The Adaptive Response of Protein Turnover to the Energetic Demands of Reproduction in a Cephalopod

N. A. Moltschaniwskyj[1,*]
C. G. Carter[2]
[1]School of Environmental and Life Sciences, University of Newcastle, P.O. Box 127, Ourimbah, New South Wales 2258, Australia; [2]Institute for Marine and Antarctic Studies, University of Tasmania, Locked Bag 49, Hobart, Tasmania 7001, Australia

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ABSTRACT

Sourcing energy for reproduction is a major driver of the life-history characteristics of animals. Unlike other molluscs, cephalopods do not appear to have significant glycogen stores, and energy is either sourced directly from ingested food or mobilized from protein stores in the muscle. Given the importance of protein to cephalopods, this study quantified changes in protein turnover in the muscle tissue in reproductively immature and maturing/mature individuals. Quantifying protein accretion and protein synthesis allowed an assessment of protein turnover in immature and maturing individuals of the southern dumpling squid (*Euprymna tasmanica*), which has fast nonasymptotic growth, has a short generation time, and does not use lipid stores. This study found that protein turnover slowed in the mantle muscle tissue with gonad growth, suggesting an adaptive response to the energy demands associated with reproduction but one that allows for continued somatic growth and muscle function in these animals. However, the cost of reproduction may be indirect, with less energy available for somatic repair, and therefore may be responsible for the rapid senescence typical of many cephalopod species.

Introduction

Periods of intense energy requirements associated with morphological development, rapid somatic growth, and reproduction occur naturally during the lifetime of animals. Energy required for reproduction is often regarded as a cost, although using stored energy reserves (capital energy) for reproduction is not a direct cost given that the reserves are accrued explicitly for reproduction when surplus energy is available (Houston et al. 2007). However, storing and carrying energy reserves have indirect costs, especially for mobile species (Houston et al. 2007), but are strategies adopted by animals that require energy for reproduction to be on tap, for example, the coordinated timing of gamete production and release by broadcast spawners. In animals for whom the cost of storing energy is too great, or food is predictably available, or gamete production does not need to be coordinated, energy for reproduction can be sourced directly from ingested food (income energy; Jonsson 1997). Cephalopod life-history models suggest that different groups use different strategies in sourcing energy (capital vs. income) for reproduction.

Most molluscan species store energy as glycogen rather than lipid (Suryanarayanan and Alexander 1971), but evolution of cephalopods from the molluscan lineage appears to have resulted in the loss of glycogen as a stored energy reserve (Suryanarayanan and Alexander 1971; O'Dor and Webber 1986). While it is proposed that lipid stored in the digestive gland may support reproductive growth, evidence for this is largely based on morphometric analysis of the digestive gland (Arkhipkin and Bjorke 1999). There is evidence that cephalopods have a protein-based metabolism (O'Dor and Webber 1986; Lee et al. 1994) and surplus energy is used for somatic growth and not stored as lipid. Yet the protein in mantle muscle may be viewed as a form of stored energy (e.g., cuttlefish; Castro et al. 1992) and used to fuel reproduction (e.g., oceanic squid *Moroteuthis ingens* [Jackson and Mladenov 1994; Jackson et al. 2004] and *Gonatus onyx* [Seibel et al. 2000] and *Octopus* species [Rosa et al. 2004; Semmens et al. 2004]). An energy model based on mobilizing protein rather than lipid or glycogen is evident in other molluscs (Hawkins and Bayne 1985) and allows animals to respond to periods of intense energy demands, for example, stress and physical damage (Hawkins 1991). When cephalopod species mobilize protein as an energy source during egg production or egg brooding, this compromises the structure and integrity of the mantle muscle, causing the females to die shortly after spawning or hatching of the eggs.

Not all cephalopods are terminal spawners, and a number of squid and sepiolid species will produce multiple batches of eggs over their adult lifetimes (Harman et al. 1989; Moltschaniwskyj 1995; Steer et al. 2004). Many of these species show no evidence of significant changes in morphometric or proximal composition of the digestive gland and mantle muscle tissue associated with reproduction, appearing to fuel reproduction directly through ingested food (i.e., income energy). Use of income energy sources in cephalopods is proposed across a wide range of species—ommastrephid squids (McGrath and

* Corresponding author; e-mail: natalie.moltschaniwskyj@newcastle.edu.au.

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Jackson 2002; Rosa et al. 2005), loliginid squid (Hatfield et al. 1992; Moltschaniwskyj and Semmens 2000; Semmens and Moltschaniwskyj 2000), and octopus (Rosa et al. 2004)—and may explain the variability in the age and size at reproductive maturation typical of many cephalopods (Arkhipkin and Laptikhovsky 1994; Pecl et al. 2004; Tafur et al. 2010). However, identifying the best metrics to use to determine the use of capital versus income reproduction is challenging (Houston et al. 2007). Many metrics assume that energy resources are limited, and as such they focus on changes in biological processes that require energy, for example, whole-body somatic and reproductive growth, or track changes in tissue composition associated with these processes.

One cost of reproduction not measured by these metrics is naturally occurring somatic damage that is not repaired because energy is being directed to reproductive activity (Harshman and Zera 2006). A short life span, typically 12 mo or less, is a very distinctive life-history characteristic of cephalopods, with patterns of somatic and reproductive growth suggesting physiological progenesis, characterized by accelerated reproductive maturation and no extended adult phase (Rodhouse 1998). There is evidence in octopus species of a strong mechanistic link between reproduction and senescence; individuals that are prevented from reproducing do not undergo senescence and live longer (O'Dor and Wells 1978). Therefore, for cephalopods that fuel any or all reproductive activities directly from ingested energy, the cost of reproduction could be a reduction in energy available to repair biological systems, which contributes to rapid senescence and death.

Protein turnover describes the processes of protein renewal, replacement (repair), and accretion through the synthesis of new proteins and degradation of existing proteins (Carter and Houlihan 2001). Rates of protein turnover depend on protein growth; protein turnover is equal to protein synthesis in animals losing protein and equal to protein degradation in animals accreting protein (Houlihan 1991; McCarthy et al. 1994). The relative rates of protein synthesis and degradation in the mantle muscle tissue of cephalopods, the major site of structural protein, should adapt to the physiological needs of the animal (Hawkins 1991). Most cephalopods have indeterminate growth; therefore, if reproduction is fueled by income energy and excess food is ingested, then the relative rates of protein synthesis and degradation in somatic tissue and reproductive tissues should be independent. Accordingly, we would expect that rates of protein synthesis and degradation in somatic tissue would not change; protein accretion and protein content of somatic tissue would therefore stay the same. Furthermore, fractional rates of protein synthesis and degradation in somatic tissue would be independent of protein turnover in reproductive tissue. If, however, diversion of energy to reproductive growth from somatic growth occurs, this could affect the mantle muscle protein turnover. Exactly how the fractional rates of protein synthesis and degradation in muscle tissue respond to reproductive maturation is unknown. If the rate of protein accretion slows (i.e., slower growth), this may be a function of either slower fractional rates of protein synthesis as less energy is allocated to somatic growth or an increase in fractional rates of protein degradation as protein is mobilized as an energy and nutrient source for reproductive growth. In either case, we would expect fractional rates of protein synthesis and degradation in muscle tissue to correlate with reproductive growth.

The temperate dumpling squid *Euprymna tasmanica* is a useful laboratory cephalopod in which to explore the costs of reproduction, the most critical reason being that the method to estimate rates of protein turnover are established for this species (Carter et al. 2009; Moltschaniwskyj and Carter 2010). Additionally, because *Euprymna* is a relatively small and solitary benthic species, it is tolerant to handling and being held individually in captivity without removal of social structure (Sinn and Moltschaniwskyj 2005; Sinn et al. 2006). This species produces multiple batches of eggs (Steer et al. 2004) and has indeterminate growth (Moltschaniwskyj and Carter 2010), and although lipid can be digested and used as an energy source (Swift et al. 2005), there is no evidence that lipid is a stored energy substrate (Moltschaniwskyj and Johnston 2006). The rapid growth rates and the apparent rapidity of the physiological responses of this species allow for collection of data on temporal scales that are interpretable with respect to rates of protein turnover. The aim of this study was to determine the adaptive response of protein turnover in the muscle tissue due to the energy demands associated with reproductive growth. We achieved this by measuring fractional rates of protein synthesis, degradation, and accretion in mantle muscle and gonad tissue in immature and mature individuals.

Material and Methods

Experimental Design

Experimental individuals were hatched from egg masses produced by three wild females caught on the north coast of Tasmania. Females, their egg masses, and all experimental individuals were held within a 1,000-L recirculating seawater system maintained at 18°C. Females were fed mysid shrimps (*Tasmanomysis oculata*, *Paramesopodopsis rufa*, *Tenagomysis* spp.) ad lib. and mated until they deposited egg masses. As hatchlings emerged from the egg, they were transferred to a new container and held in isolation while they grew. For the first 5 d of their lives, individuals were provided with cultured *Artemia* (boosted with SuperSelco) before being weaned onto mysid shrimps; all prey items were provided ad lib.

From 19 individuals, we estimated protein synthesis, protein accretion, and proximal analysis for the mantle muscle tissue and, where present, gonad (ovary and oviducal gland for females, testis for males). A gonosomatic index (GSI) was calculated for each individual as a percentage of total gonad (ovary and oviduct for females and testis and spermatophores for males) weight to total body weight. The GSI values showed two distinct groups of individuals: a group with small gonads (GSI < 9%) were classified as immature (13 individuals; 0.26–6.39 g and 44–126 d old), while animals with larger, more

developed gonads (GSI > 30%) were classified as maturing/mature (6 individuals; 3.94–7.21 g and 105–140 d old). No individuals across the group had GSI values of 9%–30%.

Estimating Fractional Rates of Protein Accretion and Synthesis

Protein accretion rates (k_g, % d^{-1}) for mantle muscle tissue and gonad were calculated from the following equation (Ricker 1979; McCarthy et al. 1994):

$$\text{protein accretion rate (\% protein d}^{-1}) = \frac{\log W_2 - \log W_1}{t} \times 100,$$

where W_1 is the tissue protein weight of the organs 7–14 d before death (g), W_2 is the tissue protein weight of the organs at the time the individual was killed (g), and t is the length of the growing period (d). Final tissue-specific protein content (W_2) was determined directly using the tissue weight and protein concentration (mg protein g^{-1} fresh wt). Initial protein weight (W_1) was estimated using the allometric relationship between organ weight and total body weight, and it was assumed that the concentration of protein in the organ was constant over the growing period (Houlihan et al. 1990). Total body weight at W_1 was estimated using digital pictures of the dorsal view of the individual in the tank. A relationship between dorsal surface area, digitized from the photograph, and the total weight was determined for individuals of a known weight (total wet weight = 0.028 × [surface area]^2 + 0.84 × surface area, $R^2 = 0.95$, weight range of 0.79–8.01 g). The protein content at W_1 was estimated using the protein concentration of the organs at time of death. Gonad protein weight of each individual at the start of reproductive growth (W_1) was estimated to be 0.1 g. When estimating W_1 for both tissue types, it was assumed that the protein concentration of the tissue for each individual was constant as tissue grew; this assumption was based on the fact that there was no significant correlation between tissue weight and protein concentration (mantle muscle: $R = 0.45$, $n = 19$, $P = 0.051$; gonad: $R = 0.54$, $n = 12$, $P = 0.072$).

Protein synthesis for each individual was determined by a single injection of L-(2, 6-^{3}H)phenylalanine (Amersham Pharmacia Biotech) at the base of the arms using the flooding dose method (Garlick et al. 1980; Carter et al. 2009). Incorporation times of 1–2.5 h following injection of 1.6 and 1.8 mL ^{3}H-phenylalanine 100 g^{-1} into the base of the arms are suitable for using the flooding dose method with dumpling squid (Carter et al. 2009). Therefore, each individual was weighed immediately before injection to calculate dose (1.8 mL 100 g^{-1}) and injected with a solution of 150 mM L-phenylalanine and L-(2,6-^{3}H)phenylalanine (Amersham Pharmacia Biotech) in 0.2 μm filtered seawater at pH 7.4 (Carter et al. 2009, 2012). Following injection, animals were returned to aerated seawater at 20°C for 96–115 min to allow incorporation of L-(2,6-^{3}H)phenylalanine into the tissues. Incorporation time was based on previous validations and selected to ensure all tissue free pools remained flooded and there was linear incorporation (Carter et al. 2009). The gonad free-pool phenylalanine-specific activity (1,528 ± 92 dpm $nmol^{-1}$ phenylalanine) was equivalent to the injection solution and remained elevated over the period of incorporation ($R^2 = 0.12$, $F = 0.388$, df = 1, 10, $P = 0.266$).

Individuals were killed by decapitation; weights of total body, mantle muscle tissue, and gonad (testis and accessory organs for males or ovary and oviduct but not oviducal gland for females) were recorded before tissues were stored in liquid nitrogen. Frozen tissue was homogenized and the phenylalanine-specific radioactivity of the free pool, the free-pool phenylalanine concentration, and the protein-bound phenylalanine-specific radioactivity were determined as previously described for dumpling squid (Carter et al. 2009). Indices of protein turnover were calculated according to Garlick et al. (1980). Fractional rates of protein synthesis (k_s, % d^{-1}) were calculated as $k_s = 100 \times [(S_b/S_a) \times (1{,}440/t_1)]$, where S_b is the protein-bound phenylalanine-specific radioactivity at time t_1 (min) and S_a is the free-pool phenylalanine-specific radioactivity (Garlick et al. 1980). Protein concentration was determined using a modification of the folin-phenol method (Lowry et al. 1951), and RNA concentration was estimated using dual-wavelength absorbance (Ashford and Pain 1986). Fractional

Table 1: Average values of parameters in the mantle muscle tissue of immature and mature southern dumpling squid

Variable	Immature	Mature	T	df	P
k_s (% d^{-1})	4.56 ± .90	1.67 ± .24	3.12	13.6[a]	.001
k_d (% d^{-1})	4.23 ± .94	1.02 ± .28	3.27	13.9[a]	.006
k_g (% d^{-1})	.33 ± .23	.65 ± .19	−.88	17	.394
C_s[a]	24.98 ± 3.82	11.69 ± 1.31	3.29	14.5[a]	.005
Protein retention efficiency (%)	9.28 ± 9.54	41.12 ± 13.89	−1.88	17	.077
k_{RNA} (% d^{-1})	1.69 ± .10	1.43 ± .11	1.26	17	.225

Note. Mean ± SE. Formal comparisons of average values of each parameter between immature ($n = 13$, GSI < 9%) and mature ($n = 6$, GSI > 30%) individuals were made using independent-sample t-tests for unequal sample sizes. k_s = fractional rates of protein synthesis, k_d = fractional rates of protein degradation, k_g = fractional rates of protein accretion, C_s = protein capacity for protein synthesis, and k_{RNA} = RNA activity.

[a]Equal variances were not assumed, and df was adjusted accordingly.

rates of protein degradation (k_d, % d^{-1}) were calculated as $k_d = k_s - k_g$ (Millward et al. 1975). RNA was expressed both as the capacity for protein synthesis (C_s, mg RNA g $protein^{-1}$) and as RNA activity (k_{RNA}, k_s g^{-1} RNA d^{-1}; Sugden and Fuller 1991). Note that RNA concentration could not be determined in any of the ovaries of dumpling squid.

Statistical Analysis

Differences in mean mantle muscle tissue fractional rates of protein synthesis (k_s), degradation (k_d), and accretion (k_g); RNA activity (k_{RNA}); protein retention efficiency; and capacity for protein synthesis (C_s) were compared between immature and maturing individuals using independent-sample *t*-tests. To determine whether protein turnover in the mantle muscle tissue changed with protein turnover in the gonad, the relationships between (as the predictor) GSI and (as the response) gonad fractional rates of protein synthesis, degradation, and accretion and capacity for protein synthesis were examined using Type II regression (McArdle 1988). For mature individuals, comparisons between fractional rates of protein synthesis, degradation, and accretion measured in gonad and mantle muscle tissue in the same individuals were performed using paired *t*-tests. All analyses were carried out in SPSS (ver. 17).

Results

Immature versus Mature Individuals

Mantle muscle tissue average fractional rates of protein synthesis (k_s) and degradation (k_d) were approximately four times slower in maturing/mature individuals than in immature individuals (table 1). Associated with these slower rates was evidence that the mantle muscle of maturing/mature individuals had less capacity for protein synthesis (C_s); they had approximately half the capacity for protein synthesis estimated for the mantle muscle of immature individuals (table 1). Despite this, there was no evidence that the protein retention in efficiency in the muscle tissue significantly differed between immature and maturing/mature individuals (table 1). In part, this was due to substantial variability in the efficiency of protein retention in the immature individuals (coefficient of variation = 371%) and the maturing/mature individuals (coefficient of variation = 86%). Protein growth (k_g) and RNA activity (k_{RNA}) were very similar between the two groups of animals (table 1). There was no evidence that changes in the rates of protein synthesis, protein degradation, or protein growth in the gonad had a significant relationship with the same processes in the mantle muscle tissue (fig. 1).

Tissue-Specific Rates in Mature Individuals

Gonad tissue had substantially faster fractional rates of protein synthesis and degradation, approximately 11% and 8% d^{-1}, respectively, compared with the mantle muscle (table 2). This resulted in the gonad tissue–synthesized protein accretion rate

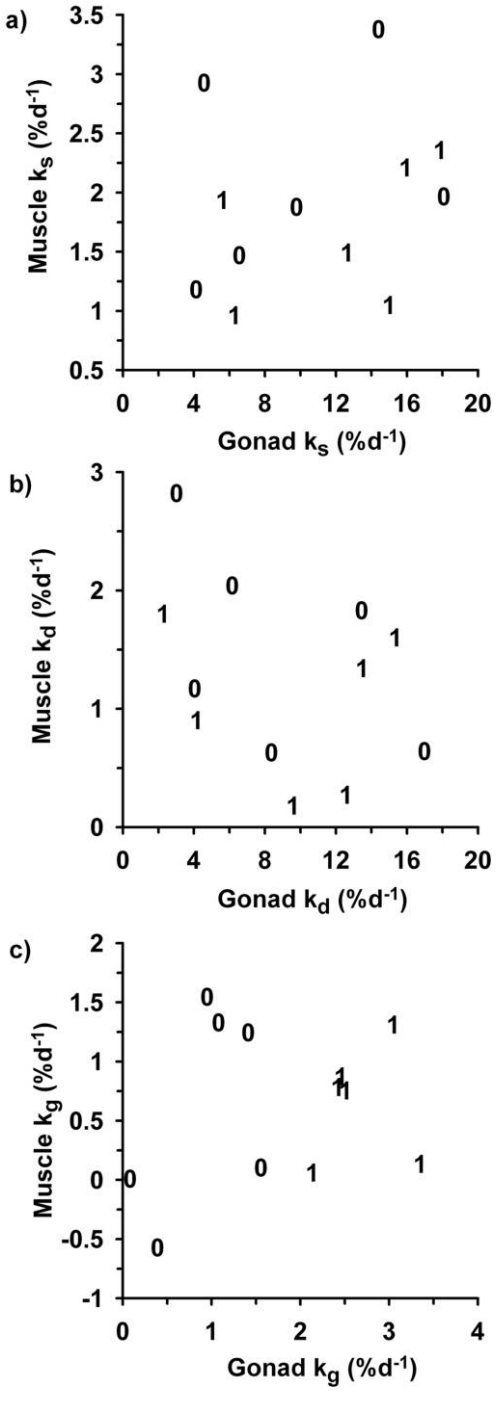

Figure 1. Relationships between fractional rates of (*a*) protein synthesis, (*b*) protein degradation, and (*c*) protein accretion in the gonad and mantle muscle tissue of maturing/mature southern dumpling squid *Euprymna tasmanica*. Fractional rates were determined by injecting each individual at the base of the arms with a solution of 150 mM L-phenylalanine and L-(2,6-^{3}H)phenylalanine in 0.2 μm filtered seawater at pH 7.4. Regression analysis determined that no significant linear relationships were evident (k_s: $F = 0.65$, df = 1, 10, $P = 0.442$; k_d: $F = 1.59$, df = 1, 10, $P = 0.236$; k_g: $F = 0.46$, df = 1, 10, $P = 0.515$). Data are coded as follows: 0 = immature individuals that had GSI < 9%; 1 = maturing/mature individuals that had GSI > 30%.

Table 2: Summary of muscle and gonad fractional rates of protein synthesis, degradation, and accretion in reproductively maturing/mature dumpling squid

Fractional rates (% d^{-1})	Gonad	Muscle	t	P
Protein synthesis	12.23 ± 2.10	1.67 ± .24	−5.26	.003
Protein degradation	9.58 ± 2.16	1.02 ± .28	−3.85	.012
Protein accretion	2.66 ± .18	.65 ± .19	−7.93	.001

Note. Mean ± SE. The six maturing/mature individuals used in these comparisons had undergone substantial reproductive growth (GSI > 30%). Paired-sample t-tests were used to test the null hypothesis that the average difference between muscle and gonad fractional rates of protein synthesis, degradation, and accretion equaled 0 (df = 5) for all tests.

being 3% d^{-1} (SE = 0.62%) faster than in mantle muscle tissue (table 2). However, there was no evidence of a significant relationship between gonad mass and the fractional rates of protein synthesis and degradation in the gonad (fig. 2*a*, 2*b*). However, there was a positive asymptotic relationship between gonad size and gonad protein accretion ($F = 43.39$, df = 1, 10, $P < 0.001$), with immature individuals increasing accretion rates with gonad size and maturing/mature individuals showing no change (fig. 2*c*). Fractional rates of protein synthesis and degradation in the gonad were strongly positively related ($F = 256.28$, df = 1, 10, $P < 0.0001$), but fractional rates of protein degradation were slower than fractional rates of protein synthesis (fig. 3). On average, fractional rates of protein degradation in the gonad were 1.79% d^{-1} (SE = 0.30%) slower than fractional rates of protein synthesis (paired-sample t-test, $t = 5.98$, df = 11, $P < 0.001$).

Discussion

Overall, this study produced a model of protein turnover that indicates that when *Euprymna tasmanica* allocates energy to reproductive growth, there was no difference in retention of protein for somatic growth between mature and immature individuals. Significantly slower mean fractional rates of mantle protein degradation in mature females (this study) and no evidence that lipid is used as a storage product (Moltschaniwskyj and Johnston 2006) support an income energy model for reproduction in some cephalopod species (Hatfield et al. 1992; Moltschaniwskyj 1995; Moltschaniwskyj and Semmens 2000; Semmens and Moltschaniwskyj 2000). This study has revealed that both fractional rates of mantle protein synthesis and degradation were slower in mature individuals, suggesting that less energy is available for protein synthesis but slower rates of protein degradation allowed somatic growth to continue (Moltschaniwskyj and Carter 2010); this supports an adaptive response in mantle protein turnover (Hawkins 1991). Although at the whole-animal level continuing somatic growth suggests no cost of reproductive maturation, slower protein synthesis and degradation in mature individuals are an indirect cost because less energy is available for somatic repair and may contribute to the short life spans and rapid senescence typical of cephalopods (Houlihan et al. 1990; Rodhouse 1998).

The fourfold-slower fractional rates of protein synthesis and degradation in the mantle muscle of reproductive adults explains the nonasymptotic growth form of many cephalopod species (Jackson 1989; Jackson et al. 1993). It may also explain the rapid senescence and short life span, suggesting that maintaining viability in somatic tissue is less important once production of gametes begins; the oceanic squid *Moroteuthis ingens* is an extreme example (Jackson and Mladenov 1994; Jackson et al. 2004). Protein degradation is an important process in maintenance of animals, and turnover of protein allows for the breakdown and removal of older and damaged proteins (Makrides 1983). In general, the short life span of cephalopods, death shortly before or just after attaining an asymptotic size, and the often relatively short reproductive phase suggest that senescence occurs rapidly in cephalopods. Although the direct mechanistic relationship between protein degradation and senescence has not been established (Makrides 1983), these data do provide some insights into senescence processes in cephalopods with multiple spawning strategies.

Indeterminate growth in *E. tasmanica* is mechanistically a function of the efficiency with which synthesized protein is retained (Moltschaniwskyj and Carter 2010). The slowing of growth associated with reproduction, a characteristic of many cephalopods, appears to be due to the slower fractional rates of protein synthesis in mature *Euprymna*. Rates of protein synthesis slow in response to energy limitation, that is, starvation (Houlihan et al. 1989). Short-lived cephalopods must undertake egg production, spawning, and in some cases egg brooding over a few months of adulthood; therefore, the allocation of energy for reproduction is a form of energy limitation to somatic growth. There is a positive relationship between fractional rates of protein synthesis and metabolic rate (Houlihan 1991); therefore, the slower growth in mature cephalopods may be associated with slowing metabolic rates, supporting the idea of a "reproductive drain hypothesis." The very fast rates of fractional protein synthesis in the ovary and the slowing of mantle muscle fractional rates of protein synthesis suggest that energy was allocated to reproductive growth and away from the mantle muscle tissue. The patterns of growth of squid would be strongly determined by such changes in tissue-specific rates of protein synthesis. However, the efficiency in the retention of synthesized protein for growth was very variable in dumpling

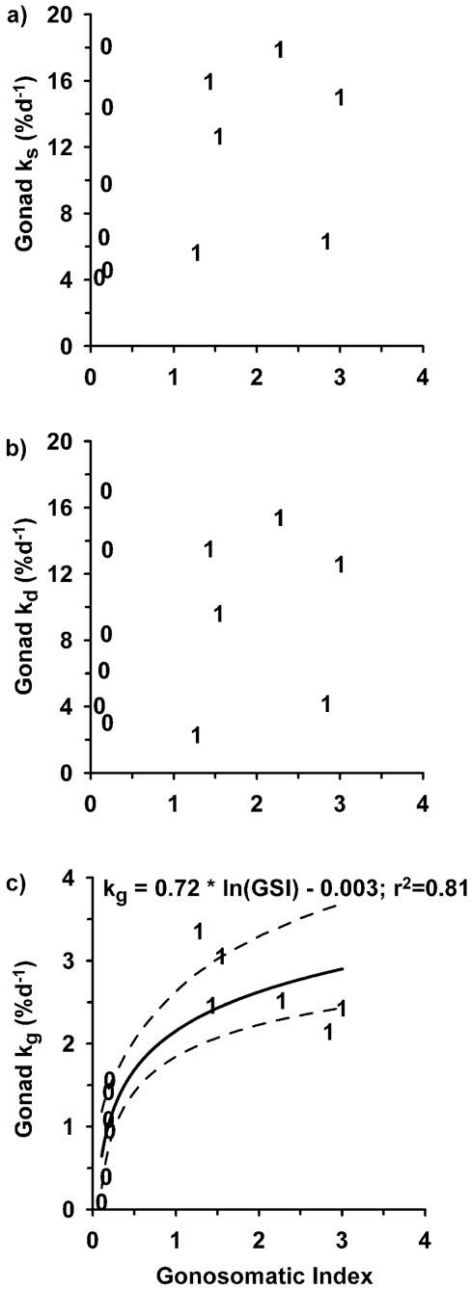

Figure 2. Relationships between gonosomatic index and gonad tissue rates of (*a*) protein synthesis, (*b*) protein degradation, and (*c*) protein accretion in southern dumpling squid *Euprymna tasmanica*. Fractional rates were determined by injecting each individual at the base of the arms with a solution of 150 mM L-phenylalanine and L-(2,6-^{3}H)phenylalanine in 0.2 μm filtered seawater at pH 7.4. Regression analysis determined that no significant linear relationships were evident for protein synthesis and degradation (k_s: $F = 1.40$, df $= 1, 10$, $P = 0.263$; k_d: $F = 0.43$, df $= 1, 10$, $P = 0.525$). There was a positive asymptotic relationship between gonad size and gonad protein accretion ($F = 43.39$, df $= 1, 10$, $P < 0.001$). The equation for protein accretion includes the standard error for the slope in brackets, and the dashed lines are the 95% confidence limits around the fitted (solid) line. Data are coded as follows: 0 = immature individuals that had GSI < 9%; 1 = maturing/mature individuals that had GSI > 30%.

squid, particularly immature individuals. In calculating the efficiency in the retention of synthesized protein, an assumption is made that the concentration of protein in the tissue is constant; however, rates of protein synthesis may be affected by the time of the last meal. It is possible that the large differences among individuals in their efficiency in the retention of synthesized protein for growth may be due to this assumption being incorrect. However, such variability in efficiency in the retention of synthesized protein for growth is worth exploring in greater detail because it may well provide a mechanistic explanation of the highly variable growth rates typical of many squid species (Moltschaniwskyj 2004).

There is little doubt that for *E. tasmanica* there was a somatic cost associated with reproduction; a reduction in mantle muscle protein synthesis with the onset of reproduction suggests lower availability of amino acid and/or energy-restricted protein synthesis in the muscle tissue (Carter and Houlihan 2001). Given that *E. tasmanica* shares a number of life-history and ecological characteristics with the reef loliginid squid and cuttlefish, it is highly likely that for most cephalopod species for whom the cost of reproduction is not apparent at the whole-animal level (Rodhouse et al. 1988; Hatfield et al. 1992; Moltschaniwskyj and Semmens 2000; Semmens and Moltschaniwskyj 2000), the cost may be evident at the suborganismal level. Highly variable retention of synthesized protein for somatic growth would also explain why, for many cephalopod species, proximal analysis fails to detect changes in mantle muscle protein concentrations associated with reproduction (Moltschaniwskyj and Semmens 2000; McGrath and Jackson 2002; Rosa et al. 2005). For *Octopus* species and some ommastrephid squid, rates of protein degradation are correlated with reproduction (O'Dor and Wells 1978; Jackson and Mladenov 1994; Arkhipkin and Bjorke 1999); the loss of muscle protein is dramatic in these species and results in almost complete loss of structural protein. A teleost analogy is maturing salmon species that cease feeding during maturation; while there is extensive loss of tissue mass, the extent of the loss differs among the tissues (Mommsen et al. 1980; Martin et al. 1993).

Overall, this study supports the current developing premise that in squid an indeterminate growth pattern is a function of allocating energy to both somatic and reproductive growth during the adult phase. It was not possible in this study to determine differences between sexes because of the small number of mature individuals. Although the energy committed to gonad production is greater for females than males, the need to maintain somatic growth and muscle function is the same for both sexes. Furthermore, many of the parameters measured showed little variation among mature animals, suggesting that if there were differences between the sexes, they would have been small. This study provides evidence that the cost of reproduction was manifested as a slowing in the rates of both protein degradation and synthesis, expressed at the whole-animal level as continuous growth and a short life span (Moltschaniwskyj and Carter 2010). *Euprymna tasmanica*, like all of the modern-day cephalopods, has life-history characteristics of short life and rapid growth, reproduction income energy, and multiple spawning.

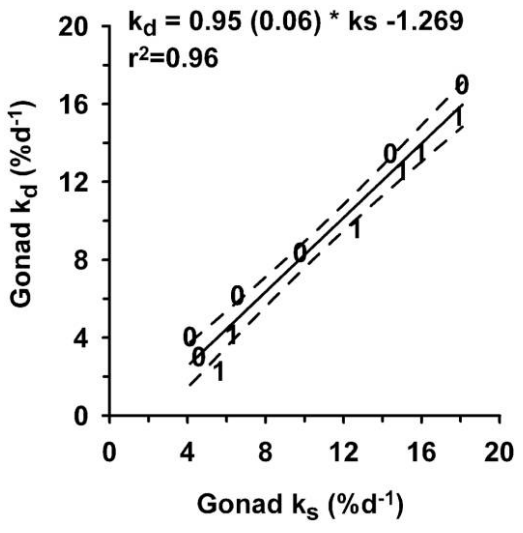

Figure 3. Linear relationship between rates of protein synthesis (k_s) and protein degradation (k_d) in gonad tissue ($F = 256.28$, df = 1, 10, $P < 0.0001$). The value in brackets is the standard error of the slope of the equation, and the dashed lines are the 95% confidence limits around the fitted (solid) line. Data are coded as follows: 0 = immature individuals that had GSI < 9%; 1 = maturing/mature individuals that had GSI > 30%.

Therefore, the conclusions drawn in this study support the patterns of reproductive and somatic growth seen in a range of cephalopod species.

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