis weight from stage 5 (15.3 g) to stage 6 (1.6 g) (Fig. 1F). While the weight of the other parameters in males showed a gradual increase with maturity stage, the weight of the PSC showed a marked increase (229.4%) in weight from stage 5 (27.76 g) to stage 6 (91.45 g) individuals. This was not necessarily related to the production and movement

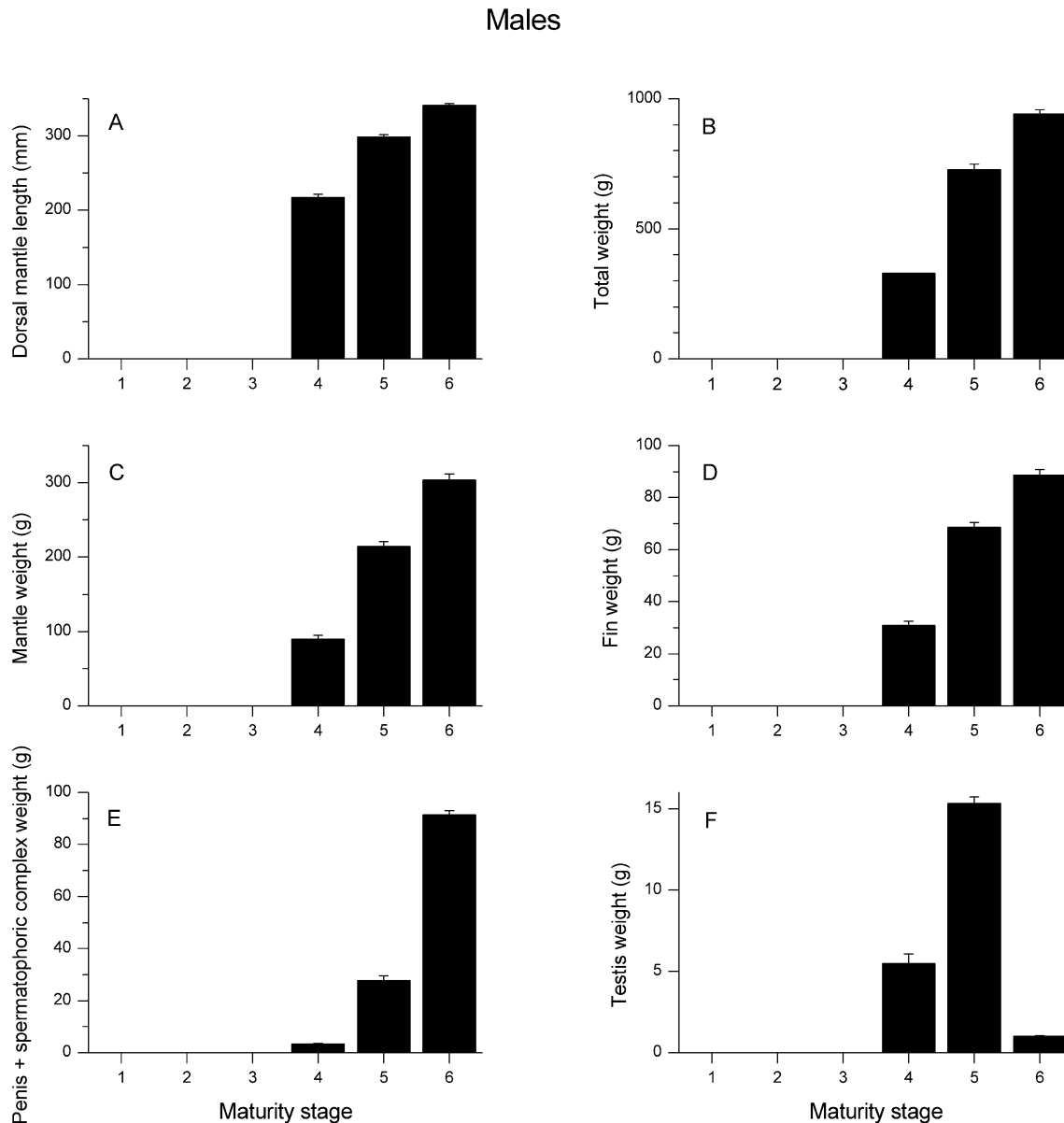
of spermatophores into the penis, as some individuals had few spermatophores. Increase in the weight of the PSC would largely be due to thickening and lengthening of the penis in the late stage males. Thus, the increase in the growth of the PSC occurred with a concomitant and marked regression of the testis.

Testis weight increased in concert with the weight of the PSC until approximately 24 g (Fig. 2). Testis weight then dropped to < 2 g while the PSC increased to > 100 g. At the greatest testis weight, ~24g, the testis was only slightly less than the weight of the PSC (~30 g). However, when the PSC reached their maximum weight (> 120 g), testis weight could be as small as 1/100 of the PSC.

Females

Mean mantle length increased up to stage 4 with little change after that stage (Fig. 3A). In contrast, total

Fig. 1 The change in mean mantle length and weight of selected parameters with maturity stage for male individuals of *M. ingens*



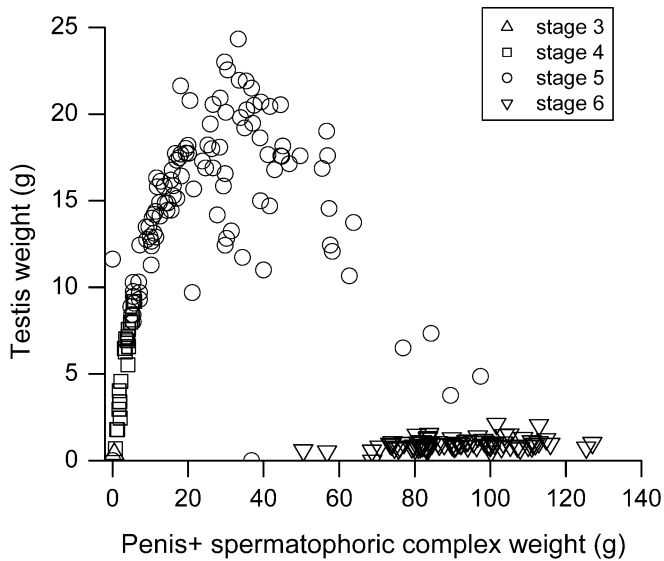


Fig. 2 The relationship between the penis + spermatophoric complex weight and testis weight for male individuals of *M. ingens*

weight, fin weight and nidamental gland weight all showed a steady increase up to maturity stage 5 with a drop to spent, stage 6 individuals. (Fig. 1B,D,F). Mantle weight showed substantial growth up to stage 4, which was then followed by a decline from stages 4–6. (Fig. 3C). Mean nidamental gland length and oviducal gland weight did not decrease with increasing maturity stage (Fig. 3E,G). Mean ovary weight increased substantially from stage 4 to 5 (with some ovaries exceeding 1 kg) followed by a remarkable drop in weight occurring from stage 5 to stage 6 (Fig. 3H). Mean ovary weight dropped by 92.6% from stage 5 individuals (789.37 g) to stage 6 individuals (58.42 g). Mature females had a range in GSI values from 13.5 to 40.5% with a mean GSI of 26.2%, SE 2.35.

Oviducts were generally small and hard to distinguish on many females. Careful dissection was required to remove oviducts for weighing, even for stage 5 and stage 6 individuals, although stage 6 females had larger stretched oviducts. For stage 4–6 females, weight of the oviducts were generally < 10 g and the majority of these contained no eggs or only very few eggs. Two stage 6 individuals did have heavier oviducts (31.8 g, 143.9 g) with some eggs present (Fig. 4). It is possible that these individuals were captured near the end of egg-laying. The evidence suggests that there is no storage of eggs within the oviducts of *M. ingens*.

Condition

The regressions between ML; and gonad, mantle and fin weights were all strong except for ML-testis weight, that was very low due to the small testis size in stage 5 and stage 6 males, because of testis degeneration. Coefficients of determination were relatively high (> 0.78) for all other relationships (Table 1). The residuals of these

regressions were then used to examine investment in both somatic and reproductive tissues. We examined the relationship between ML-gonad residuals and the relative investment in both mantle tissue (ML-MW residuals) and fin tissue (ML-fin weight residuals). Any trend in these comparisons can provide information on the allocation of energy during the maturation process.

Males

There was no relationship between the ML-testis residuals and ML-MW residuals (Fig. 5A). This suggested that testis growth was not developing at the expense of mantle growth, but rather energy for testis development was coming from feeding. There was a positive correlation between ML-testis residuals and ML-fin weight residuals ($P < 0.05$, $n = 198$; Fig. 5B), suggesting some drop in fin condition in concert with testis regression, however, the relationship was weak ($r = 0.24$). There was also a positive correlation between ML-MW residuals and ML-fin weight residuals ($r = 0.50$, $P < 0.001$, $n = 198$; Fig. 5C). Stage 6 males had the lowest condition in both mantle and fins, suggesting that these structures lose condition in concert in post spawning animals.

Females

Residual analysis of the females showed a more defined pattern than the males. There was a positive correlation between ML-ovary residuals and ML-MW residuals ($r = 0.40$, $P < 0.001$, $n = 174$; Fig. 6A). Although stage 5 females were in relatively good condition with large ovaries, the spent stage 6 females were in very poor condition and had exhausted ovaries that were light in weight. This indicates that females invest in mantle tissue up until maturity, and then switch to a rapid breakdown of mantle tissue with mobilisation of mantle protein in association with a cessation in feeding. There is thus no trade-off between somatic and reproductive investment prior to maturity. Virtually no food was ever present in the stomachs of stage 5 and 6 females, indicating that feeding had ceased.

The relationship between ML-ovary residuals and ML-fin weight residuals showed a similar pattern to that described above for ML-ovary : ML-MW residuals, with a significant positive correlation ($r = 0.40$, $P < 0.001$, $n = 174$; Fig. 6B). This suggests that there is also a loss of fin condition after spawning.

There was also a strong positive relationship between ML-MW residuals and ML-fin weight residuals ($r = 0.77$, $P < 0.001$, $n = 181$, Fig. 6C). This indicates that the loss of condition in these two structures occurred in concert, with spent (stage 6) females having both poor mantle and fin condition.

Condition in relation to maturity stage

Comparing the mean somatic condition residual (ML-MW) for each maturity stage revealed the extent of

Females

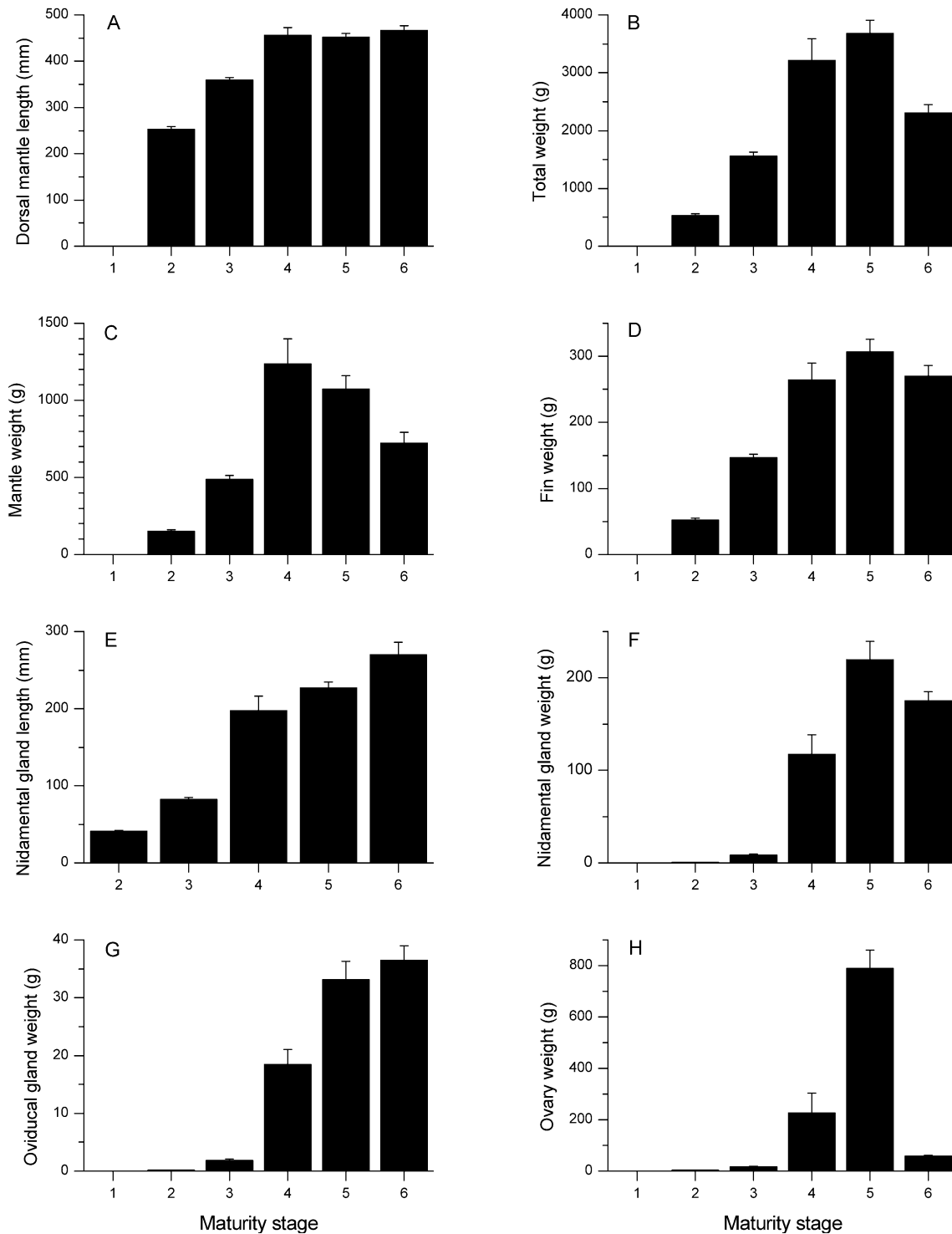


Fig. 3 The change in mean mantle length, nidamental gland length and weight of selected parameters with maturity stage for female individuals of *M. ingens*

change in condition with the maturation process for both sexes. Males were in good condition at stage 4 (Fig. 7A), condition then dropped precipitously

from stages 4–6 with stage 6 individuals in relatively poor condition. Females, likewise, showed an increase in mean condition from stages 2–4 followed by drop in condition from stages 4–6 (Fig. 7B). Stage 6 females were likewise in relatively poor condition.

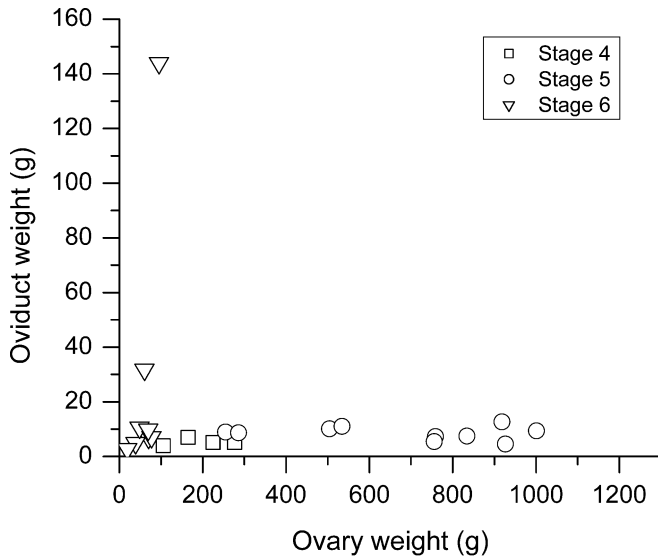


Fig. 4 The relationship between ovary weight and oviduct weight for stage 4, 5 and 6 females of *M. ingens*

Table 1 Geometric-mean (type II) regression statistics for mantle length; and gonad, mantle and fin weights for both males and female individuals of *M. ingens*

	Intercept	Slope	95% Confidence intervals	r^2	n
Females					
ML-ovary wt	-16.37	6.91	6.22-7.59	0.78	178
ML-mantle wt	-5.80	3.30	3.17-3.43	0.93	180
ML-fin wt	-5.41	2.95	2.87-3.04	0.96	180
Males					
ML-testis wt	-19.65	8.19	6.70-9.68	0.08	203
ML-mantle wt	-4.83	2.89	2.80-2.97	0.93	198
ML-fin wt	-4.44	2.53	2.41-2.65	0.89	197

Proximal analysis

Our study of proximal composition was hindered by the lack of stage 6 females available for analysis along with very few stage 5 females. Thus, the results can provide only preliminary trends, and greater sample sizes are needed for a more robust analysis. The situation was better for males, where there were adequate samples from immature through to spent individuals.

General trends observed in the biochemical analysis suggest that the mantle muscle of *M. ingens* was composed predominantly of water, with the dry component mainly protein and to a lesser extent lipid, with relatively low levels of carbohydrate (Tables 2, 3). The digestive gland was also predominantly comprised of water, however, lipid and carbohydrate were present in concentrations up to 3.7 times and 11 times higher, respectively, than those observed in muscle tissue. Conversely, the protein concentration of the digestive gland was up to 4.5 times less than that of muscle. Both

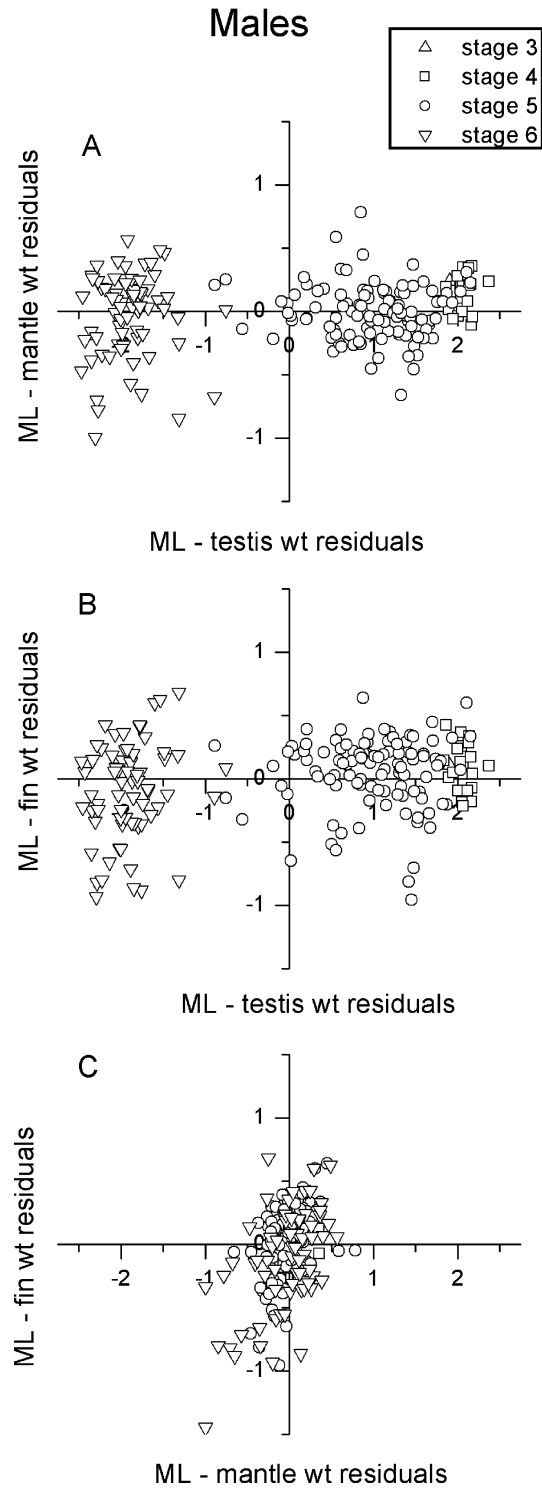


Fig. 5 The relationship between **A** ML-testis weight residuals : ML-mantle weight residuals; **B** ML-testis weight residuals : ML-fin weight residuals; and **C** ML-mantle weight residuals : ML-fin weight residuals for stage 3-6 males of *M. ingens*

testis and ovary were similar in composition to that of the mantle, with relatively high levels of water and lipid and low levels of carbohydrate, although both lipid and carbohydrate were present in approximately twice the

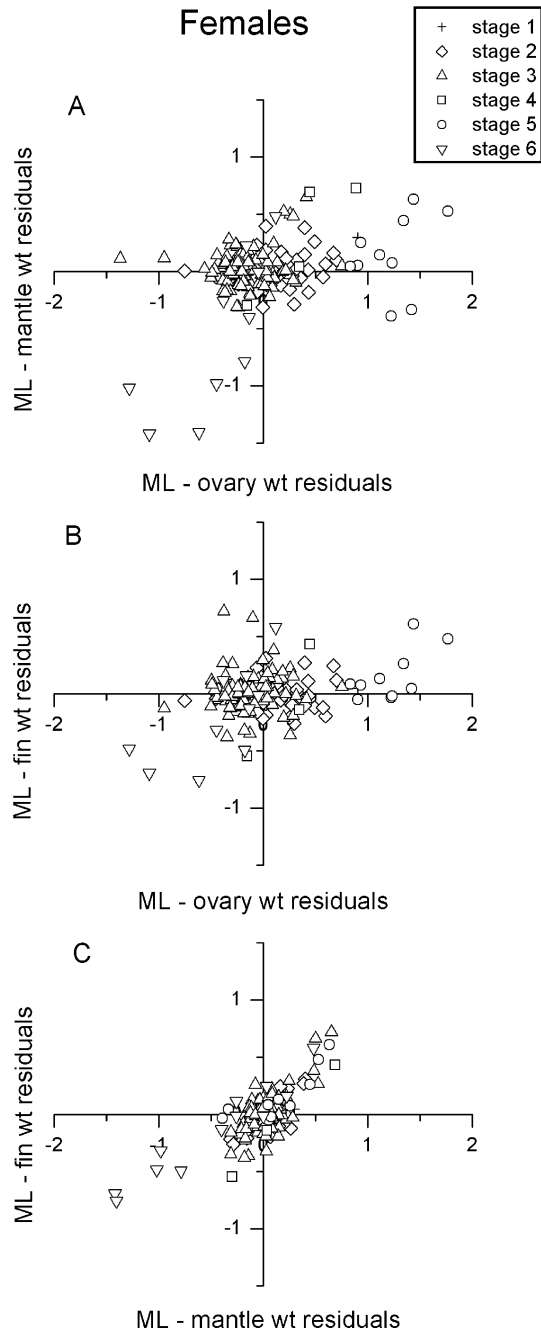


Fig. 6 The relationship between **A** ML-ovary weight residuals : ML-mantle weight residuals; **B** ML-ovary weight residuals : ML-fin weight residuals; and **C** ML-mantle weight residuals : ML-fin weight residuals for stage 1–6 females of *M. ingens*

concentration of that of the muscle. Males and females showed a general trend of water increasing in the mantle, while protein, lipid (except for our single value for a mature female) and carbohydrate all decreased with maturity, suggesting a transfer of energy from the muscle tissue during reproductive maturation. Male digestive glands showed an increase in lipid in the mature and spent individuals, with a small decrease in carbohydrate. Water and protein fluctuated in male

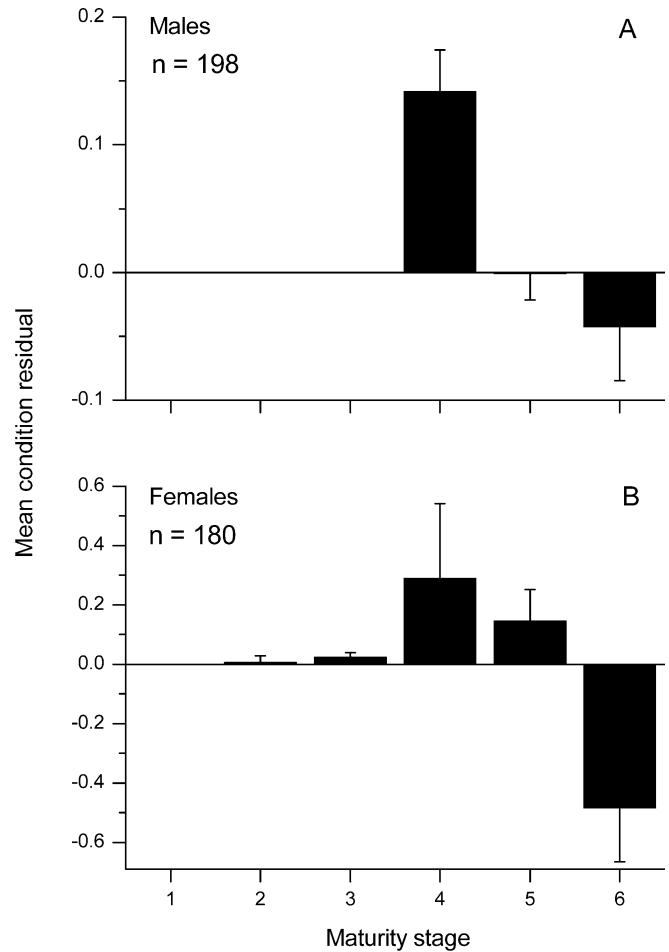


Fig. 7 The mean somatic condition residual (ML-MW) for each maturity stage for **A** males, stage 4–6 and **B** females, stage 2–6 of *M. ingens*

digestive glands with maturity. Water in male testis also showed an increase from immature to spent individuals, while carbohydrate remained relatively unchanged. Similarly, there were no clear trends in carbohydrate in female ovaries with maturity, but in contrast to the male testis, water increased in ovaries with maturity. Lipid levels in male testis showed a slight drop from immature to mature individuals, but were then followed by a rise from stage 5 to stage 6 individuals, with spent testes having a mean lipid concentration that was 2.3 times greater than mature testes. The data also suggested a rise in ovary lipid with maturity in females.

Biochemical correlations

Males

Mantle weight had a strong positive correlation with mantle water ($r=0.78$, $P<0.001$, $n=22$), digestive gland lipid ($r=0.78$, $P<0.001$, $n=16$) and testis water ($r=0.66$, $P=0.001$, $n=22$), suggesting that the mantle and testis lose condition with growth. There were also

Table 2 Proximal composition of male mantle, digestive gland and testis with maturity stage. Asterisk indicates trend not discernable due to lack of replication (values are percentage \pm SE), n is given in parentheses for each analysis. The variation in n is due to removal of outliers

Element	Immature/preparatory, stage 2/3	Mature, stage 5	Spent, stage 6
Mantle			
Water	79.13 \pm 0.72 (8)	82.27 \pm 0.59 (11)	86.5 \pm 1.19 (4)
Protein	52.8 \pm 4.01 (6)	51.08 \pm 5.12 (7)	55.27*
Lipid	31.33 \pm 6.3 (7)	23.17 \pm 1.54 (11)	22.72 \pm 2.44 (4)
Carbohydrate	0.89 \pm 0.20 (7)	0.67 \pm 0.12 (11)	0.65 \pm 0.07 (4)
Digestive gland			
Water	52.45 \pm 2.6 (8)	46.56 \pm 3.15 (11)	63.91 \pm 5.33 (4)
Protein	20.79 \pm 1.63 (5)	19.31 \pm 2.97 (6)	33.46 \pm 8.26 (2)
Lipid	37.04 \pm 8.21 (4)	71.17 \pm 4.96 (10)	79.29 \pm 14.55 (2)
Carbohydrate	4.90 \pm 0.87 (7)	4.15 \pm 0.56 (10)	2.98 \pm 0.88 (4)
Testis			
Water	72.8 \pm 2.15 (10)	78.72 \pm 0.47 (11)	87.5 \pm 1.55 (4)
Lipid	34.68 \pm 5.45 (6)	28.41 \pm 3.44 (10)	64.78 \pm 16.05 (4)
Carbohydrate	1.12 \pm 0.34 (7)	1.54 \pm 0.46 (11)	1.017 \pm 0.53 (4)

Table 3 Proximal composition of female mantle, digestive gland and ovary with maturity stage. Asterisk indicates trend not discernible due to lack of replication (values are percentage \pm SE), n is given in parentheses for each analysis. The variation in n is due to removal of outliers

Element	Immature, stage 2	Preparatory, stage 3	Maturing, stage 4	Mature, stage 5
Mantle				
Water	82.0 \pm 1.21 (8)	85.4 \pm 0.98 (10)	86.2 \pm 2.08 (5)	92.67 \pm 0.88 (3)
Protein	57.17 \pm 0.94 (5)	46.04 \pm 4.06 (8)	34.5 \pm 5.39 (5)	29.47 \pm 5.07 (3)
Lipid	25.4 \pm 1.98 (6)	23.35 \pm 1.71 (9)	18.57 \pm 5.4 (4)	35.20*
Carbohydrate	0.7 \pm 0.19 (8)	0.5 \pm 0.1 (10)	0.44 \pm 0.44(5)	0.35 \pm 0.06 (3)
Digestive gland				
Water	48.63 \pm 2.92 (9)	45.09 \pm 2.09 (9)	61.71 \pm 4.59 (5)	72.00*
Protein	14.06 \pm 0.98 (4)	10.29 \pm 3.09 (2)		19.54*
Lipid	53.3 \pm 9.23 (8)	70.85 \pm 2.56 (7)	62.24 \pm 4.75 (4)	54.99*
Carbohydrate	3.43 \pm 0.28 (8)	4.19 \pm 0.79 (9)	3.7 \pm 0.79 (3)	4.00*
Ovary				
Water	77.17 \pm 1.76 (6)	76.63 \pm 2.17 (8)	67.8 \pm 2.13 (5)	71.00*
Protein			9.87 \pm 0.53 (2)	
Lipid	34.18 \pm 3.7 (6)	46.11 \pm 4.79 (9)	48.45 \pm 5.86 (6)	42.77*
Carbohydrate	1.61 \pm 0.33 (6)	1.0 \pm 0.16 (9)	0.55 \pm 0.14 (6)	2.08*

relatively strong negative partial correlations when controlling for total weight, between digestive gland weight and digestive gland water ($r = -0.85$, $P < 0.001$, $n = 20$), digestive gland protein ($r = -0.62$, $P < 0.05$, $n = 10$), testis lipid ($r = -0.58$, $P < 0.05$, $n = 15$) and mantle water ($r = -0.45$, $P < 0.05$, $n = 20$). This suggests that the digestive gland, while it is losing protein, is maintaining high lipid levels and not losing water as in the other structures.

Mantle water was moderately negatively correlated with digestive gland carbohydrate ($r = -0.46$, $P < 0.05$, $n = 21$) but moderately positively correlated with digestive gland lipid ($r = 0.52$, $P < 0.05$, $n = 16$), while digestive gland carbohydrate was moderately negatively correlated with testis water ($r = -0.49$, $P < 0.05$, $n = 20$). This suggests that the digestive gland was not losing condition to the extent of other structures. Testis water was strongly correlated with mantle water ($r = 0.81$, $P < 0.001$, $n = 22$), while testis lipid was moderately negatively correlated with mantle lipid ($r = -0.50$, $P < 0.05$, $n = 17$), which highlights the fact that the mantle and testis were replacing lost constituents with water.

Females

The correlations among tissue constituents were not as clear for females due to the fact that we had few mature females and no spent females. However, mantle weight was positively correlated with mantle water ($r = 0.64$, $P = 0.001$, $n = 25$) and negatively correlated with mantle protein ($r = -0.68$, $P = 0.001$, $n = 21$), which also suggested that protein was being mobilised from the mantle during growth. There were also significant positive partial correlations when controlling for body weight, between digestive gland weight and both digestive gland carbohydrate ($r = 0.51$, $P < 0.05$, $n = 17$) and mantle protein ($r = 0.94$, $P < 0.001$, $n = 16$). This may suggest that the majority of carbohydrate in the digestive gland is structural and that this organ becomes lighter relative to body weight, as protein is mobilised from the mantle and energy is directed to maturation. There appears to be no obvious explanation for the negative correlations between digestive gland water and both mantle lipid ($r = -0.57$, $P < 0.05$, $n = 17$) and ovary carbohydrate ($r = -0.52$, $P < 0.05$, $n = 20$), except that the concentra-

tions of these constituents do not appear to be related to the composition of the digestive gland. Overall, as was the case for males, there is no evidence to suggest that the digestive gland was being broken down to fuel reproduction in females. There was a negative partial correlation between ovary weight and ovary water ($r = -0.55$, $P < 0.05$, $n = 17$), which suggests that the ovary is increasing in condition with growth.

Discussion

This study has provided a broader understanding of the cost of reproduction in *M. ingens* as described by Jackson and Mladenov (1994). The process of tissue breakdown with maturity is more dramatic in females than males (with the exception of the testis). However, both sexes show a marked drop in condition in stage 5 and stage 6 individuals. Significantly, even in the females, the condition of the fins is maintained in preference to the mantle. This suggests that the fins of *M. ingens* continue to function and are used as a means of locomotion despite a concomitant decrease in mantle condition.

Both male and female individuals of *M. ingens* share in the marked regression of the gonad. In the case of females this is due to egg-release, while in males it represents a cessation of sperm production. In both instances, this results in an organ that is only a fraction of the size seen in mature stage 5 individuals and represents an irreversible process in reproduction. However, growth of the accessory reproductive organs continue (ie., PSC in males, nidamental gland length and oviducal weight in females), even in stage 6 individuals. This highlights how *M. ingens* is able to maintain the integrity of organs essential to the reproductive process.

The reproductive strategy of *M. ingens* appears to be similar to octopus, where brooding females undergo tissue breakdown and remobilisation of protein to fuel reproduction during starvation (O'Dor and Wells 1978). Fields (1965) also noted severe tissue breakdown in the terminal spawning *Loligo opalescens*, with some females losing more than 50% of their total body weight. Fields (1965) described females with mantles 'thin and limp' and with reproductive structures becoming 'small and flaccid'. The observed changes in *M. ingens* are similar to some oceanic gonatids (Arkhipkin and Bjorke 1999; Okutani et al. 1995; Bjorke et al. 1997; Seibel et al. 2000) where the females also go through a process of gelatinisation. The features exhibited by female individuals of *M. ingens* are very similar to those described for female individuals of *G. fabricii* by Arkhipkin and Bjorke (1999), where females become transformed to gelatinous 'floating dirigibles' in preparation to support their negatively buoyant egg masses until hatching of the paralarvae. However, unlike in *M. ingens*, these gonatids increase in weight as they go from mature to spent and gelatinous. The mechanics of egg laying in *M. ingens* is unknown, but it has been suggested that this species

could also 'brood' their egg masses (Jackson 2001) and the tissue breakdown and 'gelatinisation' may be related to this.

The loss of condition in *M. ingens* contrasts to oceanic ommastrephids and other species that maintain their condition, continue to grow and fuel their reproduction from feeding rather than energy stores (Harman et al. 1989; Rodhouse and Hatfield 1992; Hatfield et al. 1992). Recent investigations on somatic condition in concert with gonad development, in the ommastrephid *Nototodarus gouldi* in Australian waters (McGrath and Jackson 2002), provide a useful parallel to our work with *M. ingens*. As is common for other ommastrephids, McGrath and Jackson (2002) could detect no evidence of energy being diverted away from somatic growth during sexual maturation. *N. gouldi* continues to feed and grow and maintain tissue integrity throughout the maturation process. Eggs are released in small batches, with no current evidence for spent (stage 6) individuals with marked tissue degeneration. Work summarised in McGrath and Jackson (2002) noted how females of other multiple spawning ommastrephids have GSI values $< 16\%$. According to life history theory, individuals with the greater percentage investment in gonads are thought to be near the terminal end of the spawning continuum (Pecl 2001). For example, the terminal spawning *Todarodes pacificus* can have GSI values reaching almost 50% (Ikeda et al. 1993). Likewise, the reproductive organs of *Loligo opalescens* can range between 25% and 50% of the total body weight (Fields 1965). The mean GSI value in mature females (26.2%), along with the maximum value of 40.5%, highlights the terminal reproductive strategy of *M. ingens*.

Research into the use of energy reserves to fuel reproduction for tropical *Photololigo* sp. likewise indicated some loss of condition during the maturation process (Moltschaniwskyj and Semmens 2000), and even some localised tissue breakdown (Moltschaniwskyj 1997). However, this was minimal compared to *M. ingens* and it has been suggested that *Photololigo* sp. fuels its reproduction solely from its food intake. Furthermore, work on the temperate *Loligo gahi* has found that the water content of somatic tissues did not increase with maturation (Guerra and Castro 1994), suggesting that tissue integrity was maintained. In contrast to these studies, the temperate loliginid *Loligo forbesi* appears to lose condition in association with maturation (Collins et al. 1995), and although feeding was observed to continue, there appeared to be transfer of energy from the soma to the gonad.

Generally, it is thought that terminal spawning squid accumulate eggs in their oviducts in preparation for a single massive spawning event (Harman et al. 1989; Moltschaniwskyj 1995). Consequently, evidence for a terminal spawning squid, therefore suggests that in mature female individuals, weight of the oviducts should exceed ovary weight (Pecl 2001; McGrath and Jackson 2002). We found no suggestion that *M. ingens* accumulates eggs in the oviducts prior to spawning. On the

contrary, we propose that *M. ingens* uses the oviducts as ducts rather than egg storage devices, where eggs move directly from the ovary to egg-release with little or no intervening time in the oviducts. The immense size of the ovary may make it virtually impossible for all the eggs to be maintained in the oviducts. Therefore, oviduct weight relative to ovary weight may not necessarily provide supplementary evidence for a terminal spawning strategy, although it is possible that *M. ingens* is an exception.

Our proximal composition data do suggest a biochemical constituent cost in reproduction, although drawing conclusions for females was hindered by the lack of stage 6 females in our analysis. However, given the observed trends, we expect that analysis of stage 6 females will reveal an even greater cost than we documented in males. The loss of constituents in the mantle with maturation suggests these are remobilised to help fuel reproduction in absence of feeding.

Interestingly, there were no obvious tradeoffs detected between the soma and digestive gland, suggesting that this organ is not used as an energy store for reproduction. In fact, preliminary results suggest an increase in lipids in immature to stage 6 males. This concurs with earlier conclusions by Semmens (1998), that the digestive gland of loliginid squids is not a storage organ but an organ for excreting excess lipids. This is further supported by fatty acid analyses of the digestive gland, demonstrating that lipids are not greatly modified when passed to the digestive gland of *M. ingens* (Phillips et al. 2001, 2002, 2003), and suggesting an excretory rather than storage role like that proposed for loliginids (Semmens, 1998). Arkhipkin and Bjorke (1999) suggest that the digestive gland of *Gonatus fabricii* is used to fuel reproduction. Alternatively, the decrease in digestive gland weight in spent individuals of *G. fabricii* could be due to the digestive gland ceasing to function and breaking down following the cessation of feeding, as it is an autonomous system using nutrients from intracellular digestion for its own cellular processes (Boucaud-Camou and Yim 1980). The digestive gland would also decrease in weight at the beginning of starvation if the majority of lipid in the gland were excreted as waste.

Conclusion

The reproductive strategy of *M. ingens* presents an interesting scenario of how to fuel a single massive spawning event. *Moroteuthis ingens* is one of the more important squid species within the sub-Antarctic ecosystem (Jackson et al. 1998a, 1998b; Jackson et al. 2000b; Phillips et al. 2003). Despite the extremely large digestive gland possessed by this species (that can exceed 2.5 kg in females, Phillips et al. 2001), we have no evidence that this evidently rich store of calories is of any use in fueling the reproductive process. This would be due to the constraints of a protein-based metabolism

used by squids (O'Dor and Webber 1986; Jackson and O'Dor 2001). Indeed, research on the role of the digestive gland in oceanic squids, as has been carried out with loliginids (Semmens 1998) is needed. The predominant energy store in *M. ingens* is the mantle. When the ovary increases in size to the extent that feeding is physically impossible (Jackson and Mladenov 1994), females then turn to the most abundant energy store, the mantle, resulting in a dramatic loss of condition for the females. While this process is also observed in males, the fact that it is not as marked as in the females is probably due to the huge ovary that is produced in contrast to the testis. Analyses on stage 6 females would also greatly assist in understanding the biochemical changes associated with maturation and spawning in females. Of particular interest is how *M. ingens* deposits its eggs and whether there is any care or brooding of the eggs. The widespread distribution of this species suggests that direct video observations with a remotely operated vehicle could be feasible for such a study.

Acknowledgements We thank staff at NIWA New Zealand who supplied squid samples and allowed J.M.S. to join two research cruises. We are also grateful to the crew of the "Adriatic Pearl" who enthusiastically collected squid in Tasmanian waters. Thanks also to Justin Ho and Louise Ward from the University of Tasmania, who assisted with the biochemical analysis and Tertia McArthur, Eliza Green and Chester Sands who assisted with dissections. Thanks to Belinda McGrath-Steer for the interesting discussions on reproductive strategies. This work was funded by an Australian Research Council Large Grant (no. A19933031). We thank two anonymous reviewers who helped improve an earlier draft of the manuscript.

References

- Arkhipkin AI, Bjorke H (1999) Ontogenetic changes in morphometric and reproductive indices of the squid *Gonatus fabricii* (Oegopsida, Gonatidae) in the Norwegian Sea. *Polar Biol* 22:357–365
- Bjorke H, Hansen K, Sundt RC (1997) Egg masses of the squid *Gonatus fabricii* (Cephalopoda, Gonatidae) caught with pelagic trawl off northern Norway. *Sarsia* 82:149–152
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37:911–917
- Boucaud-Camou E, Yim M (1980) Fine structure and function of the digestive cell of *Sepia officinalis* (Mollusca: Cephalopoda). *J Zool* 191:89–105
- Calow P (1973) The relationship between fecundity, phenology, and longevity: a systems approach. *Am Nat* 107:559–574
- Cherel Y, Weimerskirch H (1999) Spawning cycle of onychoteuthid squids in the southern Indian Ocean: new information from seabird predators. *Mar Ecol Prog Ser* 188:93–104
- Collins MA, Burnell GM, Rodhouse PG (1995) Reproductive strategies of male and female *Loligo forbesi* (Cephalopoda: Loliginidae). *J Mar Biol Ass UK* 75:621–634.
- Fields WG (1965) The structure, development, food relations, reproduction, and life history of the squid *Loligo opalescens* Berry. *Calif Dep Fish Game Fish Bull* 131:1–108
- Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* 82:1473–1483
- Guerra A, Castro BG (1994) Reproductive-somatic relationships in *Loligo gahi* (Cephalopoda: Loliginidae) from the Falkland Islands. *Antarct Sci* 6:175–178

- Harman RF, Young RE, Reid SB, Mangold KM, Suzuki T, Hixon RF (1989) Evidence for multiple spawning in the tropical oceanic squid *Stenoteuthis oualaniensis* (Teuthoidea: Ommastrephidae). *Mar Biol* 101:513–519
- Hatfield EMC, Rodhouse PG, Barber DL (1992) Production of soma and gonad in maturing female *Illex argentinus* (Mollusca: Cephalopoda). *J Mar Biol Ass UK* 72:281–291
- Ikeda I, Sakurai, Y, Shimazaki K (1993) Maturation process of the Japanese common squid *Todarodes pacificus* in captivity. In: Okutani T, O'Dor R, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 179–187
- Jackson GD (2001) Confirmation of winter spawning of *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) in the Chatham Rise region of New Zealand. *Polar Biol* 24:97–100
- Jackson GD, Mladenov PV (1994) Teminal spawning in the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae). *J Zool* 234:189–201
- Jackson GD, O'Dor RK (2001) Time, space and ecophysiology of squid growth, life in the fast lane. *Vie Milieu* 51:205–215
- Jackson GD, George MJA, Buxton NG (1998a) Distribution and abundance of the squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) on the Patagonian Shelf region of the South Atlantic. *Polar Biol* 20:161–169
- Jackson GD, McKinnon JF, Lallas C, Ardern R, Buxton NG (1998b) Food spectrum of the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) in New Zealand waters. *Polar Biol* 20:56–65
- Jackson GD, Shaw AGP, Lallas C (2000a) Distribution and biomass of two squid species off southern New Zealand: *Notodarus sloanii* and *Moroteuthis ingens*. *Polar Biol* 23:699–705
- Jackson GD, Buxton NG, George MJA (2000b) The diet of the southern Opah *Lampris immaculatus* on the Patagonian Shelf; the significance of the squid *Moroteuthis ingens* and anthropogenic plastic. *Mar Ecol Prog Ser* 206:261–271
- Lewis AR, Choat JH (1993) Spawning mode and reproductive output of the tropical cephalopod *Idiosepius pygmaeus*. *Can J Fish Aquat Sci* 50:20–28
- Lipinski MR (1979) Universal maturity scale for the commercially important squids. The results of maturity classification of the *Illex illecebrosus* populations for the years 1973–77. ICNAF Res Doc 79/2/38, Serial 5364, International Commission for the Northwest Atlantic Fisheries, Dartmouth, Canada
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Mann R, Gallagher SM (1985) Physiological and biochemical energetics of larvae of *Teredo navalis* L. and *Namkia gouldi* (Bartsch) (Bivalve: Teredinidae). *J Exp Mar Biol Ecol* 85:211–228
- Maxwell MR, Hanlon RT (2000) Female reproductive output in the squid *Loligo pealeii*: multiple egg clutches and implications for a spawning strategy. *Mar Ecol Prog Ser* 199:159–170
- McGrath BL, Jackson GD (2002) Egg production in the arrow squid *Nototodarus gouldi* (Cephalopoda: Ommastrephidae), fast and furious or slow and steady? *Mar Biol* 141:699–706
- Melo YC, Sauer WHH (1999) Confirmation of serial spawning in the chokka squid *Loligo vulgaris reynaudii* off the coast of South Africa. *Mar Biol* 135:307–313
- Moltschaniwskyj NA (1995) Multiple spawning in the tropical squid *Photololigo* sp.: what is the cost in somatic growth? *Mar Biol* 124:127–135
- Moltschaniwskyj N (1997) Changes in mantle muscle structure associated with growth and reproduction in the tropical squid *Photololigo* sp. (Cephalopoda: Loliginidae). *J Moll Stud* 63:290–293
- Moltschaniwskyj NA, Semmens JM (2000) Limited use of stored energy reserves for reproduction by the tropical loliginid squid *Photololigo* sp. *J Zool* 251:307–313
- Nesis KN, Nigmatullin ChM, Nikitina IV (1998) Spent females of deepwater squid *Galiteuthis glacialis* under the ice at the surface of the Weddell Sea (Antarctic). *J Zool* 244:185–200
- O'Dor RK, Webber DM (1986) The constraints on cephalopods: why squid aren't fish. *Can J Zool* 64:1591–1605
- O'Dor RK, Wells NJ (1978) Reproductive vs. somatic growth; hormonal control in *Octopus vulgaris*. *J Exp Biol* 77:15–31
- Okutani T, Nakamura I, Seki K (1995) An unusual egg-brooding behavior of an oceanic squid in the Okhotsk Sea. *Venus* 54:237–239
- Peel G (2001) Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids. *Mar Biol* 138:93–101
- Phillips KL, Jackson GD, Nichols PD (2001) Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Islands: stomach contents and fatty acid analyses. *Mar Ecol Prog Ser* 215:179–189
- Phillips KL, Nichols PD, Jackson GD (2002) Lipid and fatty acid composition of the mantle and digestive gland of four Southern Ocean squid species: implications for food-web studies. *Antarct Sci* 14:212–220
- Phillips KL, Nichols PD, Jackson GD (2003) Dietary variation of the squid *Moroteuthis ingens* at four sites in the Southern Ocean: stomach contents, lipid and fatty acid profiles. *J Mar Biol Ass UK* 83:523–534
- Rocha F, Guerra A, Gonzalez AF (2001) A review of reproductive strategies in cephalopods. *Biol Rev* 76:291–304
- Rodhouse PG, Hatfield EMC (1992) Production of soma and gonad in maturing male *Illex argentinus* (Mollusca: Cephalopoda). *J Mar Biol Assoc UK* 72:293–300
- Sands CJ, Jarman SN, Jackson GD (2003) Genetic differentiation in the squid *Moroteuthis ingens* inferred from RAPD analysis. *Polar Biol* 26:166–170
- Sauer WHH, Lipinski MR (1990) Histological validation of morphological stages of sexual maturity in chokka squid *Loligo vulgaris reynaudii* D'Orb (Cephalopoda: Loliginidae). *S Afr J Mar Sci* 9:189–200
- Seibel BA, Hochberg FG, Carlini DB (2000) Life history of *Gonatus onyx* (Cephalopoda: Teuthoidea): deep-sea spawning and post-spawning egg care. *Mar Biol* 137:519–526
- Semmens JM (1998) An examination of the role of the digestive gland of two loliginid squids, with respect to lipid: storage and excretion? *Proc R Soc Lond B* 265:1685–1690