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Female reproductive output in the squid *Loligo pealeii*: multiple egg clutches and implications for a spawning strategy

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ABSTRACT: We examined actual and potential reproductive output with particular attention to the females' abilities to lay multiple clutches of eggs. Combining the results of 2 summer spawning seasons, 28 of 47 females that laid eggs in captivity produced substantial clutches (i.e., 5 or more egg capsules per clutch) at least twice. Multiply-ovipositing females exhibited a variety of patterns of oviposition, ranging from relatively small clutches at short intervals to large clutches several weeks apart. Actual reproductive output varied greatly between females. In both years, the number of egg capsules and ova laid showed a negative relationship with the combined mass of the ovary and oviduct at the time of death. Separate correlations between the number of ova laid and the combined number of oocytes and ova remaining in the reproductive tract at death revealed a similarly negative, although statistically weaker, relationship in both years. Most importantly, the number of ova laid in captivity (mean = 11 800 in 1997 and mean = 15 293 in 1998) exceeded the combined number of ova and oocytes remaining at death (mean ca 4500 in both years) by roughly 3×, providing an indication of the extent to which only counting remaining oocytes and ova can underestimate fecundity. The ages of ovipositing females spanned 4 to 6 mo. Interestingly, neither age nor mantle length consistently affected reproductive output, i.e., short young females could be just as fecund as longer older females. A supplementary feeding experiment failed to demonstrate an effect of feeding regime on captive lifespan or reproductive output. The females in one year (1998) were maintained in isolation without access to males; these females laid fertilized eggs, some over periods of 15 or more days, demonstrating the use of stored sperm. For females that had oviposited in both years, the oocytes remaining in the ovary always ranged greatly in size and structure. Thus, the 'spawning strategy' of *Loligo pealeii* appears to involve multiple ovipositions over weeks or months, with oocytes possibly being developed continually. Placing the results of this study in a larger context, reproduction by females in this and other loliginids most likely entails copulation with multiple males and the laying of multiple clutches of eggs, possibly in different locations.

KEY WORDS: Cephalopod · Ecology · Fecundity · Fishery · Life history · Reproduction · Squid · *Loligo pealeii*

INTRODUCTION

Early ecological theory recognized the central role that female fecundity plays in the dynamics of populations (Leslie 1945, Birch 1948, reviewed in Caswell 1989). Because females in many species make large energetic investments in reproduction (reviewed in Stearns 1992), the spatial and temporal patterning of female reproductive activities (e.g., food acquisition, gamete development, fertilization, birthing) largely shape mating systems and the processes of sexual selection (Bateman 1948, Emlen & Oring 1977, reviewed in Andersson 1994). Thus, understanding the ways in which females parcel out reproductive effort over their lifetimes is crucial to understanding the behavior of both populations and individuals.

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In squids (Cephalopoda: Teuthoidea), many studies have examined female fecundity through measures of potential reproductive output, such as the biomass or number of oocytes in the ovaries and ova in the proximal oviducts of dissected females (reviewed in Voss 1983, Mangold 1987). For species within the Loliginidae, especially *Loligo* spp., a common finding is that ovarian oocytes range greatly in size and structure (e.g., Sauer & Lipinski 1990, Lum-Kong et al. 1992, Lum-Kong 1993, Boyle et al. 1995, Collins et al. 1995, Moltschaniwskyj 1995, Rocha & Guerra 1996). These observations challenge the traditional view, based in part on studies on *Loligo opalescens*, that female coleoid cephalopods lay all of their eggs in a brief period before death (reviewed in Mangold 1987, Mangold et al. 1993, Rocha & Guerra 1996), and seem more compatible with the notion that females of some species lay multiple clutches of eggs over a substantial portion of their life cycle.

Here, we focus on female reproductive output in the longfin inshore squid *Loligo pealeii* by examining the ability of females to lay multiple clutches of eggs in captivity; initial data were reported by Maxwell et al. (1998). Measures of the actual reproductive output of female loliginids are largely anecdotal (e.g., Roper 1965, Hixon 1980, Macy 1980), although Moltschaniwskyj's (1995) study of *Photololigo* sp. stands as a notable exception. *L. pealeii* females lay their eggs in gelatinous capsules, 1 of which can contain up to several hundred ova. To distinguish the egg capsule from the unfertilized and fertilized ova that it contains, we use the term 'ova' to refer to the individual ova within the egg capsule. A female can lay a clutch of 50 or more egg capsules over the course of several hours (Drew 1911). For the purposes of our study, we quantify a female's actual reproductive output in 2 ways: (1) the number of egg capsules that she lays in captivity and (2) an estimation of the number of ova that are within these capsules. We use the terms 'actual reproductive output' and 'reproductive output' synonymously. By contrast, 'potential reproductive output' refers to the gametes that remain in a female's reproductive tract at death. We call the gametes in the ovary 'oocytes' and those in the oviduct and oviducal gland 'ova.' Throughout this paper, we distinguish between ova that are laid in egg capsules and those that remain in the females' bodies at death. By summing actual and potential reproductive output, we provide a 'total estimated fecundity' of the females.

We also address a second issue. Measures of potential reproductive output, such as examinations of ovaries and oviducts, are 'snap-shots' of the females' reproductive conditions at death. What these measures indicate about the females' reproductive histories is unclear. Thus, we quantify the relationship between potential and actual reproductive output through regression and correlational analyses. In addition to analyzing both the mass and number of oocytes and ova, we include mantle length and age as measures of potential output. For example, mantle length correlates positively with the combined number of oocytes and ova in the ovary and oviduct in several *Loligo* spp. (Coelho et al. 1994, Boyle et al. 1995, Collins et al. 1995).

This study was motivated in part by the desire to introduce a more accurate combination of methods to assess fecundity in female squids. The challenges of combining experiments on live squids with measurements and biometric correlations of anatomical features are formidable and expensive. Yet, fisheries managers and cephalopod biologists require live animals to understand the recruitment dynamics and life history strategies of some of the commercially valuable squid species (Rodhouse et al. 1998). Historically, squids have been thought to be terminal spawners in which females would have available to them a limited number of mature eggs. In such a system, one would predict a strong negative relationship between the number of eggs laid and the number of ova remaining in the female's reproductive tract; furthermore, remaining ova should be mature. Here we attempt to provide insight into the 'spawning strategy' of *Loligo pealeii*, which we define as the coordination of egg development and egg laying.

METHODS

Collection and maintenance. Squids were jig-caught in Nantucket and Vineyard Sounds during May to September 1997 and May to September 1998. We placed single females into large tanks (2 to 4 m diameter) maintained at ambient temperature, and daily checked each tank for egg capsules. New egg capsules were removed, wrapped loosely in a mesh bag, and put into a holding tank at ambient temperature.

In 1997, each of the 36 females was maintained separately with a male throughout her captive life. Each pair received 2 to 10 fish (*Fundulus heteroclitus*) per day. Fish offered to the squid were 5 to 8 cm in total length (snout to end of tail); *F. heteroclitus* of this size averaged 3.56 g in body mass (SE = 0.50 g, n = 16). In an attempt to increase the females' lifespans in captivity, we kept 36 females in isolation in large tanks in 1998 (i.e., no other conspecific was added to the tank). Of these females 24 were each provided with 6 *F. heteroclitus* per day ('High' diet). The remaining 12 females were provided with 2 fish per day ('Low' diet). To investigate the effect of feeding regime on survivorship and reproductive output, 6 females on the High

diet were compared statistically with females on the Low diet. The females of each of 6 pairs were matched in terms of mantle length and date of capture. To conduct this experiment throughout the season, we used relatively short females (i.e., less than 15 cm in mantle length), because longer females become scarce as the season progresses in Nantucket and Vineyard Sounds (M.R.M. unpubl. data).

Reproductive output and anatomy. Egg capsules that were laid in captivity were allowed to incubate before their ova were counted. In May and the first half of June, the ambient water was 10 to 15°C; the ova were counted 20 d after being laid. After mid-June, the ambient water was 15 to 23°C, and the ova were counted 7 d after laying. Unfertilized ova within the capsules remained clear, small, and spherical (ca 1 to 2 mm in greatest diameter) throughout the incubation period. The fertilized ova developed beyond the point when the yolk sac was distinguishable from the embryonic head/mantle and when the optic lobes were evident (i.e., Stages 20 to 21 in Arnold 1990).

We sampled egg capsules from each female to estimate the number of ova laid in captivity. In 1997, we randomly selected 10 capsules that were laid over the course of each female's captive life. To estimate the number of ova laid by a female in captivity, we counted the ova within her sampled capsules, calculated the mean value, and multiplied this mean value by the number of egg capsules laid. In 1998, we randomly selected 10 egg capsules each time each female oviposited; thus, singly ovipositing females had the ova of 10 capsules counted, whereas multiply-ovipositing females had the ova of 20 or more capsules counted. For each female, we calculated the mean number of ova per capsule for all capsules counted, and multiplied this mean by each female's number of egg capsules laid.

Once a female died, we measured her dorsal mantle length and extracted her statoliths and her ovary, proximal oviduct, and oviducal gland. We preserved the statoliths in 70% ethanol. The rings of each female's left statolith were counted by William K. Macy using the method described in Brodziak & Macy (1996). Two counts were performed on the statolith, and the mean number of rings was taken for the 2 counts. Based on studies on other squid species, including *Loligo* spp. (reviewed in Jackson 1994, Brodziak & Macy 1996) we assumed that the number of statolith rings equaled age in days. We preserved the ovary, oviduct, and oviducal gland in 10% formalin; once fixed, we weighed each organ to 0.01 g.

We counted all of the ova that remained in the oviducal gland. We estimated the number of oocytes and ova remaining in the ovary and oviduct by the following procedure. For the ovary, we broke off two 0.10 g pieces, one from the anterior and the other from the posterior region. We counted the oocytes in these 2 pieces with the aid of a dissecting microscope (4× maximum magnification). We divided the number of oocytes in each piece by the mass of the piece. For each female, we averaged these 2 ratios and multiplied this average value by the mass of the ovary to estimate the number of oocytes in the ovary. We verified this sampling technique by counting all of the oocytes in the ovaries of 10 females in 1998. For the oviduct, we broke off one 0.10 g piece because the ova were much more uniform in size and appearance than the ovarian oocytes. We counted the ova in the piece, divided the number of ova by the mass of the piece, and multiplied this value by the mass of the oviduct. We verified this procedure for 9 of the 10 females in 1998. For both the ovary and oviduct, we calculated the percent difference between the actual and estimated number of oocytes or ova by the equation:

$$\text{Percent difference} = \frac{\text{estimated} - \text{actual}}{\text{actual}} \times 100\% \quad (1)$$

where a positive value indicates overestimation (i.e., estimated greater than actual) and a negative value indicates underestimation (i.e., estimated less than actual). This estimation method tended to overestimate the actual number. The mean ± SE percent difference for oocytes in the ovary was 22.4 ± 10.9% of the actual (n = 10), and that for the ova in the oviduct was 13.2 ± 11.1% of the actual (n = 9). We view these deviations from the actual as being slight, and do not adjust our estimated numbers.

Data analysis. All statistical tests were performed with the Statview (Version 4.0, Abacus Concepts Inc., Berkeley, CA) and JMP (Version 2.0.4, SAS Institute Inc., Cary, NC) software packages; all tests were 2-tailed. Through multiple regression, we examined the effects of female mantle length, female age (i.e., days = number of statolith rings), and the combined mass of the ovary and oviduct on female reproductive output in captivity. Of the 20 females that oviposited in 1997, the statoliths of one were in poor condition, and the organs of another were damaged. Thus, we used 18 females for analysis. For the 1998 females, we restricted our analysis to the females maintained on the High quality diet. Unlike the females kept on the Low quality diet, the females on the High quality diet more fully encompassed the size range of mature females in this population; their mantle lengths ranged from 11 to 22 cm. Of the 19 females on the High quality diet that oviposited, the statoliths of 3 were in poor condition, resulting in 16 females for analysis. For both years, some of the independent variables were moderately correlated with each other, but the largest coefficient in any year was 0.66, so

correlation among the independent variables was not a concern in the multiple regression tests (Tabachnick & Fidell 1989).

RESULTS

General results

In 1997, 22 of the 36 females lived for at least 3 d in the tanks, and the mean ± SE survival of these 22 females was 13 ± 2 d (range = 3 to 33 d). Twenty of these 22 females laid egg capsules. In 1998, 34 of the 36 females lived for at least 3 d in the tanks; their mean ± SE survival in captivity was 16 ± 2 d (range = 4 to 50 d). In total, 27 of these 34 females laid egg capsules (19 females on the High diet, 8 females on the Low diet). Defining a 'clutch' as the laying of at least 1 egg capsule in a day, each female laid an average of 2.9 separate clutches in 1997 (SE = 0.5, n = 20) and 2.7 separate clutches in 1998 (SE = 0.4, n = 27). Pooling these clutches for each year, the mean ± SE number of newly-laid egg capsules per clutch was 20 ± 3 in 1997 (maximum = 104 egg capsules per clutch, n = 60 clutches) and 39 ± 3 in 1998 (maximum = 107 egg capsules per clutch, n = 73 clutches).

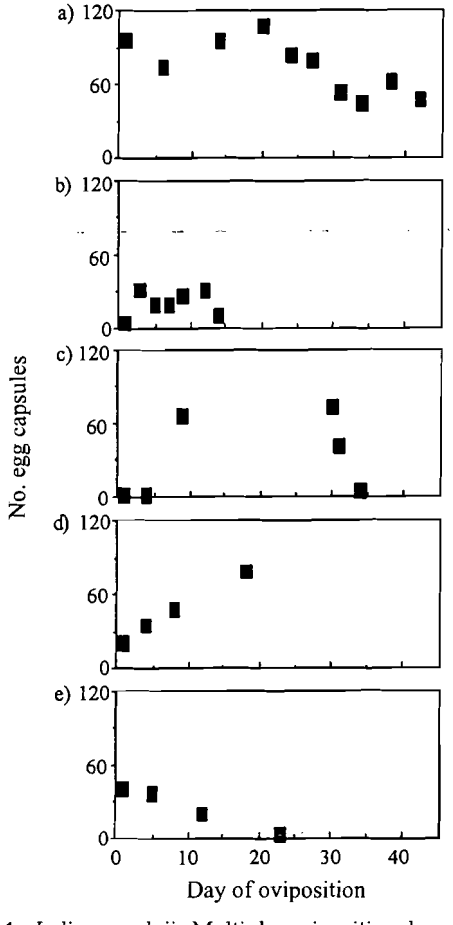

Fig. 1. *Loligo pealeii*. Multiple oviposition by selected females. Each frame represents the egg-laying activity of 1 female; each data point represents the laying of 1 or more egg capsules. (b,c,e) 1997 females; (a,d) 1998 females. Day 1 = each female's first day of oviposition in captivity

During the 2 seasons, 28 females laid substantial clutches (i.e., 5 or more egg capsules) on at least 2 separate days. In 1997, 12 females did so; in 1998, 16 females did so. The temporal patterning of these multiple clutches varied between the females. The reproductive histories of a few females illustrate this variation (Fig. 1). Some females laid clutches frequently, with either many capsules per clutch (e.g., Fig. 1a) or relatively few capsules per clutch (e.g., Fig. 1b). Another pattern was the spacing of large clutches by 2 to 3 wk (e.g., Fig. 1c). The output of some females increased over time (e.g., Fig. 1d); for others, output decreased over time (e.g., Fig. 1e).

In both years, most of the females that lived for at least 3 d and oviposited were found to have eggs remaining in the ovary and/or oviduct at death. Of the 20 females in 1997, 10 had oocytes and ova present, 5 had oocytes only, and 2 had ova only. Of the 27 females in 1998, 20 had oocytes and ova present, 3 had oocytes only, and 1 had ova only. For both years, oocytes in the ovary always ranged greatly in size and structure. Small oocytes, less than 1 mm in greatest diameter and veined in appearance, were found among larger, translucent, amber-colored oocytes (1 to 2 mm in greatest diameter). The amber-colored oocytes resembled the fully mature ova of the oviduct and oviducal gland.

Variation in reproductive output within females

The number of egg capsules within a clutch could change remarkably over a female's life (Fig. 1). For a given clutch of egg capsules, the number of ova within each capsule showed little variation, as evidenced by the small error bars for the clutches shown in Fig. 2. For a given female, the number of ova per egg capsule appeared to hover around a certain value, although this value differed between females (Fig. 2). For at least 1 female, however, there was an indication that the number of ova per capsule changed with time (Fig. 2a). Thus, our method of estimating actual ova production in captivity by sampling egg capsules over the females' reproductive careers is appropriate to capture this within-female variation.

The degree of fertilization of the egg capsules differed between the 1997 females, who were continuously kept with a male, and the 1998 females, who

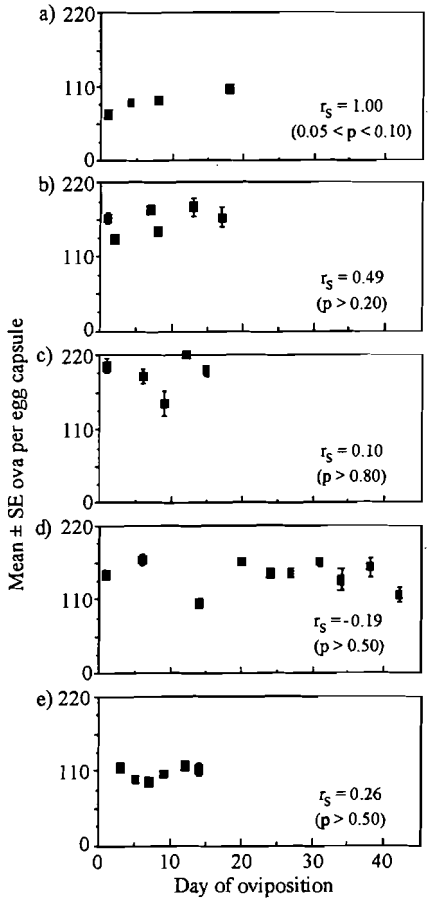

Fig. 2. *Loligo pealeii*. Ova per egg capsule laid by multiply-ovipositing females. For each day of oviposition, the ova within 10 randomly selected egg capsules were counted. The Spearman correlation coefficient indicates how ova production changed over time for each female. (a–d) females from 1998, (e) a female from 1997. Data points are mean ± SE number of ova per capsule. Day 1 = each female's first day of oviposition in captivity

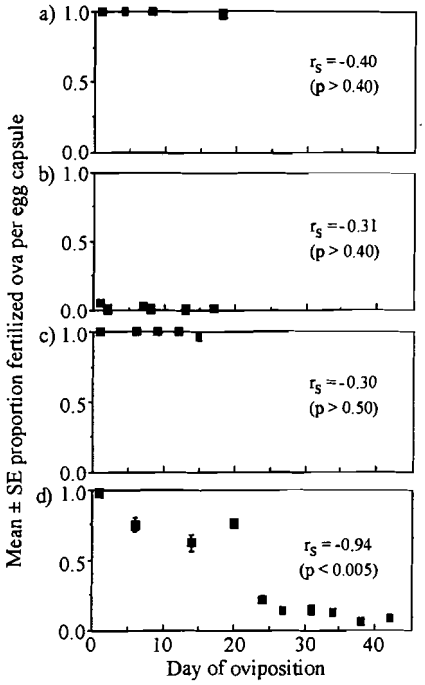

Fig. 3. *Loligo pealeii*. Proportion of fertilized ova per egg capsule for the multiply-ovipositing females in 1998. Data shown are for the egg capsules in Fig. 2. The Spearman correlation coefficient gives an indication of how the proportion of fertilized ova changed over time for each female. (a–d) correspond to the females in Fig. 2a–d. Data points are mean ± SE proportion of fertilized ova per capsule. Day 1 = each female's first day of oviposition in captivity

were maintained in isolation. For example, a female in 1997 (Fig. 2e) had all of the ova in her counted egg capsules fertilized. The 1998 females show variation in the degree of fertilization (Fig. 3), with 2 females showing very low degrees of fertilization on several days of oviposition (Fig. 3b,d).

An examination of the first clutches of the 1997 and 1998 females further demonstrates this difference in the degree of fertilization. The 1997 females laid their first egg capsules 3.8 ± 0.7 d (mean ± SE, n = 20) after first being housed with the males. Of these clutches, 14 contained 10 or more egg capsules; the ova were counted for 10 randomly-selected capsules from 9 of these first clutches. The mean percentage of fertilized ova per egg capsule ranged from 91.4 to 100% for these clutches (Fig. 4a), and the overall mean ± SE was 98.9 ± 0.9% (n = 9). The 1998 females laid their clutches 5.0 ± 0.5 d after first being isolated (mean ± SE, n = 27). Of these clutches, 24 clutches were of 10 or more egg capsules. The mean percentage of fertilized ova per egg capsule varied from 1.0 to 100.0% (Fig. 4b), with the overall mean ± SE being 74.7 ± 6.1% (n = 24). It is likely that a greater proportion of the ova in 1998 would have been fertilized if the females had been allowed access to males. Thus, the following analyses focus on the total number of ova laid by the 1997 and 1998 females.

Variation in reproductive output between females

1997: multiple regression analysis

The 18 females each produced an average of 61 egg capsules in captivity (SE = 12, range = 1 to 185 capsules); each capsule contained 162 ova on average (SE = 4, range = 91 to 294 ova, n = 122 capsules censused from a subset 13 females). The 3 independent variables (Table 1) showed an overall significant effect

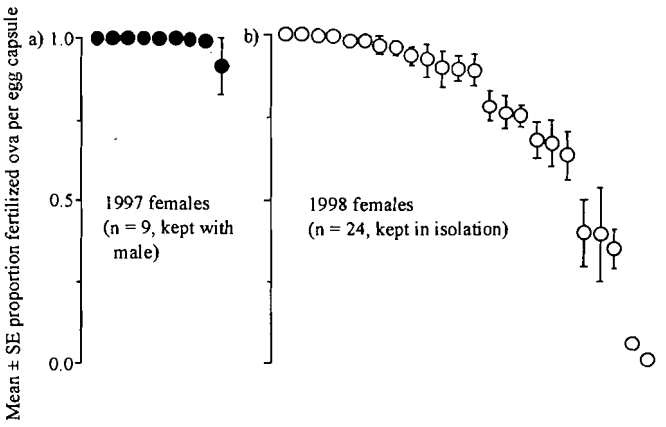

Fig. 4. *Loligo pealeii*. Proportion of fertilized ova per egg capsule for the first clutch laid in captivity. All clutches shown were of 10 or more egg capsules; 10 egg capsules were randomly selected and censused for analysis. Each data point represents the mean ± SE proportion of fertilized ova per capsule for a female's first clutch; females are arranged in descending order of proportion ova fertilized

on the number of egg capsules laid, with the combined ovary and oviduct mass showing a negative effect. To standardize each female's output with respect to her captive lifespan, we divided each female's number of egg capsules by her lifespan. This analysis also yielded a significant overall effect (ANOVA whole-model test: $F_{3,14} = 7.10$, $p < 0.01$), with the combined ovary and oviduct mass showing a negative effect ($p < 0.001$). The number of ova laid in captivity was estimated for 13 of these 18 females; the set of independent variables failed to show a significant effect on the number of ova laid in captivity (Table 1). Dividing each female's number of ova laid by her captive lifespan yielded a similarly non-significant result (ANOVA whole-model test: $F_{3,9} = 1.99$, $p > 0.10$).

The average number of ova laid in captivity by the above 13 females was 11 800 (Table 2). These females had, on average, a combined total of 4406 oocytes and ova remaining in their bodies at death (Table 2). For these females, the number of laid ova correlated weakly and negatively with the combined number of remaining oocytes and ova (Spearman correlation: $r_S = -0.16$, $p > 0.60$). The exclusion of a particularly fecund female (Female 7-M) slightly affected this correlation ($r_S = -0.42$, $p > 0.10$). The 'total estimated fecundity' for these females ranged from 5755 to 58 490 (Table 2).

1998: multiple regression analysis

The 16 females laid an average of 114 egg capsules in captivity (SE = 45, range = 14 to 765 capsules); each capsule contained 134 ova on average (SE = 2, range = 5 to 256 ova, n = 396 capsules censused from these 16 females). The 3 independent variables showed a significant overall effect on the number of egg capsules laid in captivity, with the combined ovary and oviduct mass showing a negative effect (Table 3). Dividing each female's number of egg capsules laid by captive lifespan did not change the statistical significance of these results (ANOVA whole-model test: $F_{3,12} = 4.18$, $p < 0.05$). The number of ova laid in captivity was estimated for all 16 females; the set of independent variables showed a significant overall effect, with the combined ovary and oviduct mass showing a negative effect and mantle length showing an indication of a positive effect (Table 3). Dividing the number of ova by the females' captive lifespans weakened the overall effect (ANOVA whole-model test: $F_{3,12} = 3.39$, $0.05 < p < 0.10$), although the combined ovary and oviduct mass continued to show a significant, negative effect ($p < 0.01$).

Table 1. Multiple regression: effects on reproductive output (1997 females). Separate multiple regression tests performed for each dependent variable

	Dependent variable: Number of egg capsules laid n = 18 $R^2 = 0.42$ $F_{3,14} = 3.59$, $p < 0.05$[a]		Dependent variable: Number of ova laid n = 13 $R^2 = 0.37$ $F_{3,9} = 1.77$, $p > 0.20$[a]	
Independent variable	Coefficient ± SE	p-value	Coefficient ± SE	p-value
Mantle length	5.2 ± 3.8	$p > 0.10$	2442.9 ± 1269.2	$0.05 < p < 0.10$
Age	1.1 ± 0.6	$0.05 < p < 0.10$	66.8 ± 224.0	$p > 0.70$
Oviduct + Ovary mass[b]	−68.5 ± 23.1	$p < 0.05$	−11850.3 ± 8510.8	$p > 0.10$

[a]ANOVA whole-model test
[b]Log-transformed to correct for non-normality

Table 2. Biometric measurements and 'total estimated fecundity' for females of 1997 for which the number of ova laid in captivity was estimated (n = 13 females). ML = mantle length, Og = number of ova remaining in oviducal gland at death (directly counted), Od = number of ova remaining in oviduct (estimated), Oy = number of oocytes remaining in ovary (estimated), Ova laid = number of ova laid in captivity (estimated)

ID	ML (cm)	Age (d)	Og	Od	Oy	'Potential reproductive output' Og + Od + Oy	'Actual reproductive output' Ova laid	'Total estimated fecundity'
7-A	9.6	138	0	0	0	0	14705	14705
7-B	10.8	118	0	0	0	0	6501	6501
7-C	12.1	141	1	0	3134	3135	7625	10760
7-D	13.4	168	3	0	1863	1866	22001	23867
7-E	13.6	174	7	631	0	638	10461	11098
7-F	13.7	151	0	2894	4202	7096	260	7356
7-G	13.7	180	6	6758	865	7629	9942	17571
7-H	14.3	164	0	2762	792	3554	6977	10532
7-I	15.3	170	6	310	1257	1572	9483	11055
7-J	15.8	165	2	49	1148	1199	13912	15111
7-K	16.2	177	24	0	1701	1725	4030	5755
7-L	20.2	181	6	1417	10103	11526	6348	17874
7-M	22.5	181	12	0	17323	17335	41155	58490
Mean	14.7	162	5	1140	3261	4406	11800	16206
SE	1.0	5	2	551	1392	1438	2866	3799

Table 3. Multiple regression: effects on reproductive output (1998 females). Separate multiple regression tests performed for each dependent variable

	Dependent variable: Number of egg capsules laid[b] n = 16 $R^2 = 0.52$ $F_{3,12} = 4.38$, $p < 0.05$[a]		Dependent variable: Number of ova laid[b] n = 16 $R^2 = 0.53$ $F_{3,12} = 4.49$, $p < 0.05$[a]	
Independent variable	Coefficient ± SE	p-value	Coefficient ± SE	p-value
Mantle length	0.03 ± 0.03	$p > 0.40$	0.08 ± 0.04	$0.05 < p < 0.10$
Age	0.01 ± 0.01	$p > 0.10$	0.01 ± 0.01	$p > 0.20$
Oviduct + Ovary mass	−0.09 ± 0.03	$p < 0.01$	−0.12 ± 0.03	$p < 0.01$

[a]ANOVA whole-model test
[b]Log-transformed to correct for non-normality

The average number of ova laid in captivity by the 16 females was 15 293 (Table 4). These females had, on average, 4559 oocytes and ova remaining in their bodies at death (Table 4). The number of ova laid showed an indication of correlating negatively with the number of oocytes and ova remaining (Spearman correlation: $r_S = -0.46$, $0.05 < p < 0.10$). The exclusion of Female 8-H affected the statistical significance of this correlation ($r_S = -0.62$, $p < 0.05$). The influence of Female 8-H in the above multiple regression analyses was diminished by the log-transformations of the dependent variables; the exclusion of Female 8-H from the regression analyses did not affect the statistical significance of the independent variables. The 'total estimated fecundity' for these females ranged from 6036 to 116 994 (Table 4).

Effect of feeding regime: High versus Low quality diet

Females on the High quality diet consumed more food (mean ± SE fish eaten per day: 3.1 ± 0.4, n = 6) than the females on the Low quality diet (mean ± SE fish eaten per day: 1.6 ± 0.1, n = 6). Captive lifespan, the number of egg capsules laid, the number of ova laid, and a measure of reproductive investment failed

Table 4. Biometric measurements and 'total estimated fecundity' for females of 1998 that were kept on the 'High quality' diet and for which the number of ova laid in captivity was estimated (n = 16 females). Codes for columns given in Table 2

ID	ML (cm)	Age (days)	Og	Od	Oy	'Potential reproductive output' Og + Od + Oy	'Actual reproductive output' Ova laid	'Total estimated fecundity'
8-A	11.7	172	4	719	1436	2159	3877	6036
8-B	12.3	166	0	294	3460	3754	8743	12497
8-C	12.3	149	0	152	0	152	13538	13689
8-D	13.2	166	1	0	1691	1692	19729	21421
8-E	13.5	169	2	128	1895	2026	4372	6397
8-F	14.9	161	2	151	3704	3857	5674	9531
8-G	15.4	171	0	89	604	693	14806	15499
8-H	16.2	175	27	649	4452	5128	111866	116994
8-I	16.4	162	5	281	3625	3912	7274	11186
8-J	16.8	170	9	4044	5455	9508	721	10229
8-K	18.6	165	0	679	4127	4805	6046	10851
8-L	18.7	188	6	1888	3307	5201	12550	17751
8-M	19.2	163	3	3617	5251	8871	6571	15442
8-N	19.3	160	6	965	4049	5019	4225	9245
8-O	20.7	158	8	359	1663	2029	21025	23054
8-P	22.0	185	13	346	13783	14141	3669	17810
Mean	16.3	168	5	898	3656	4559	15293	19852
SE	0.8	2	2	309	785	910	6605	6592

to differ significantly between the 2 feeding regimes (Fig. 5). Dividing the number of egg capsules and the number of ova laid by the females' lifespans did not change the statistical significance of the results.

DISCUSSION

Reproductive output

Females in both years laid substantial clutches of egg capsules on multiple days: 12 of the 36 females in 1997 and 16 of the 36 females in 1998. Noticeable variation in reproductive output existed within and between females. A female's clutch size could vary over time (e.g., Fig. 1c,d), although the number of ova that she packed into each egg capsule tended to remain around a certain value (Fig. 2). Between females, the numbers of egg capsules and ova laid in captivity varied greatly, by as much as an order of magnitude (Tables 2 & 4).

Our estimated values of reproductive output are generally consistent with those for other *Loligo* spp. With regard to potential output, females in both years had a combined average of roughly 4500 oocytes and ova remaining in their bodies at death (Tables 2 & 4). In other studies of *Loligo* spp., the mean combined numbers of oocytes and ova in the ovary and oviduct are similarly on the order of several thousand (e.g., Coelho et al. 1994, Boyle et al. 1995, Collins et al. 1995; see Mangold 1987 for earlier references). Females of the present study laid 11 800 and 15 293 ova on average during their captivity in 1997 and 1998, respectively. Only 1 female (8-H) exceeded Hixon's (1980) estimated 55 000 ova produced by a female (15.5 cm ML) that laid 220 egg capsules in captivity; female 8-H was 16.2 cm ML and laid 111 866 ova (Table 4). The maximum number of egg capsules was 185 in 1997 and 765 in 1998. Other data on egg output for *L. pealeii* further demonstrate the females' reproductive capacities. Macy (1980) maintained a female (8.6 cm ML) that laid 73 egg capsules in captivity, and 1 female in our tanks (19.1 cm ML, not included in our analyses) was caught in October 1998 and lived for 91 d in captivity, during which time she laid over 500 egg capsules (J. A. Cavanaugh unpubl. data).

In our study, the mean number of laid ova exceeded the mean combined number of oocytes and ova remaining in the reproductive tract by roughly 3× in both years. These data provide an indication of the extent to which counting the oocytes and ova in the bodies of dead females can underestimate true fecundity. To our knowledge, the only other similar estimate was made by Boletzky (1987), who found in 1 female *Sepia officinalis* that the number of ova laid was about 4× that of ovarian counts in comparably sized females. For *Loligo pealeii*, the mean 'total estimated fecundity' of our females was 16 206 in 1997 and 19 852 in 1998 (Tables 2 & 4). The number of ova laid did not correlate strongly with the number of oocytes and ova remaining

in the reproductive tract at death, however. In both years, only a weak and negative correlation existed between these 2 variables; in 1998, this correlation was sensitive to the data from the 1 extremely fecund female mentioned above.

Variation in reproductive output between females was explained more reliably by the combined mass of the ovary and oviduct. In 1998, the combined oviduct and ovary mass showed a significant negative effect on the number of egg capsules and ova laid (Table 3). In 1997, the combined oviduct and ovary mass showed a negative effect on the number of egg capsules laid (Table 1).

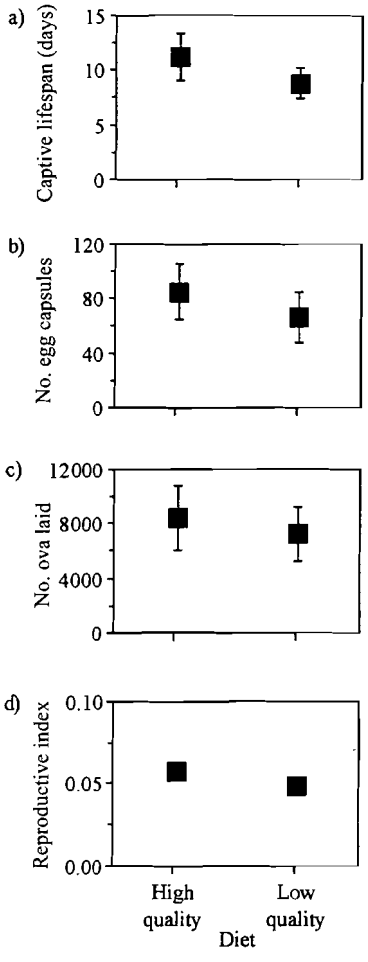

Fig. 5. *Loligo pealeii*. Effect of feeding regime on lifespan and reproduction (1998 females, n = 6 pairs). Data points are mean ± SE. (a) Lifespan in captivity. Wilcoxon paired-sample test: smallest sum of signed ranks = 3.5, $p > 0.20$; $0.20 < \text{Power} < 0.50$ (calculated retrospectively). (b) Number of egg capsules laid. Wilcoxon paired-sample test: smallest sum of signed ranks = 5.0, $p > 0.20$; $0.20 < \text{Power} < 0.50$ (calculated retrospectively). (c) Number of ova laid. Wilcoxon paired-sample test: smallest sum of signed ranks = 6.0, $p > 0.50$; $\text{Power} < 0.20$ (calculated retrospectively). (d) Reproductive index (oviduct + ovary mass/body mass). Wilcoxon paired-sample test: smallest sum of signed ranks = 3.0, $p > 0.20$; $\text{Power} < 0.40$ (calculated retrospectively)

Neither age nor mantle length consistently showed significant effects on reproductive output. Thus, small young females did not necessarily lay fewer egg capsules or fewer ova than did longer, older females. This result applies to the absolute numbers of laid capsules and laid ova, as well as when these data were divided by the females' captive lifespans. Our failure to detect a consistent effect of mantle length suggests that the actual reproductive output of shorter females may not be constrained by body length or size (Peters 1983, Reiss 1989), as biometric correlations might suggest. In this study, mantle length correlated positively with the combined mass of the ovary and oviduct (Pearson correlation, 2-tailed: $r = 0.65$, $n = 18$, $p < 0.05$ [1997]; $r = 0.66$, $n = 16$, $p < 0.05$ [1998]). In other *Loligo* spp., mantle length correlates positively with the combined number of oocytes and ova in the oviduct and ovary (Coelho et al. 1994, Boyle et al. 1995, Collins et al. 1995).

We cannot readily dismiss the possibility that mantle length affects the reproductive success of female *Loligo pealeii*, because other factors might have influenced reproduction. For example, each female in 1997 had constant access to a male, and differences in male phenotype or behavior might have contributed to variation in reproductive output between females. Female mantle length may affect other components of fitness, such as egg size. Boyle et al. (1995) found a positive correlation among mantle length and the diameter of oviduct eggs in *L. forbesi*. The relationships among ovum size, hatchling size and hatchling survivorship warrant further investigation in squids.

The females of 1998, who were kept in isolation without access to males, laid fertilized ova over several weeks (Fig. 3). These females were almost certainly using sperm stored in the seminal receptacle. We did not find clusters of sperm or spermatophores within the mantle cavities of any of the 36 females that we isolated in 1998; this includes 2 females that lived for only 1 or 2 d in captivity. In contrast, we frequently found clusters of sperm around the opening of the distal oviduct when dissecting females that had access to males (e.g., the 1997 females and newly captured females from both years). Drew (1911) estimated that spermatophores placed within the female's mantle cavity do not persist for more than a few days, but this requires experimental verification. Sperm depletion seemed to occur in some of the 1998 females. For example, the proportion of fertilized ova significantly decreased over time for one female (Fig. 3d), and another female consistently laid clutches with low degrees of fertilization (Fig. 3b).

Spawning strategy

Results from the present study have implications for the spawning strategy of this population of *Loligo pealeii*. Most of the females that oviposited over the course of 3 to 50 d in captivity had oocytes remaining in the ovary and/or ova remaining in the oviduct: 17 of 20 females in 1997 and 24 of 27 females in 1998. The oocytes in the ovary always ranged greatly in size, from small and veined in appearance (some much less than 1 mm in greatest diameter) to large and amber-colored (ca 1 to 2 mm in greatest diameter). The presence of the small, veined oocytes suggests that the females had developed oogonia that had yet to undergo vitellogenesis (Cowden 1968, Selman & Arnold 1977, Baeg et al. 1993).

Such a range in the size of ovarian oocytes suggests that the females do not develop and lay a single batch of eggs. Rather, the production of mature ova may have continued during the females' periods of egg-laying (Mangold 1987). The presence of the apparently pre-vitellogenic oocytes in the ovaries of ovipositing females calls for careful interpretation. As other authors have cautioned (e.g., Lum-Kong et al. 1992, Lum-Kong 1993), it is not certain whether these small oocytes typically develop for later fertilization, or whether they remain undeveloped during the egg-laying period. Additionally, it is unclear at what time the females in our study initially formed these small oocytes. Lacking histological examinations of the ovaries, we cannot determine whether the females formed primary oogonia during the egg-laying period. In the loliginid *Photololigo* sp., Moltschaniwskyj (1995) found primary oogonia in the ovaries of females that had oviposited. Other studies, however, indicate that spawning females of some loliginids do not always continue to form primary oogonia (Knipe & Beeman 1978, Lopes et al. 1997). How females coordinate the processes of primary oogonia formation, oocyte development, fertilization, and oviposition remains an outstanding question in cephalopod research.

Our analyses point to a negative relationship between reproductive output and the biomass of the remaining gametes (i.e., combined mass of the ovary and oviduct). This result suggests a trade-off between ova that have been laid and oocytes and ova remaining in the reproductive tract. While this suggestion might imply that *Loligo pealeii* females have a finite, non-renewing supply of gametes, we emphasize that our study examined only recent reproductive output. Not only could these females have oviposited in nature before we captured them, but the captive lifespans of the females might have been shorter than the time required to develop fully mature ova that would have replaced ova laid in the laboratory. This comment notwithstanding, if one accepts the hypothesis that the females' gametes are indeed non-renewing, then, given that longer females would have a larger initial supply of gametes, one would predict that reproductive output would show a positive relationship with mantle length. This prediction, however, is not strongly supported statistically (Tables 1 & 3).

Investigations of a cephalopod's spawning strategy also include the question of whether females undergo somatic growth during the egg-laying period (e.g., Harman et al. 1989, Lewis & Choat 1993, Moltschaniwskyj 1995). The females in the present study fed before and after producing clutches of egg capsules. In the feeding experiment, females on the High quality diet consumed more food than did females on the Low quality diet, but feeding regime failed to significantly affect captive lifespan, reproductive output, or the reproductive index; we note that these tests were of low to moderate statistical power. Still, it is possible that the High-diet females converted their more abundant food into somatic effort if they did not significantly increase their reproductive output, as in *Sepia pharaonis* (Gabr et al. 1999). However, we did not measure squid growth in our study.

This controlled study of multiple oviposition demonstrates considerable variation in reproductive output, both within and between females. Actual reproductive output was not tightly constrained by mantle length or age. This result points to possible behavioral or social influences on reproduction. We restricted the females in our laboratory trials by allowing them only limited social interactions with conspecifics (1997: 1 male; 1998: no males), yet we know from extensive field observations that squids commonly (but not exclusively) lay eggs in communal egg masses in which hundreds of squids may participate in reproductive behavior (Griswold & Prezioso 1981, Hanlon 1996, Hanlon et al. 1997, 1999). Although our maintenance of the isolated females indicates that females do not necessarily require the presence of males to lay eggs, it is possible that the spawning aggregations of *Loligo pealeii* and several other loliginids stimulate females to increase reproductive output. It would be worthwhile to extend our laboratory observations by subjecting females to differing levels of social interactions to see whether social context influences the amount or the patterning of egg laying.

The overall reproductive strategy of female *Loligo* is likely to include multiple mates, the storage of sperm from these mates, and the laying of multiple egg clutches possibly in different locations (Hanlon & Messenger 1996, Hanlon 1998). Considering that *L. pealeii* females oviposit at least over several weeks of their brief life span, they apparently engage in reproductive behavior for a large portion of their lives. The change

in thought regarding cephalopod reproduction is that some loliginid squids appear to lay eggs over a longer period than previously envisioned. We hope that future research on actual reproductive output combined with detailed analysis of oocyte production and development will help clarify how female squids allocate reproductive effort over their lifetimes.

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