

G. Pecl

## Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids

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**Abstract** A major difficulty confronting the determination of cephalopod reproductive life history is assessing over what portion of the life span an individual is reproductively mature and actively depositing eggs. This paper assesses the potential of the tropical *Sepioteuthis lessoniana* and two genetic types of the temperate *Sepioteuthis australis*, to spawn multiple batches of eggs at discrete times throughout the adult life span. This is achieved by histological examination of the ovarian gametogenic cycle and detailed morphological assessments of the reproductive system, in conjunction with other biological information. The genetic type of *S. australis* found at the northern limits of its Australian distribution showed evidence of a high correlation between body size and quantity of mature eggs, suggesting that eggs may be accumulating to be laid in a single batch. Although maturation was also a size-related process in *S. lessoniana* and Tasmanian *S. australis*, oviduct size was not correlated with body weight in mature females, which is indicative of multiple spawning. Further supporting evidence includes relatively low gonadosomatic indices, the heavier weight of the ovary relative to the oviduct, and the feeding activity of mature animals. Mature *S. lessoniana* and *S. australis* individuals were present at each location over very wide age and size ranges. In Tasmanian waters, there were

distinct seasonal differences in the reproductive biology of *S. australis*. Summer-caught individuals had much higher gonadosomatic indices and may have been laying larger batches of eggs compared with winter-caught individuals. Summer-caught females also showed a negative correlation between egg size and egg number within the oviduct, suggesting that some individuals were producing fewer, larger eggs and others many smaller eggs. Evidence suggests that considerable flexibility is inherent in the reproductive strategy of both *S. lessoniana* and *S. australis*.

### Introduction

An individual's or population's "fitness" in the evolutionary sense is determined by both absolute fecundity and generation interval (Roff 1986). Both of these life-history traits are dependent on the reproductive strategy of a species, and indeed are likely to be divergent between those species or individuals that breed once and die, and those that breed over an extended time frame. Optimal age or size at maturity, and the associated age- and size-specific schedules of reproductive investment, are just some of the life-history characteristics that are also intimately related to the reproductive strategy of a species (Stearns 1977). As a consequence of the very short life span typical of most cephalopods, annual recruitment is essential to sustain populations (Boyle 1990), and as such an understanding of the reproductive strategy of a cephalopod fisheries resource is crucial.

Determining how long an individual takes to complete egg maturation and deposition, and assessing what portion of the life span this period represents, is a major difficulty confronting detailed examinations of cephalopod reproductive biology in field populations (Collins et al. 1995b). Although coleoid cephalopods have traditionally been viewed as semelparous organisms that reproduce once and die (Arnold and Williams-Arnold 1977; Calow 1987; e.g. *Illex illecebrosus*, O'Dor 1983; *Todarodes pacificus*, Okutani 1983), it is increasingly

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G. Pecl  
School of Marine Biology and Aquaculture,  
James Cook University, Townsville,  
Queensland 4811, Australia

*Present address:*  
Marine Research Laboratories,  
Tasmanian Aquaculture and Fisheries Institute,  
University of Tasmania, GPO Box 252-49,  
Tasmania 7053, Australia

Tel.: +61-3-62277277; Fax: +61-3-62278035  
e-mail: Gretta.Pecl@utas.edu.au

apparent that many species are not restricted to this strategy. Reproductive strategies that involve spawning within one season only but in which reproductive effort is distributed over space and time over a portion of the life span are now known to be common among cephalopods (Villanueva 1992; Hun Baeg et al. 1993; Wada and Kobayashi 1995; González and Guerra 1996; Maxwell et al. 1998).

In species with non-asymptotic growth patterns, growth and reproduction necessarily proceed together over much of the life cycle, and it is likely that the diversity of spawning modes is related to growth patterns (Mangold et al. 1993). In general, the growth and life spans of tropical and temperate species differ. Temperate and subtropical loliginids live for approximately 1 year (Natsukari et al. 1988; Collins et al. 1995a) or 2 years (Hanlon et al. 1989), whereas tropical species have life spans of <6 months (Jackson 1990; Jackson and Choat 1992; Jackson and Yeatman 1996). There is, however, little information about how the spawning biology of closely related species from disparate temperature zones may differ. Within a population the growth characteristics of cephalopod species are highly plastic, with small changes in temperature producing large changes in growth rates and final size (Forsythe 1993). Consequently, in cephalopod populations where spawning takes place all year round there may be seasonal influences on age- and size-specific schedules of reproductive investment and subsequent patterns in egg maturation and deposition.

*Sepioteuthis lessoniana* and *S. australis* are both relatively large, neritic squids of commercial importance in Australia. The maximum life span of *S. lessoniana* in Australian tropical and subtropical waters is 6–7 months (Jackson and Choat 1992). *S. australis* achieves a similar life span of around 6–10 months in temperate waters (Pecl, unpublished data). Both these species show rapid growth (4–8% body wt day<sup>-1</sup>) during their short life spans.

The mature eggs of *Sepioteuthis* spp. are very large compared to those of other squids (Hanlon 1990), in the range of 5–10 mm. Large eggs cannot be matured all at once, and *S. lessoniana* is known to have the capacity, at least in the laboratory, to lay multiple batches of eggs over a significant portion of the life span (Wada and Kobayashi 1995). Back-calculated hatching dates of both *S. lessoniana* and *S. australis* (Pecl, unpublished data) suggest that hatching, and consequently spawning, take place all year round, although there is little infor-

mation on the temporal patterns of spawning intensity. Extended spawning seasons tend to be associated with multiple spawning (Lum-Kong et al. 1992; Porteiro and Martins 1994); however, it is often unclear if prolonged spawning is due to extended individual spawning or asynchronicity in the population (Boyle et al. 1995).

This paper uses a combination of histological and morphological assessment measures, in conjunction with other biological information, to assess the reproductive strategies of the tropical *S. lessoniana* from two locations in northern Australian waters and the temperate *S. australis* from two locations in southern Australia. An examination of the patterns in egg maturation between seasonal extremes was also conducted for *S. australis* at the most southern location. Recent research has demonstrated that *S. australis* from the two regions examined in this study constitute two distinct genetic stocks (Triantafillos and Adams, in press). Differences in the reproductive biology have been put forward as a reason to support the hypothesis of these two genetic types as distinct taxa, so the examination of regional differences in the reproductive biology of *S. australis* is particularly pertinent.

## Materials and methods

### Collection and processing

*Sepioteuthis lessoniana* individuals were obtained from waters adjacent to Townsville (Table 1) by jigging, and from Brisbane by a combination of tunnel-netting by the commercial sector and jigging. *S. australis* was obtained in Newcastle by jigging, and in Tasmania by a combination of jigging and modified purse-seine. The 196 females from Tasmania included 42 females caught during winter and 154 caught in summer.

Most individuals were refrigerated or placed on ice within a few hours of capture and processed within 12 h. Some individuals obtained from the commercial sector had been frozen: 37 from Brisbane, 24 from Townsville and 10 from Tasmania. Dorsal mantle length (ML) was measured to the nearest millimetre and total body weight to the nearest 0.01 g. The mantle muscle, ovary, oviduct, nidamental and oviducal glands were dissected out and weighed separately. A gonadosomatic index (GSI) was derived for each individual as follows:

$$\text{GSI} = \frac{\text{OW} + \text{NW} + \text{ODW} + \text{OV}}{\text{BW} - \text{RW}}$$

where OW = ovary weight, NW = nidamental gland weight, ODW = oviducal gland weight, OV = oviduct weight; BW = total body weight and RW = total reproductive weight (combined weight of ovary, oviduct, nidamental and oviducal glands).

**Table 1** *Sepioteuthis lessoniana* and *S. australis*. Sample sizes, collection locations and dates

Species, location	Location	Collection dates	Total sample size	No. of females	
<i>S. lessoniana</i>	Townsville	19°10'S; 146°55'E	Feb 1995–Oct 1997	116	50
	Brisbane	27°20'S; 153°3'E	Aug 1995–Apr 1997	173	83
<i>S. australis</i>	Newcastle	32°45'S; 152°10'E	Aug 1995–Dec 1995	131	51
	Tasmania	42°15'S; 148°10'E	Jan 1996–Jul 1997	493	196

Weights could not be measured for 12 *S. lessoniana* caught in Townsville; however, total body weight was estimated for these from the relationship:  $\text{weight} = 0.00042 \times \text{ML}^{2.6045}$  ( $r^2 = 0.99$ ,  $n = 34$ ).

All squid were assigned to a maturity stage according to the relative size and colour of reproductive organs, following the six-stage maturity scale of Lipinski (1979). Under this scheme stages IV and V are mature and are hence part of the spawning stock. Each individual was thoroughly examined for external skin lesions on the head, mantle or fins, and any signs of deterioration of the reproductive organs. Feeding in spawning individuals of some semelparous species is reduced or may even stop completely (Mangold et al. 1993). The presence or absence of food in the stomach, and spermatophores in the buccal pouch, was determined for all individuals to assess if females had fed, or mated recently.

#### Analysis of oocyte maturation

For histological inspection of the ovaries, tissue was fixed in a formalin acetic-acid calcium-chloride solution (FAACC), sectioned at 6  $\mu\text{m}$  and stained with Young's haematoxylin and eosin. Frozen squid were not used for histology. Ovarian oocyte stage–frequency distributions were obtained for each female by allocating 50 randomly selected oocytes to one of five oocyte maturation stages as per Moltshaniwskyj (1995). The number of ovulated oocytes in the oviduct of mature individuals was estimated by weighing 20 eggs and scaling this value by total oviduct egg weight. Twenty oviduct eggs, or all oviduct eggs if less than 20 were present, from each individual were also measured. Oviduct eggs were oval in shape, so eggs were measured along the long axis using an ocular micrometer and a stereomicroscope. Damaged or deformed eggs were not measured.

Degree of oviduct fullness was estimated for Tasmanian-caught *S. australis* following the method described by Harman et al. (1989), modified for oviduct weight rather than volume. Briefly, maximum oviduct weight in each 10 mm ML size class was noted and plotted against mantle length (linear relationship:  $r^2 = 0.88$ ,  $n = 17$ ,  $P < 0.0001$ ). An equation to predict the potential maximum oviduct weight for any maturing female was derived from the linear regression through these maximum values. Percent oviduct fullness was calculated for each female by dividing the actual oviduct weight by the maximum predicted by the equation:  $\text{maximum oviduct weight} = (0.157 \times \text{ML}) - 22.566$ . As this method involves regression analyses, some estimates of oviduct fullness consequently exceeded 100%.

#### Age estimation

Age information was determined from increments in the statolith, validated as daily for both *S. lessoniana* (Jackson 1990) and *S. australis* (Pecl, unpublished data). Statoliths were stored in 70% ethanol, rinsed thoroughly in distilled water and dehydrated in 100% ethanol prior to mounting in crystal bond. Smaller *S. lessoniana* statoliths were ground on the anterior (concave) surface only; larger *S. lessoniana* and all *S. australis* statoliths were ground on both surfaces. Increments in very large *S. australis* statoliths were enhanced by heat treatment, whereby the statolith was placed on a hot plate for 1–2 min until light brown in colour. Statoliths were ground on wet 1200  $\mu\text{m}$  carborundum paper and polished with 0.05  $\mu\text{m}$  alumina powder on wet suede polishing cloth. Daily rings were counted from the hatching check under a light microscope with a polarised light source. Statoliths were discarded as unreadable if the hatching check was unclear, or if two consecutive counts differed by more than 10%, or less than 90% of the rings were visible.

## Results

### *Sepioteuthis lessoniana*

The relationships examined in this study did not differ between locations for *S. lessoniana*, thus individuals

from both locations were combined to simplify analyses. Early stage *S. lessoniana* females with small developing ovaries showed a predominance of primary and secondary oocytes, with the range of oocyte stages present in the ovary increasing with the progression of female maturation stage (Fig. 1). This confirms that the macroscopic features used in the allocation of maturation stage are consistent with microscopic changes occurring within the ovary. The ovarian oocyte stage–frequency distribution showed little variability between females of the same maturity stage. In fully mature stage V females all ovaries had a low proportion of hydrated stage 5 oocytes and at least 65% of oocytes were in the first three stages of oocyte development.

Maturation in female *S. lessoniana* individuals was a size-related process. Weight of the ovary, nidamental glands and oviducal glands were all highly correlated with total body weight, more so than with age (Table 2). However, total body weight of mature females was not related to oviduct weight ( $r = 0.18$ ,  $n = 35$ ,  $P = 0.30$ ), or the number of eggs present in the oviduct ( $r = 0.22$ ,  $n = 21$ ,  $P = 0.33$ ; Fig. 2). In all but one individual the oviduct was lighter than the ovary, and oviduct weight never exceeded 15% of mantle wet weight in any individuals.

The size of oviduct eggs ranged from 5.33 to 7.45 mm (mean  $6.21 \pm 0.12$  SE,  $n = 171$ ), and the maximum number of ovulated eggs present in any one oviduct was 298. Although there was variation in oviduct egg size between females, within a female the size of oviduct eggs was very consistent. Average size of ovulated oocytes was not related to weight of the oviduct ( $r = -0.21$ ,  $n = 21$ ,  $P = 0.36$ ), or number of eggs within the oviduct ( $r = -0.40$ ,  $n = 21$ ,  $P = 0.076$ ), suggesting that oocytes were not continuing to grow within the oviduct and were therefore ready to be laid. Average size of the oviduct eggs was also not related to total body weight ( $r = 0.30$ ,  $n = 21$ ,  $P = 0.19$ ) or mantle wet weight ( $r = 0.38$ ,  $n = 20$ ,  $P = 0.10$ ).

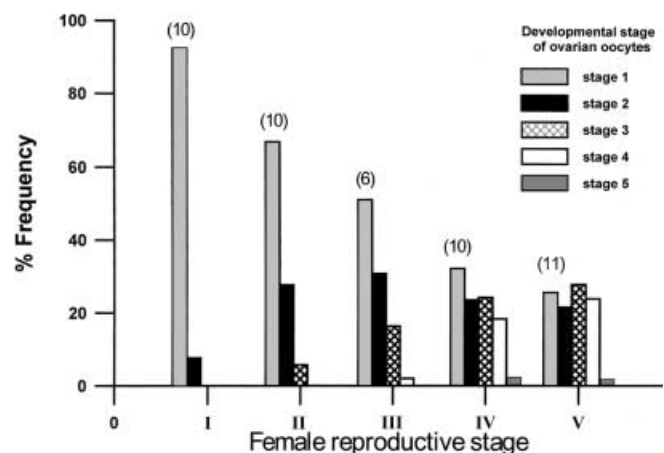
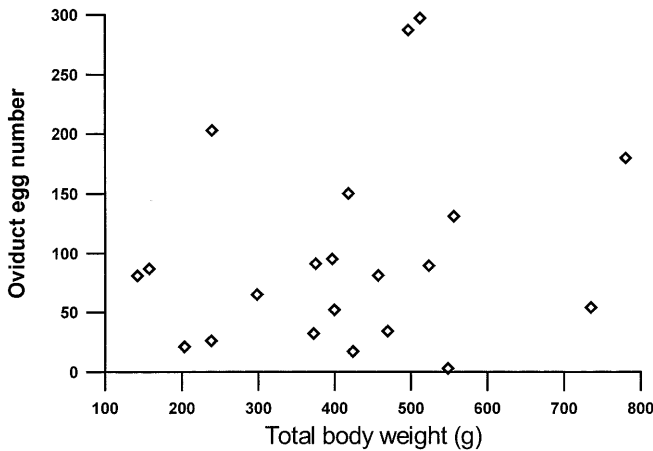


Fig. 1 *Sepioteuthis lessoniana*. Ovarian oocyte stage–frequency distribution for females in each of the five reproductive stages. Number of females in each reproductive stage in parentheses

**Table 2** *Sepioteuthis lessoniana*. Correlations of the weights of the major components of the female reproductive system with total wet weight and age

	Ovary			Nidamental glands			Oviducal gland		
	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>
Weight (g)	114	0.60	<0.001	95	0.63	<0.001	93	0.66	<0.001
Age (days)	50	0.33	0.018	44	0.34	0.022	43	0.37	0.015

Although maturation appeared to be a size-dependent process, mature females were still present in the population over very wide age, weight and length ranges as were maturing individuals (Table 3). There was no macroscopic evidence of any female being spent or dying, as there was no deterioration of any of the reproductive organs or exterior lesions on the head, mantle or fins. However, three of the 38 mature individuals were found with large, stretched empty oviducts and may have spawned previously as they were quite distinct from stage IV females, in which the unused oviduct is visible as a thin tube or strip lying over the ovary. These individuals had lower gonadosomatic ratios (range 3–6%, average  $4.1 \pm 1.2\%$  SE) relative to other mature females (range 5.1–19.6%, average  $12.7 \pm 0.7\%$  SE). All mature females had mated recently, except for one of the individuals with a very large, stretched empty oviduct. Mature females were still feeding, with the percentage of mature females with food in their stomachs similar to that of immature and maturing females (Brisbane:  $\chi^2 = 0.22$ , *df* = 1, *P* = 0.64; Townsville:  $\chi^2 = 0.99$ , *df* = 1, *P* = 0.32).



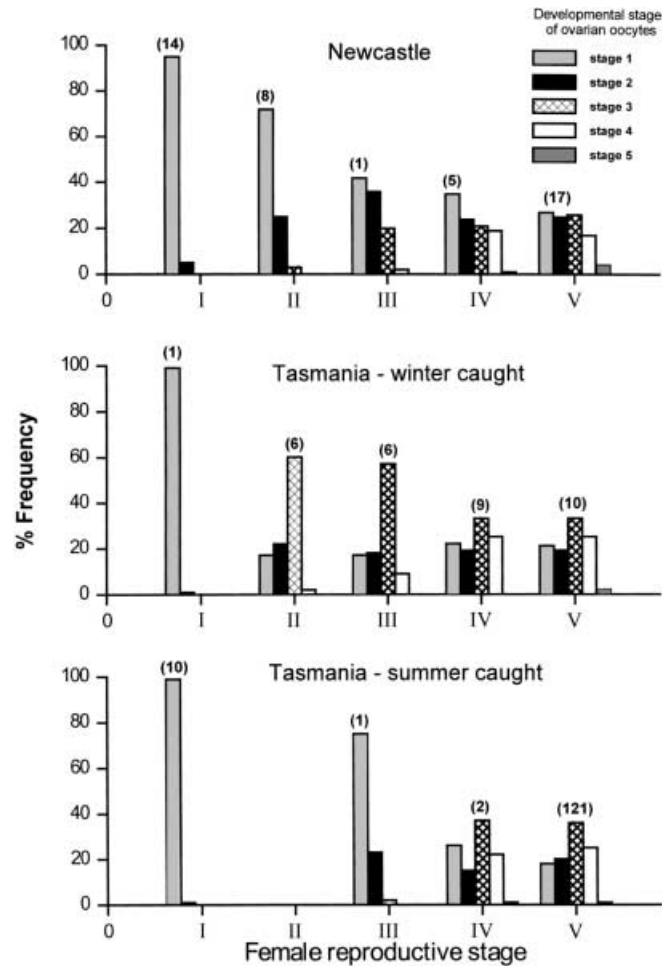
**Fig. 2** *Sepioteuthis lessoniana*. Number of mature eggs in the oviduct with total body weight of mature females

**Table 3** *Sepioteuthis lessoniana*. Age, weight and mantle length ranges for mature and immature females

	<i>n</i>	Age (days)	Weight (g)	Mantle length (mm)
Mature	38	107–187	108–960	118–252
Immature and maturing	144	71–178	46–567	84–200

*Sepioteuthis australis*

There were substantial differences evident in the reproductive biology of *S. australis* individuals from the two regions, and between seasons in Tasmania, thus data were graphed and analysed separately. In Tasmanian winter-caught females the microscopic condition of the ovary was not consistent with the macroscopic features used in the allocation of maturation stage, as immature females had substantial numbers of stage 3 and 4 oocytes in their small ovaries (Fig. 3). However, at least 50% of oocytes were in the first three stages of development in the ovaries of all mature *S. australis* indi-



**Fig. 3** *Sepioteuthis australis*. Ovarian oocyte stage–frequency distribution for females in each of the five reproductive stages, caught in Newcastle, and summer and winter in Tasmania. Number of females in each reproductive stage in *parentheses*

viduals from both Tasmania and Newcastle (Fig. 3). Maturation in female *S. australis* from both locations was a size-related process (Table 4), with the weights of the ovary, nidamental and oviducal glands all highly correlated with female total weight. Weights of the major components of the reproductive system of individuals from Newcastle and summer-caught Tasmanian individuals also showed correlations with age (Table 4).

The GSI was positively correlated with female size in Newcastle-caught *S. australis* ( $r = 0.70$ ,  $n = 22$ ,  $P < 0.001$ ), and the maximum GSI values for mature individuals (7.4–22.7%, average  $15.9 \pm 1\%$  SE) were only marginally higher than those of *S. lessoniana*. GSIs for mature summer-caught Tasmanian squid spanned a very broad range (8.8–35.4%, average  $18.7 \pm 0.3\%$  SE) and were much higher than the GSI values in winter-caught individuals (3.4–13.6%, average  $9 \pm 0.7\%$  SE;  $t$ -test,  $t = 12.98$ ,  $df = 158$ ,  $P < 0.0001$ ). In summer-caught squid, smaller individuals tended to have higher GSI values ( $r = -0.20$ ,  $n = 134$ ,  $P = 0.026$ ).

The oviduct was lighter than the ovary in all *S. australis* individuals. The weight of the oviduct was 16.8% of the mantle weight in the smallest and youngest mature Tasmanian summer-caught individual; however, it did not exceed 15% of mantle weight in all other *S. australis*. The weight of the oviduct and number of eggs within the oviduct in mature *S. australis* from Newcastle and summer-caught Tasmanian squid were both correlated with total body weight (Table 5; Fig. 4). In Tasmanian-caught females there was a great deal of variation in oviduct fullness; mature females ranged

from having either very full, heavy oviducts to oviducts that were completely empty (Fig. 5). There was no correlation between body size and degree of oviduct fullness for summer- (Spearman rank,  $r_s = 0.048$ ,  $n = 129$ ,  $P = 0.42$ ) or winter- (Spearman rank,  $r_s = -0.049$ ,  $n = 15$ ,  $P = 0.80$ ) caught individuals.

The maximum number of ovulated eggs in the oviduct of Tasmanian summer-caught females was double that of females caught in winter or Newcastle-caught females (Table 6). As with *S. lessoniana*, although average egg size varied between *S. australis* females, within a female, egg size was very consistent (Fig. 6). Average egg size was not related to weight of the oviduct, or female total weight (Table 5). However, females caught in summer from Tasmania showed a weak negative correlation between egg size and number of eggs within the oviduct (Table 5), suggesting that individuals were producing fewer, but larger eggs or many smaller eggs. Newcastle individuals with heavier mantles were producing larger eggs (Table 5).

*S. australis* females were mature over very wide age and size ranges at all locations sampled, with the ranges particularly wide in summer-caught females from Tasmania (Table 7). Immature and maturing females were only found up to half the maximum weight attained in mature females. Only one mature individual caught in Newcastle had a stretched but empty oviduct. In Tasmanian-caught *S. australis*, three individuals from the winter sample and 12 from the summer sample had stretched empty oviducts. Three Tasmanian females caught in summer also had small mantle lesions occu-

**Table 4** *Sepioteuthis australis*. Correlations of the weights of the major components of the female reproductive system with total wet weight and age

	Ovary			Nidamental glands			Oviducal gland		
	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>
Weight (g)									
Newcastle	50	0.85	<0.001	50	0.90	<0.001	50	0.90	<0.001
Tasmania-summer	151	0.92	<0.001	154	0.96	<0.001	154	0.95	<0.001
Tasmania-winter	39	0.73	<0.001	41	0.80	<0.001	41	0.83	<0.001
Age (days)									
Newcastle	43	0.56	<0.001	43	0.57	<0.001	43	0.58	<0.001
Tasmania-summer	124	0.78	<0.001	127	0.78	<0.001	127	0.77	<0.001
Tasmania-winter	35	0.35	0.039	37	0.35	0.033	37	0.39	0.016

**Table 5** *Sepioteuthis australis*. Correlations of reproductive parameters in each location/season caught

Correlation	Newcastle			Tasmania-summer			Tasmania-winter		
	<i>r</i>	<i>n</i>	<i>P</i>	<i>r</i>	<i>n</i>	<i>P</i>	<i>r</i>	<i>n</i>	<i>P</i>
Oviduct and body wt	0.71	22	<0.001	0.58	131	<0.001	0.37	16	0.15
No. eggs and body wt	0.64	22	<0.001	0.49	107	<0.001	0.37	15	0.17
Egg size and oviduct wt	0.05	16	0.84	0.02	103	0.80	-0.01	7	0.99
Egg size and no. eggs	-0.14	16	0.61	-0.29	103	0.004	-0.30	8	0.48
Egg size and body wt	0.46	16	0.07	0.08	103	0.48	-0.47	8	0.24
Egg size and mantle wt	0.56	16	0.02	0.09	103	0.37	-0.48	8	0.22

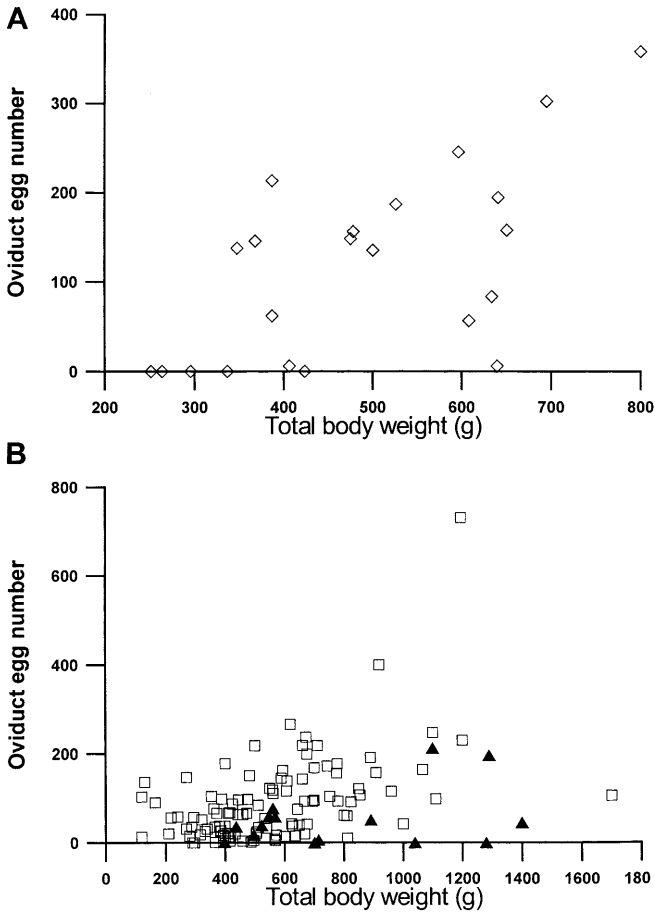


Fig. 4A, B *Sepioteuthis australis*. Number of mature eggs in the oviduct with total body weight of mature individuals caught in A Newcastle and B summer (squares) and winter (triangles) in Tasmania

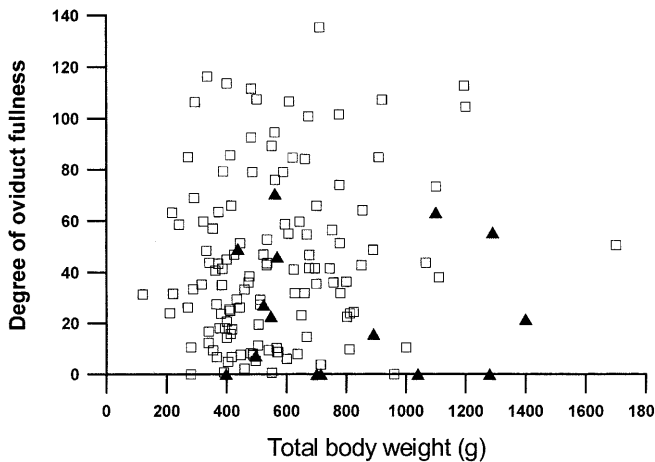


Fig. 5 *Sepioteuthis australis*. Estimated degree of oviduct fullness with total body weight in Tasmanian-caught individuals. Method of estimation involved regression analyses, hence some values exceed 100% (see “Methods” section) (squares summer-caught females; triangles winter-caught females)

pying less than 10% of the mantle area, and two individuals had stretched oviducts and small mantle lesions.

Despite the small lesions there was no macroscopic evidence of deterioration of the reproductive organs or any other tissues. In Tasmanian summer-caught females, those with stretched oviducts or lesions had no difference in the proportion found with food in the stomach ( $\chi^2 = 0.21$ ,  $df = 1$ ,  $P = 0.65$ ), or average GSI values ( $t$ -test,  $t = 2.738$ ,  $df = 132$ ,  $P = 0.007$ ) compared to other mature individuals. At least 30% of mature individuals at each location had ingested prey in the stomach, and all mature females had mated.

### Discussion

The primary evidence for multiple spawning in *Sepioteuthis lessoniana* and Tasmanian *S. australis* is the lack of a strong correlation in mature females between body size and oviduct fullness or quantity of eggs, together with evidence of continuous egg production throughout adult life. Supplementary evidence for this conclusion includes a lighter oviduct compared to the ovary, since it would be reasonable to expect the oviduct to become heavier if oocytes were accumulating (Moltschaniwskyj 1995). Individuals of both *S. lessoniana* and *S. australis* were found with large stretched but empty oviducts, thought to indicate previous spawning; however, they did not appear to be spent or in the process of dying. A multiple spawning strategy is further supported by the relatively low GSI of both *S. lessoniana* and *S. australis*, compared to known semelparous squid (e.g. up to 50% in *Loligo opalescens*, Fields 1965; 23% for the oviducal eggs alone in *Illex illecebrosus*, O’Dor 1983). The proportion of body mass committed to reproductive structures assists in the interpretation of a species’ spawning biology, as life-history theory predicts that reproductive effort of a semelparous animal should be high (Calow 1987). Alternatively, low GSIs suggest that relatively less energy is channelled into egg development at any one time during reproduction and are an indication of a non-semelparous reproductive strategy.

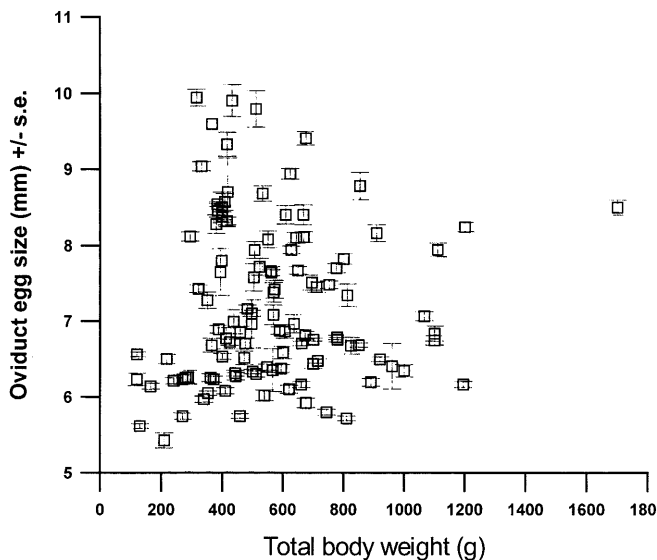
Ovarian oocyte stage–frequency distribution revealed continuous egg production in individuals at all maturity stages. Although this appears to be a necessary characteristic of cephalopods that spawn multiple times (e.g. *Idiosepius pygmaeus*, Lewis and Choat 1993; *Photololigo* sp., Moltschaniwskyj 1995), this evidence alone cannot be used to support a multiple spawning mode (Mangold et al. 1993). Many known semelparous squid, e.g. *Illex illecebrosus* (O’Dor 1983) and *Teuthowenia megalops* (Nixon 1983), show a single size mode in the ovarian oocyte–size frequency distributions. The critical measure appears to be the numerical importance of the largest oocyte size mode (Mangold et al. 1993). The proportion of mature or nearly mature oocytes was always much less than the total number of developing eggs in the ovaries of all mature *S. lessoniana* and *S. australis* individuals, as has been found in *Loligo vulgaris reynaudii* (Sauer and Lipinski 1990).

**Table 6** *Sepioteuthis australis*. Number and size of oviduct eggs in mature individuals in each location/season caught. Size and age of females with the highest number of oviduct eggs are shown in parentheses

Parameter	Newcastle	Tasmania-summer	Tasmania-winter
Max. no. of eggs	363 (800 g)	731 (1195 g and 203 days)	212 (1100 g and 175 days)
Size of oviduct eggs (mm)			
Range	6.34–7.67	5.43–9.95	6.39–7.80
Mean $\pm$ SE	6.79 $\pm$ 0.09	7.2 $\pm$ 0.10	7.07 $\pm$ 0.19

**Table 7** *Sepioteuthis australis*. Age, weight and mantle length ranges for mature females ( $n$  number of mature females; values in brackets total number of females examined; values in parentheses maxima recorded for immature and maturing females)

Location/season	$n$	Age (days)	Weight (g)	Mantle length (mm)
Newcastle	22 [51]	129–183 (162)	251–799 (404)	160–272 (197)
Tasmania-summer	134 [154]	117–263 (176)	120–1700 (550)	147–358 (255)
Tasmania-winter	26 [42]	129–212 (196)	277–1400 (636)	178–314 (240)



**Fig. 6** *Sepioteuthis australis*. Average oviduct egg size with total body weight, of each mature female caught in Tasmania. Error bars: SE

Mature eggs are stored in the oviducts, and unless spawning intervenes eggs must accumulate in the oviduct (Harman et al. 1989; Moltschaniwskyj 1995). In a simultaneous terminal-spawning species, the majority of eggs must be available for egg laying within a very brief period of time (Jackson and Mladenov 1994), and consequently the oviducts become very full and heavy. However, in *S. lessoniana* there was no relationship between oviduct weight and body size, suggesting that mature oocytes were not resident in the oviduct for long and did not accumulate to be laid in a single batch. Oviduct weight was moderately correlated with body

size in summer-caught Tasmanian *S. australis*, however oviduct fullness was not, again indicating that mature oocytes were not accumulating. In a large sample size, a certain degree of correlation between body size and oviduct weight would still be expected under this scenario, as larger animals have the capacity for a higher number of eggs even if they are not resident within the oviduct for a long time period.

Growth and reproduction appear to occur simultaneously in mature animals, with the large size range of mature females indicating that considerable growth takes place after the onset of sexual maturity. Immature *S. australis* were only found up to half the maximum weight of mature individuals. A high but variable growth rate combined with variation in the timing of sexual maturity may account for the very wide size range at maturity noted for many species (Boyle 1990). It has also been suggested that the wide variation in the total number of oocytes in the oviduct and ovary of females at the same stage of maturity and body size may also be explained by variable growth rates between individuals (Rocha and Guerra 1996). However, a high correlation between body size and weights of all the major reproductive organs, except for oviduct weight or fullness, cannot be explained solely by variability in growth between individuals. A more likely explanation for *S. lessoniana* and Tasmanian *S. australis* is a multiple spawning reproductive strategy.

*S. lessoniana* is known to spawn multiple times in captivity (Wada and Kobayashi 1995), supporting the association of these morphological and histological features to a multiple spawning capacity in wild populations. In addition, spent or dead females have not been recorded in *S. lessoniana*, or in *S. australis* on known spawning grounds, and mortality may be sporadic over a prolonged period or occur at low levels continuously. Cannibalism of weak or dying squid may account for the absence of any moribund or dead squid on spawning grounds as has been suggested for *L. vulgaris reynaudii* (Sauer and Smale 1993). The stomach contents of Tasmanian summer-caught *S. australis* suggest cannibalism (G.D. Jackson and G.T. Pecl, unpublished data), and top level predators are common in the spawning area (e.g. seals, dolphins and sharks).

*S. australis* females from South Australian waters are known to copulate more than once per season, as tagged females initially caught with spermatophores were subsequently recaptured a month later with fresh spermatophores (Triantafillos 1998). Tagged females remain on

spawning grounds for up to 2 months in South Australia, and in Tasmania females tagged towards the end of the spawning season were still present on the spawning grounds for at least 2 weeks (G.D. Jackson and G.T. Pecl, unpublished data). Examination of stomach fullness suggested that mature females were still feeding whilst in spawning condition at all locations examined for both species.

There were several distinct differences between populations of female *S. australis* caught in summer and winter in Tasmania, suggesting that the reproductive strategy may have a seasonal component in temperate waters. Reproductive and accessory reproductive tissues accounted for a much higher percentage of the total body mass in summer-caught females, with GSI values twice those of winter-caught animals. Summer-caught females appeared to be laying larger egg batches, and winter-caught females smaller batches, suggesting quite large seasonal differences in age- and size-specific schedules of reproductive investment. Batch-fecundity at size has also been shown to vary between seasons for some fish species (Kjesbu et al. 1996). As there appears to be a seasonal component in *S. australis* to both growth (Pecl, unpublished data) and levels of reproductive investment, there may be a seasonal influence on the relationships between individual size and expected lifetime fecundity (Lowerre-Barbieri et al. 1998), and at the population level, parental biomass and subsequent recruitment.

Life-history theory predicts a trade-off between egg size and fecundity. Although egg size was consistent within an individual, between females there was substantial variation in oviduct egg size, with some females laying fewer, larger eggs and other females laying more numerous, smaller eggs. Larger eggs are usually associated with a longer development time, and egg size determines the size of hatchlings and their subsequent growth and survivorship properties (Calow 1983). The relationship between egg size, hatchling size and hatchling survivorship warrants further investigation in squids (Maxwell and Hanlon 2000).

The spawning strategy of *S. australis* females caught in Newcastle is unclear. Females showed a high correlation between body size and oviduct weight, despite a small sample size, suggesting that perhaps this genetic form of *S. australis* may tend more towards the terminal end of the spawning continuum, although the relatively low GSI values do not support this conclusion. However, the largest female examined was only 800 g, and the oldest, 183 days, and thus it is unlikely that these GSI values represent the maxima achieved by this species in the temperate waters of Newcastle. The GSI was positively correlated with size, suggesting that if larger animals were caught, higher GSIs may have been found. Further confounding the situation, 75% of the mature Newcastle females were caught in winter. If the relationship between oviduct weight and body size is examined for these individuals only, an even higher correlation is found ( $n = 19$ ,  $r = 0.89$ ,  $P < 0.0001$ ).

Tasmanian winter-caught *S. australis*, for a similar sample size, did not show a significant correlation for the same relationship, and the GSIs were also lower compared with Newcastle females. Differences in the reproductive biology, although these have not been previously described, have been suggested as evidence for the two genetic types of *S. australis* examined in this study as constituting distinct taxa (Triantafillos and Adams, in press), and the preliminary results presented here support this hypothesis. Many of the individuals used in the present study are the same individuals analysed by Triantafillos and Adams in their genetic study. Further investigation of the spawning biology of the genetic type of *S. australis* found in Newcastle is warranted with larger, seasonal samples and a wider size range of individuals.

Ultimately the links between environment, individual energy reserves, and the degree to which population synchronicity occurs will determine the nature of the reproductive strategy and its inherent flexibility. Spawning strategies may not be limited simply to early maturation and the laying of many small batches, or late maturation and fewer but larger batches. It seems possible that individuals may also mature early and lay a few large batches or mature later if growing in colder seasons but lay smaller batches more frequently. Maxwell and Hanlon (2000) determined considerable flexibility in the reproduction of captive *Loligo pealeii*, whereby reproductive output was not tightly constrained by length or age. Short, young females were as fecund as large, older ones, and substantial variation was evident between females with some laying small clutches frequently and others large clutches several weeks apart. Considerable flexibility is inherent in the reproductive strategy of *S. australis* and *S. lessoniana*, which like that of *Loligo forbesi* (Boyle et al. 1995) and *L. pealeii* (Maxwell and Hanlon 2000) would provide the potential to cope with fluctuations in abiotic and biotic conditions.

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