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Description and quantification of developmental abnormalities in a natural *Sepioteuthis australis* spawning population (Mollusca: Cephalopoda)

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ABSTRACT: Eggs of the southern calamary *Sepioteuthis australis* were sampled from spawning sites off eastern Tasmania, Australia, during an austral spring/summer spawning season. At fortnightly intervals, 3 unfouled and 3 biofouled egg strands were sampled from 6 to 23 distinct egg masses (N_{eggs} = 2649). Highly significant variation was noted between sample dates in the frequency of unfertilised eggs, developmental abnormalities and egg mortalities. Unfertilised eggs were only found during late October and early November and represented a mean 2.12 ± 1.25% SE and 0.58 ± 0.58% SE eggs per strand respectively. Frequency of abnormality varied significantly between sample dates and ranged from 8.35 ± 1.86% SE eggs per strand in late November to 0.92 ± 0.41% SE in late December. Abnormalities were arbitrarily categorised as defects in external yolk sac morphology, reduced embryonic size, mantle deformities, eye deformities and arm deformities. Defects in external yolk sac morphology were found throughout the spawning season and accounted for 46.3% of all abnormalities. Incidence of mortality varied significantly between sample dates and ranged from 1.40 ± 0.68% SE per strand in late October to 11.61 ± 3.23% SE in early January. Highly significant correlation was noted between incidence of developmental abnormality and within-strand egg position. Biofouled egg strands were characterised by comparatively low incidences of unfertilised and dead eggs. The influences of environment, egg position and biofouling upon embryonic development in *S. australis* are discussed.

KEY WORDS: Squid · Embryos · Abnormality · Mortality · Temperature · Egg position · Biofouling

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INTRODUCTION

Developmental abnormalities have been identified within the eggs and embryos of many marine taxa and are typically associated with unfavourable developmental conditions. Elevated water temperature generates abnormalities in embryos of the echinoid *Arachnoides placenta* (Chen & Chen 1992), larvae of the Arctic cod *Boreogadus saida* (Graham & Hop 1995) and hatchlings of the tilapia *Oreochromis mossambicus* (Wang & Tsai 2000). Increased salinity promotes abnormalities in megalopae of the crab *Rhithropanopeus harrisii* (Gonçalves et al. 1995), and exposure to UV-B radiation induces embryonic abnormalities in the green sea urchin *Strongylocentrotus droebachiensis* (Adams & Shick 2001). *In situ* sampling of amphipod populations in the Baltic Sea indicates rates of embryonic abnormality between 1.5 and 4.9% (*Monoporeia affinis*; Sundelin & Eriksson 1998). Similar sampling of copepod nauplii off Japan suggests an incidence of abnormality between 20 and 40% (*Pseudocalanus newmani*; Ban et al. 2000). The extent of variation between and within these estimates may reflect class, species, population and/or season specificity in frequency of embryonic abnormality. Distinct seasonality has been noted in incidence of abnormal eggs, embryos and veligers within gastropod populations; abnormalities are most prevalent within broods developing outside the main reproductive season (*Littorina saxatilis*: Sokolova 1995; *L. neglecta*: Johnson et al. 2000). Irrespective of cause,

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variation in the incidence of embryonic abnormality may potentially influence rates of recruitment with flow-on effects to population size and structure. For short-lived species, such variation in successful production of young can have a significant impact. Cephalopod populations are characterised by life-history traits such as short-life spans, semelparity and rapid response to variation in environmental conditions (Rodhouse 2001). Variation in the population size and structure of cephalopod populations may, in part, reflect variation in rates of embryonic abnormality.

Mature loliginid squid typically aggregate in coastal waters to mate and spawn, e.g. *Loligo opalescens* (McGowan 1954), *L. pealei* (Arnold 1962), *L. vulgaris reynaudii* (Sauer et al. 1993), *Sepioteuthis lessoniana* (Segawa 1987) and *S. australis* (Moltschaniwskyj & Pecl unpubl.). Some of the loliginids produce multiple batches of eggs (e.g. *L. pealei*: Maxwell & Hanlon 2000), with successive batches being laid over a period of several weeks (e.g. *L. vulgaris*, *L. forbesi*: Rocha & Guerra 1996; *L. pealei*: Maxwell et al. 1998; *L.v. reynaudii*: Melo & Sauer 1999; *S. australis*, *S. lessoniana*: Pecl 2001). Individually encapsulated eggs are collectively packaged within gelatinous material to form discrete egg strands (Boletzky 1989). This gelatinous wrapping confers physical protection against mechanical abrasion and chemical protection against microbial infection (e.g. Biggs & Epel 1991). The spawning behaviour of *S. australis* is consistent with the general loliginid trend. Between October and December each year, large numbers of mature squid aggregate in coastal waters around Tasmania, Australia, to mate and spawn (Moltschaniwskyj & Pecl unpubl.). Mated females attach multiple egg strands, typically containing between 3 and 9 eggs, onto seagrass or macroalgae holdfasts. Collective deposition of egg strands leads to the formation of discrete egg masses containing <10 or >600 egg strands (Moltschaniwskyj & Pecl unpubl.). In common with other cephalopod species, developmental rate in *S. australis* is positively associated with water temperature (Steer et al. 2002). With a life expectancy of 6 to 10 mo (Pecl 2000), the embryonic phase of *S. australis* is equivalent to ~20% of the post-hatching life span and represents both a critical formative phase and a significant proportion of the entire life cycle. *S. australis* embryos undergo direct development to hatch as miniature juveniles of basic adult form. Typical for short-lived species, the success of future squid generations depends entirely upon the ability of present generations to produce viable offspring. Therefore, correct embryonic development represents an important component of this ability.

The present study provides the first *in situ* assessment of abnormalities within a natural cephalopod population. Incidence of developmental abnormality was quantified within a shallow spawning and commercially exploited *Sepioteuthis australis* population off the east coast of Tasmania. Morphological abnormalities have previously been reported in cephalopod eggs reared under laboratory conditions (e.g. Naef 1928, Ranzi 1929, Marthy 1969). Experimental elevation of temperature promotes abnormalities in embryos of the squids *Illex illecebrosus* (O'Dor et al. 1982), *Todarodes pacificus* (Sakurai et al. 1996), *Loligo vulgaris reynaudii* (Oosthuizen et al. 2002b) and *L. forbesi* (Gowland et al. 2002). Hypoxic conditions at the centre of egg masses induce retardation and abnormal embryonic development in both squid (*Loligo* spp.: Arnold 1990) and oysters (*Crassostrea virginica*: Baker & Mann 1994). Conversely, biofouling of egg capsules has positive effects upon embryonic development in some molluscs and amphibians (e.g. Cohen & Strathmann 1996, Pinder & Friet 1994). The specific aims of this study were to identify and quantify developmental abnormalities typically observed in *S. australis* embryos, and to assess factors affecting incidence of developmental abnormalities, e.g. temperature, within-mass egg position and biofouling.

MATERIALS AND METHODS

Sepioteuthis australis egg masses were sampled from spawning grounds situated in Great Oyster Bay, eastern Tasmania, Australia (42° 07′ 34″ S, 148° 17′ 51″ E). At fortnightly intervals between October 2000 and January 2001, SCUBA divers searched 3 discrete areas of the seagrass *Amphibolis antarctica* for *S. australis* egg masses (Fig. 1). All sample sites were located within 0.5 km of the shore in 4 to 8 m of water. Particular emphasis was

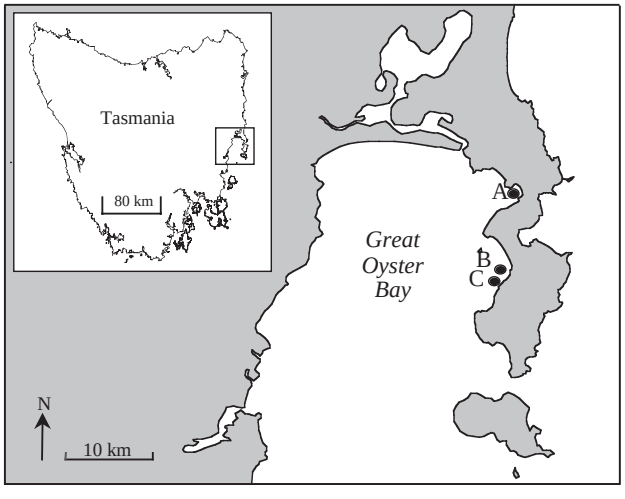

Fig. 1. *Sepioteuthis australis*. Spawning grounds from this study. Egg strings were collected from discrete areas of the seagrass *Amphibolis antarctica* (A,B,C) at 2 wk intervals between late October 2000 and early January 2001

placed upon locating egg strands that contained embryos close to hatching. Developmental stage, early or late, was estimated *in situ* based on egg capsule size and embryo morphology (primarily eye pigmentation). Three unfouled and 3 biofouled egg strands were removed from between 6 and 23 egg masses per sampling trip. Unfouled egg strands were defined as those displaying 0 to 10% surface coverage by epiphytic algal growth (Fig. 2A). Fouled egg strands were defined as those displaying 50 to 100% surface coverage (Fig. 2B). All material was bagged in fresh seawater and stored out of direct sunlight prior to dissection.

Egg strands were examined within 8 h of field collection. Embryos were dissected out and numbered sequentially according to their position within the egg strand (Position 1 = proximal/fixed end of the strand). Individuals were examined under a dissection microscope and allocated a developmental stage according to the criteria described by Steer (unpubl.) for *Sepioteuthis australis*, which follows that of Segawa (1987) for *S. lessoniana*. Incidence of unfertilised eggs, identified by an opaque appearance and lack of cell division, was calculated per egg strand as a function of sample date (normalised to account for variation in sample site and degree of biofouling). Examination of several thousand embryos generated a clear impression of normal embryonic morphology. Abnormal development was recognised in late stage embryos by an absence of specific organs (e.g. arms or eyes), or by distortions in character shape, size or position relative to the individual (e.g. inversion of the mantle). Abnormalities were arbitrarily allocated into 1 of 5 categories: defects in external yolk sac morphology, pronounced reduction in embryo size, mantle deformities, eye deformities and arm deformities. Brief visual descriptions of developmental abnormalities were recorded per embryo, and illustrated examples of each category are provided (Fig. 3). Incidence of abnormally developed embryos was calculated per egg strand as a function of sample date (normalised to account for variation in sample site and degree of biofouling). Frequency occurrence of dead embryos, identified by arrested development, was similarly calculated. Dead embryos were scored exclusively as mortalities, i.e. regardless of any developmental abnormality. A total of 84 egg masses yielding 481 egg strands and 2649 embryos were examined during the spawning season.

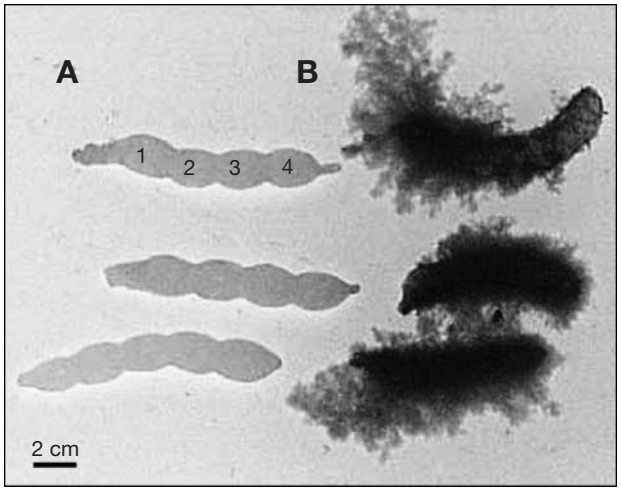

Fig. 2. *Sepioteuthis australis*. Photograph of field-collected egg strands containing late stage embryos. (A) Unfouled egg strands; (B) biofouled egg strands. Numbers denote within-strand egg position i.e. 1 = position nearest the proximal/fixed end of the strand

Information on sea-bottom temperature (SBT in °C) was collected to provide background information on ambient conditions during the sampling period. Laboratory rearing of squid eggs (Hanlon 1990) indicates that controlled temperature changes should not exceed 1°C d^{-1} (equivalent to $\Delta 0.04$°C h^{-1}). Hourly SBT was measured *in situ* during this study in order to provide detailed assessment of natural temperature fluctuations. SBT was measured via a 32K StowAway 'TidbiT' temperature datalogger secured to the seafloor (<4 m deep) at Site A, 1 mo prior to first sampling.

Frequency analyses of data revealed both normal and skewed data distributions. For example, incidence of developmental abnormality per egg strand was skewed on 3 of 6 sample dates (Ryan-Joiner $p < 0.01$; kurtosis 4.68 to 19.87; skew 2.19 to 4.11). Data transformation was prohibited by the high frequency of zero values within the data set; therefore, non-parametric techniques were employed. Variation in the relationship between egg position and developmental abnormality was investigated by converting number of abnormalities per position into percentage abnormalities per position on each sample date. Percentage abnormality per nth egg position was calculated by analysis of strands containing $\geq n$ eggs. However, as only 15% of egg strands examined contained >6 eggs, results of percentage abnormality at Positions 7, 8 and 9 should be interpreted with caution.

RESULTS

Highly significant variation was observed between sample dates in frequency of unfertilised eggs (Kruskal-Wallis: $H_5 = 20.62$, $p = 0.001$). Unfertilised eggs were only found during late October and early November and represented a mean 2.12 ± 1.25% SE and 0.58 ± 0.58% SE of eggs per strand respectively (Fig. 4). Frequency of abnormality per strand differed significantly among sample dates (Kruskal-Wallis: $H_5 = 28.48$, $p <$

0.001), with mean incidence ranging between 8.35 ± 1.86% SE and 0.92 ± 0.41% SE eggs per strand in late November and late December respectively (Fig. 4). Defects in external yolk sac morphology accounted for 46.3% of all abnormalities (Table 1) and were characterised by fissures within the yolk, pinching of the yolk, or convolution of the yolk (Fig. 3A). These defects indicated abnormal development that would not progress beyond gastrulation (Stage 12). Pronounced reduction of embryonic size, recognised on the basis of comparison with embryos of equivalent developmental stage, accounted for 27.8% of abnormalities (Table 1). Reductions in embryonic size were often accompanied by a larger than normal yolk sac (Fig. 3B), or by separation

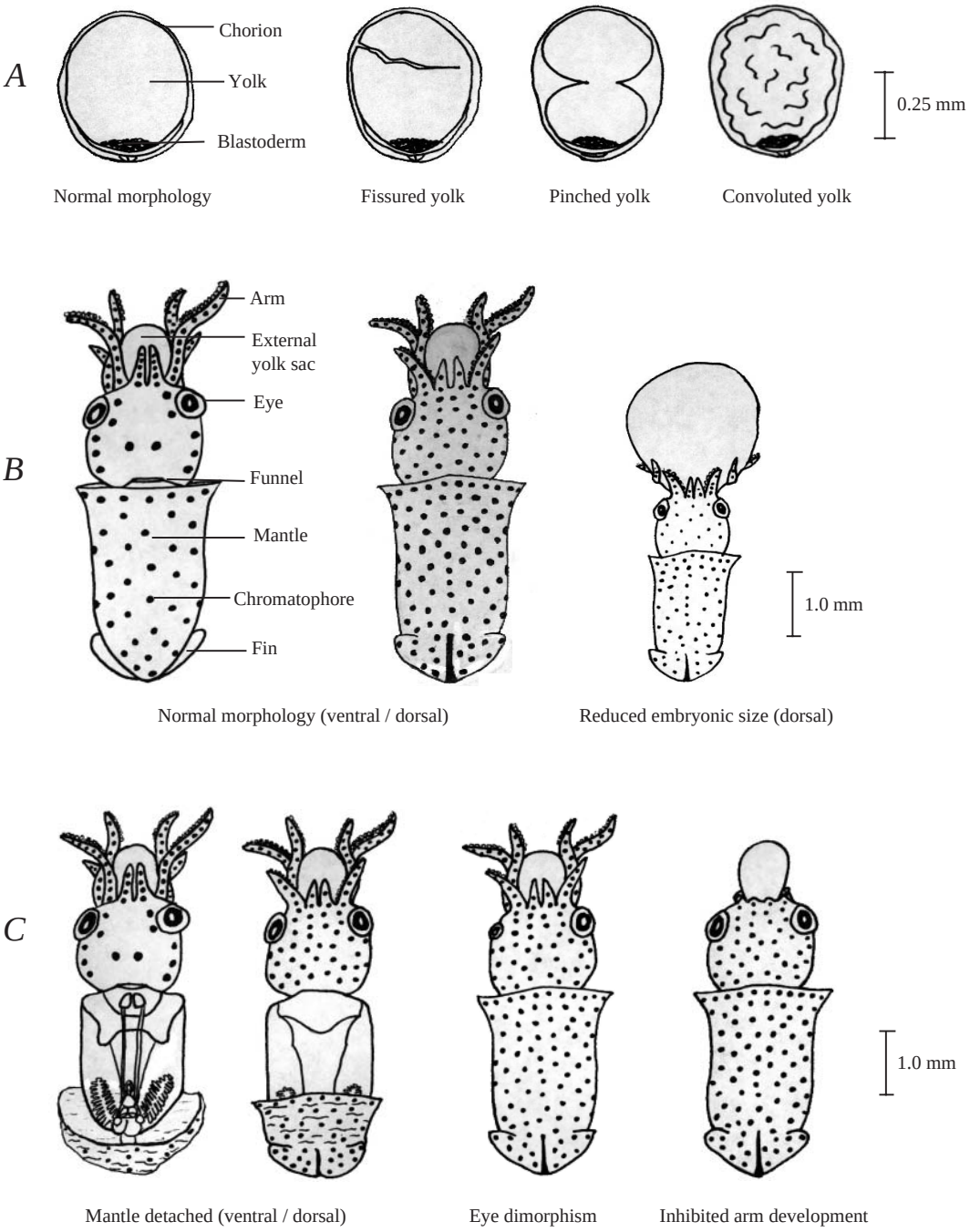

Fig. 3. *Sepioteuthis australis*. Illustrations of morphological abnormalities observed in field-sampled eggs. (A) Eggs at gastrulation (Stage 12, after Segawa et al. 1988); (B) and (C) are late stage embryos (Stage 29, after Segawa et al. 1988)

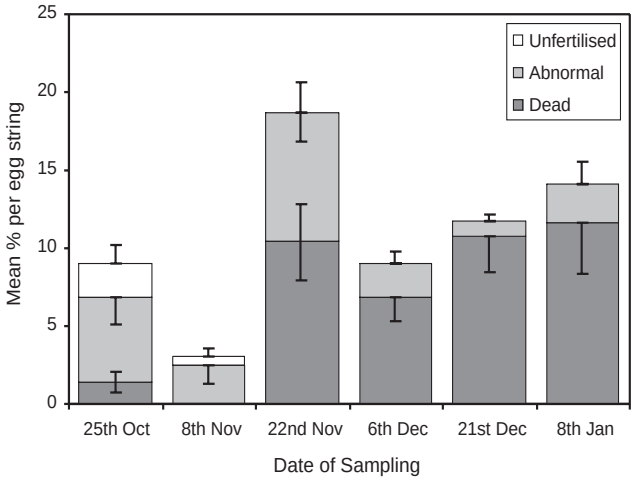

Fig. 4. *Sepioteuthis australis*. Temporal variation in the percent frequency of developmental error of egg strings. Eggs from a total of 376 egg strings were included in the analyses. Error bars: ±SE

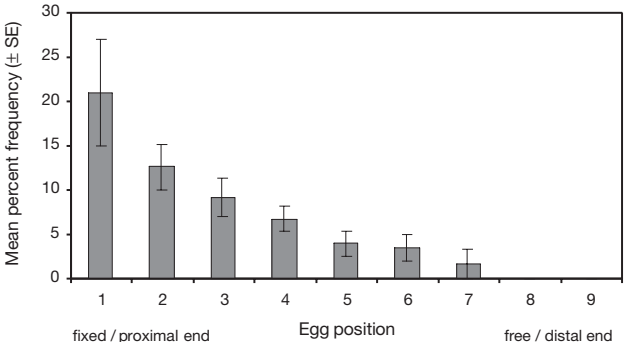

Fig. 5. *Sepioteuthis australis*. Mean frequency of embryonic abnormality per egg position when measured across the entire sampling period ($N_{strands}$ = 481, N_{eggs} = 2649)

of the sac from the developing embryo. Mantle deformities, typified by mantle detachment (Fig. 3C), accounted for 13.0% of abnormalities (Table 1). Abnormalities in eye development, largely characterised by dimorphism (Fig. 3C), accounted for 11.1% of abnormalities (Table 1). Only 1.9% of abnormalities related to errors in arm development (Table 1); these were typified by stunting or complete inhibition of arm development (Fig. 3C). Finally, mean incidence of embryonic mortality per strand varied significantly during the spawning season (Kruskal-Wallis: H_5 = 18.29, $p < 0.003$). Frequency of mortality varied between 1.40 ± 0.68% SE eggs per strand in late October and 11.61 ± 3.23% SE eggs per strand in early January (Fig. 4). No dead embryos were found during early November.

Incidence of developmental abnormality varied significantly with egg position when measured over the entire spawning season (Fig. 5; Kruskal-Wallis: $H_8 = 30.54$, $p < 0.001$). Mean percentage abnormality at each egg position decreased from 20.94% at Position 1 (fixed/proximal end) to 1.67% at Position 7 and 0.00% at Positions 8 and 9 (free/distal end). The association between frequency of abnormality and egg position strengthened as the spawning season progressed. Egg strands collected in late October and early November were characterised by a relatively even distribution of abnormalities across egg Positions 1 to 7 (Fig. 6). Frequency of abnormality was not associated with egg position during this initial phase of spawning. Correlation between developmental abnormality and egg position was first evident during late November: at this time 64% of abnormalities were located within egg Positions 1, 2 and 3 (Fig. 6). By early December 49% of abnormalities were at egg Positions 1 and 2; this decreased to 34% in late December. Towards the end of the spawning season (early January), 39% of abnormalities were located at egg Position 1 (Fig. 6).

Inter-site variation in frequency of abnormality was investigated by comparing data from unfouled egg strands collected at Sites A, B, and C during early December. No egg strands contained unfertilised eggs at this time. Mean percentage mortality per strand was not significantly associated with sample site (Kruskal-Wallis: H_2 = 2.38, $p = 0.30$). Mean values ranged from

Table 1. *Sepioteuthis australis*. Frequency of developmental error in field-sampled eggs during a spring/summer spawning season in Great Oyster Bay, Eastern Tasmania, Australia

Date (dd/mm/yy)	No. of eggs sampled	External yolk sac deformity	Reduction in embryo size	Mantle deformity	Eye deformity	Arm deformity	Total no. of abnormalities
25/10/00	241	7	2	0	2	1	12
08/11/00	133	1	0	1	2	0	4
22/11/00	385	4	9	1	1	0	15
06/12/00	519	9	3	1	0	0	13
21/12/00	502	1	1	3	1	0	6
08/01/00	134	3	0	1	0	0	4
Total	1914	25	15	7	6	1	54

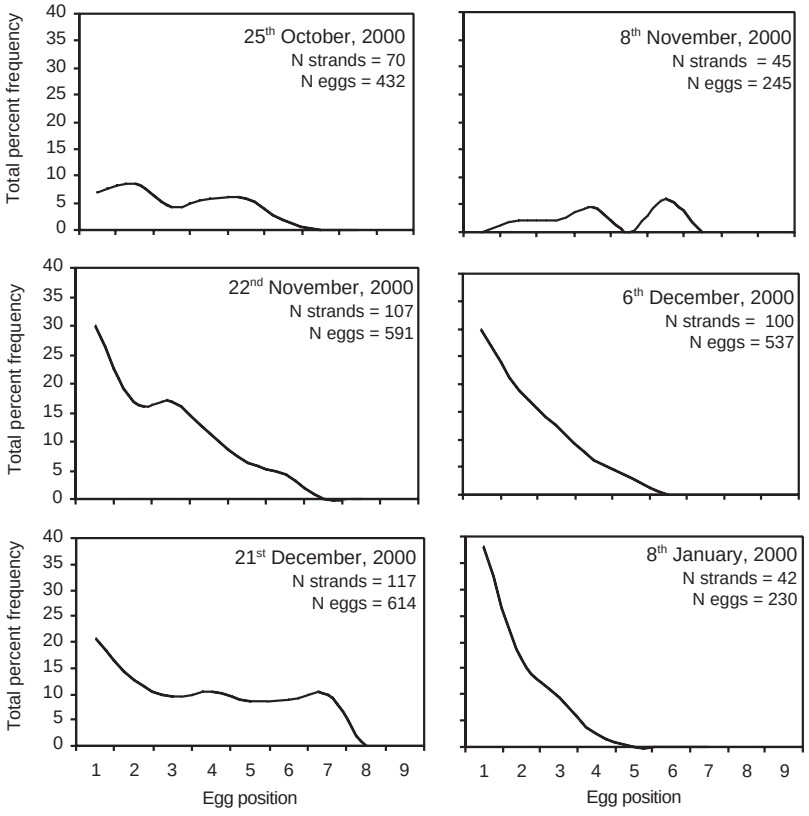

Fig. 6. *Sepioteuthis australis*. Total frequency of embryonic abnormality per egg position on each sample date ($N_{strands}$ = 481, N_{eggs} = 2649)

2.96 ± 1.61% SE at Site A to 16.1 ± 13.1% SE at Site B. In contrast, mean percentage abnormality per strand was strongly correlated with sample site (Kruskal-Wallis: $H_2 = 6.94$, $p < 0.05$). Mean values ranged from 7.50 ± 4.79% SE at Site B to 0.52 ± 0.52% SE at Site C. On this basis, Site B was characterised by a comparatively high incidence of abnormally developed embryos at that time.

Biofouled eggs strands were characterised by a comparatively lower incidence of both unfertilised and dead eggs: unfertilised eggs were never found in fouled egg strands but accounted for a mean 0.80 ± 0.46% SE eggs in unfouled egg strands. No significant correlation was found between biofouling and frequency of developmental abnormality: mean values ranged from 3.53 ± 1.00% SE eggs per strand in unfouled strands to 2.04 ± 0.69% SE eggs per strand in fouled strands (2 sample *t*-test: $t_{144} = 1.20$, $p = 0.23$). Highly significant correlation was noted between biofouling and incidence of mortality: unfouled egg strands contained a mean 11.31 ± 2.49% SE mortality compared to a mean 2.14 ± 0.94% SE in fouled egg strands (2 sample *t*-test: $t_{97} = 3.45$, $p < 0.001$).

Mean daily SBT (°C) increased steadily during the spawning season from 12.9°C in late September to 18.9°C in mid-December (Table 2). Daily fluctuations in SBT typically measured between 1 and 2°C. Fluctuations >2°C d^{-1} were most prevalent during early and mid-October and were noted on 24% of days during this period (Table 2). Hourly changes in SBT > 0.5 and >0.25°C were also most common during early and mid-October; these changes occurred ≤2 and ≤6 times per 24 h respectively (Table 2).

DISCUSSION

The results of this study suggest that non-viable eggs and embryos account for ~11% of eggs per strand in a shallow-spawning *Sepioteuthis australis* population. The incidence of non-viable eggs and embryos varied significantly during the spawning season and ranged between 3 and 19% of eggs per strand. Unfertilised eggs were only found during the first month of spawning and occurred at relatively low frequencies (≤2% eggs per strand). In contrast, egg mortality and devel-

Table 2. *Sepioteuthis australis*. Temporal changes in sea-bottom temperature (SBT, °C) during a spring/summer spawning season. Relatively pronounced daily and hourly changes in SBT (>2.0°C d^{-1} and >0.50°C h^{-1}) were most frequent in the 2 wk between 8 and 24 October 2000 (in bold)

Period (dd/mm/yy)	Mean daily SBT (°C)	Daily range in SBT > 2.0°C (%)	Daily range in SBT > 1.0°C (%)	No. of ΔSBT > 0.50°C (h^{-1})	No. of ΔSBT > 0.25°C (h^{-1})
25/09/00 – 07/10/00	12.90 ± 0.16 SE	0.0	84.6	4 (max. 2 per 24 h)	36 (max. 6 per 24h)
08/10/00 – 24/10/00	13.45 ± 0.16 SE	**23.5**	47.1	**6 (max. 2 per 24 h)**	**47 (max. 6 per 24h)**
25/19/00 – 07/11/00	14.19 ± 0.17 SE	0.0	71.4	2 (max. 1 per 24 h)	36 (max. 8 per 24 h)
08/11/00 – 21/11/00	15.69 ± 0.14 SE	7.1	42.9	4 (max. 3 per 24 h)	16 (max. 4 per 24 h)
22/11/00– 05/12/00	18.06 ± 0.14 SE	7.1	85.7	5 (max. 3 per 24 h)	46 (max. 7 per 24 h)
06/12/00 – 20/12/00	18.93 ± 0.11 SE	6.7	66.7	1 (max. 1 per 24 h)	31 (max. 7 per 24 h)
21/12/00 – 07/01/01	17.70 ± 0.23 SE	5.9	64.7	1 (max. 1 per 24 h)	31 (max. 4 per 24 h)

opmental abnormalities were noted throughout the entire season with frequency of mortality approximately double that of abnormality. Defects in external yolk-sac morphology accounted for nearly 50% of all abnormalities. Illustrated reductions in embryo size and deformities of the mantle, arms and eyes are consistent with developmental abnormalities reported in other squid species (*Todarode pacificus*: Sakurai et al. 1996; *Loligo forbesi*: Murray 1999; *Loligo vulgaris reynaudii*: Oosthuizen et al. 2002a). The intra-season variation in frequency of abnormalities within field-sampled *S. australis* eggs may reflect changes in (1) environmental conditions, (2) egg mass size and/or (3) levels of biological fouling.

Cephalopod development is intrinsically linked to water temperature (e.g. Hamabe 1960, Arnold 1965, Boletzky 1974, 1979). Incubation of eggs at temperatures outside the optimal species range results in increased levels of mortality and abnormality (O'Dor et al. 1982, Sakurai et al. 1996, Gowland et al. 2002, Oosthuizen et al. 2002b). Laboratory rearing of squid eggs indicates that controlled temperature changes should measure ≤1°C d^{-1} which equates to ≤0.04°C h^{-1} (Hanlon 1990). Experimental rearing of *Loligo vulgaris reynaudii* indicates that exposure to fluctuating temperatures (±3 to 9°C) elevates frequency of developmental abnormality to levels exceeding those associated with eggs maintained at constant temperatures (Oosthuizen et al. 2002b). This study additionally suggests that upward fluctuations in developmental temperature (+4.3°C h^{-1}) are comparatively more deleterious than downward fluctuations in developmental temperature (–5.5°C h^{-1}). The results of the present study on *Sepioteuthis australis* indicate a November peak in frequency of developmental abnormality (19% eggs per strand). This peak may reflect the relatively pronounced fluctuations in SBT (Δ >2°C d^{-1}) measured during the preceding 8 wk of egg development. Adaptation of Segawa's (1987) equation relating temperature and duration of embryonic phase in *S. lessoniana* generates an approximate oviposition date of 9 October for *S. australis* eggs collected on 22 November. As Segawa's equation assumes constant thermal conditions, oviposition date was tentatively estimated on the basis of mean field temperature during the preceding 8 wk. This estimate suggests that the *S. australis* eggs characterised by greatest frequency of developmental abnormality were progressing through early developmental stages when temperature fluctuations of >2°C d^{-1} were most common. Fluctuations >2°C d^{-1} occurred on 24% of days between 8 and 24 October, compared to a maximum 7.1% of days in any other 2 wk period. Experimental rearing of loliginid eggs indicates maximal thermal sensitivity during the early stages of development (*S. lessoniana*: Segawa 1995; *L.v. reynaudii*: Oosthuizen et al. 2002b). This coincidence of heightened embryonic sensitivity and relatively pronounced thermal variation in the field may account for the peak in developmental abnormalities during late November. It should be noted that downward fluctuations in temperature accounted for the majority of fluctuations measured during this period (57% Δ >0.25°C h^{-1}; 65% Δ > 0.50°C h^{-1}).

With the exception of this November peak, frequency of developmental abnormality increased steadily during the final 8 wk of the spring/summer spawning season. Analysis of environmental data suggests that ambient conditions varied in a similar manner during this period, i.e. SBT increased steadily and fluctuations were of comparable magnitude. The progressive increase in incidence of developmental abnormalities must therefore parallel another change in conditions. The relationship between incidence of developmental abnormality and within-strand egg position appeared to strengthen temporally. Developmental abnormalities became increasingly clustered towards the fixed/proximal end of egg strands as the spawning season progressed; this clustering may reflect increased hypoxia within egg masses. Hypoxic conditions have been noted within the centres of amphibian (e.g. Seymour 1995) and molluscan (e.g. Strathmann & Strathmann 1995) egg masses. Low oxygen conditions are associated with retardation of development and/or egg mortality (e.g. Baker & Mann 1994, Mills & Barnhart 1999, Woods 1999). Hypoxia has previously been linked to developmental abnormalities in loliginid squid egg masses held in the laboratory. Arnold (1990) states that the centre of a *Loligo* spp. egg mass 'tends to become fairly anaerobic' and that development becomes 'retarded or abnormal' if egg strands are not separated out. Loliginid egg-laying behaviour typically involves the deposition of new strands onto the periphery of extant egg masses (McGowan 1954, Arnold 1962). Discrete *Sepioteuthis australis* egg masses may contain >600 egg strands (Moltschaniwskyj & Pecl 2002) and large mass size is likely to restrict water flow therefore reducing aeration. Whilst developmental abnormalities in *S. australis* eggs were distributed relatively evenly across egg positions during the first month of spawning, 39% of all abnormalities were located at the most proximal egg position by the end of the season. This progressive clustering may reflect congruent increases in egg mass and hypoxic conditions. Proximal clustering of developmental abnormalities has been observed in field-sampled *Loligo forbesi* egg strands (Murray 1999). Use of oxygen probes during subsequent field assessments of cephalopod eggs will permit direct testing of the hypoxia hypothesis.

Late stage *Sepioteuthis australis* eggs strands typically support epiphytic growth. A previous study sug-

gests that biofouling may be beneficial to egg development in this species, as fouled egg strands display comparatively reduced levels of egg mortality (Steer et al. 2002). Photosynthesis of endogenous algae has been positively associated with development of amphibian eggs (Pinder & Friet 1994) and molluscan eggs (Cohen & Strathmann 1996). The results of the present study confirm the existence of a relationship between biofouling and reduced egg mortality in *S. australis*. A similar relationship is revealed between biofouling and reduced incidence of unfertilised eggs. In apparent contrast, no correlation exists between biofouling and incidence of morphological abnormality. Frequency of abnormality did not differ significantly between fouled and unfouled egg strands. If biofouling conferred significant benefit during egg development in *S. australis*, a continuum of effect might be expected, i.e. suppression of abnormality levels in tandem with suppression of mortality levels. As no such continuum was identified during the present study, an alternative explanation is necessary. Reversal of cause and effect offers one possibility, i.e. good quality *S. australis* eggs may support epiphytic growth rather than epiphytic growth ensuring good quality *S. australis* eggs. If epiphytic colonisation occurs exclusively upon capsules containing live fertilised eggs, the relationship between biofouling and reduced egg mortality may be entirely incidental. By this rationale, correlation may simply reflect an absence of epiphytic growth on dead or unfertile egg capsules and not a positive relationship between egg development and biofouling. Comparable levels of morphological abnormality in fouled and unfouled egg strands provide support for this hypothesis. Indeed, cephalopod culture experiments suggest 'unsatisfactory hatching due to the attachment of great quantities of diatoms or green algae' (Choe 1966). Further investigation is required to clarify the relationship between development and epiphytic colonisation of *S. australis* eggs.

This study provides *in situ* assessment of development in a commercially important cephalopod population. Rates of embryonic abnormality (~11%) are comparable with laboratory rates reported for other loliginids reared under natural thermal conditions: 5 to 10% in *Loligo vulgaris reynaudii* (Oosthuizen et al. 2002b) and 7 to 8% in *L. forbesi* (Gowland et al. 2002). The results of the present study suggest that rates of embryonic abnormality primarily reflect environmental conditions and consequently vary with spawning date and site. Rates may additionally reflect the quality of yolk provision. For example, deficiencies in dietary phospholipids can induce developmental abnormalities in cultured fish larvae (e.g. Kanazawa et al. 1981, Geurden et al. 1995). Irrespective of cause, quantification of embryonic abnormality within natural populations may generate valuable information on environmental conditions and population dynamics. A previous study of embryogenesis in sea urchins concludes that quantification of embryonic abnormalities has potential as a 'rapid and sensitive model bioassay' for monitoring marine pollution (*Lytechinus variegatus*; Bottger & McClintock 2001). *In situ* investigation of embryonic development in amphipods suggests that rates of abnormality are more toxicant-sensitive than measures of fecundity, reproductive success or developmental stage (Sundelin & Erikkson 1998). Assessment of embryonic abnormality in coastal spawning cephalopods may provide an indirect measure of environmental quality, and additionally, assessment of embryonic success in commercially exploited cephalopod populations may assist in fisheries management.

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