EARLY POST-CLEAVAGE STAGES AND ABNORMALITIES IDENTIFIED IN THE EMBRYONIC DEVELOPMENT OF CHOKKA SQUID EGGS *LOLIGO VULGARIS REYNAUDII*

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Six early, post-cleavage embryonic stages for chokka squid *Loligo vulgaris reynaudii* eggs that were developed in an aquarium are identified and described, expanding the embryonic stages for this species from 14 to 20. The influence of water temperature on embryonic development is described. At temperatures <12 and >15ºC, high percentages of morphological abnormalities were observed in embryonic development. Gross forms are described and illustrated.

Key words: abnormalities, aquarium, embryonic development, chokka squid

Embryonic development is a continuous process and the identification of particular developmental stages is still arbitrary (Segawa *et al.* 1988). Neaf (1923) approached this problem by describing various cephalopod embryos at equal time intervals over the developmental period. Arnold (1965), however, suggested that embryological staging should rather follow morphological characteristics that are easily recognizable. Both schemes have been used to describe embryonic development for various loliginid (Neaf 1928, Arnold 1965, Fields 1965, Segawa *et al.* 1988) and ommastrephid squid species (Hamabe 1962, O'Dor *et al.* 1982, Watanabe *et al.* 1996, Sakurai *et al.* 1996), but the majority have used the morphological approach.

In the first embryological study on chokka squid *Loligo vulgaris reynaudii* eggs established by Blackburn *et al.* (1998), 14 developmental stages were identified using a morphological scheme. By comparison, Arnold (1965) had used 30 stages to describe embryonic development for *Loligo pealei*. On investigating the effects of temperature on the embryonic development of chokka squid eggs, it was found that the stages identified by Blackburn (1998) did not clearly separate the early post-cleavage developmental stages, and therefore sufficient detail was lacking to distinguish slow development at low temperatures. It became necessary to revise the 14-stage classification scheme for chokka squid by expanding the classification of the early stages. In addition, the abnormal embryonic developments that were noted at sub-optimal temperatures are briefly described.

MATERIAL AND METHODS

Newly laid chokka squid egg strands were collected

by SCUBA divers during spawning on the inshore spawning grounds on the south-east coast of South Africa. The eggs were transported to the laboratory (in oxygen-enriched sample bags), where they were acclimatized and allowed to develop at stable temperatures of 7, 9, 12, 15, 18, 21, 24 and 28°C (Table I) in closed aquaria. Observations were made at 24-h intervals. There was no difference between the development of control egg strands (handled at the beginning and the end of the experiment only) and those handled on a daily basis. The embryological development criteria established by Blackburn *et al.* (1998) were used to confirm the later stages of development, whereas criteria from Arnold (1965), Fields (1965), Segawa *et al.* (1988) and Arnold and O'Dor (1990) were used to separate and identify early developmental stages not yet recognized. The staging scheme formulated for *L. pealei* by Arnold (1965) was used in this study, indicated by the prefix "A".

Live embryos were drawn to scale using a stereo microscope (magnification ×32–50) and camera lucida. The early stages before organogenesis were drawn by observation through the chorion, after the removal of the outer capsule layers. For the later stages, the chorion was removed before they were drawn. The early developmental stages were represented by ventral views only, because little information could be gleaned from the dorsal view. Embryonic stages were described by "+" or "-" signs if morphological development at the time of observation did not match the criteria set to distinguish stages by Arnold (1965).

RESULTS AND DISCUSSION

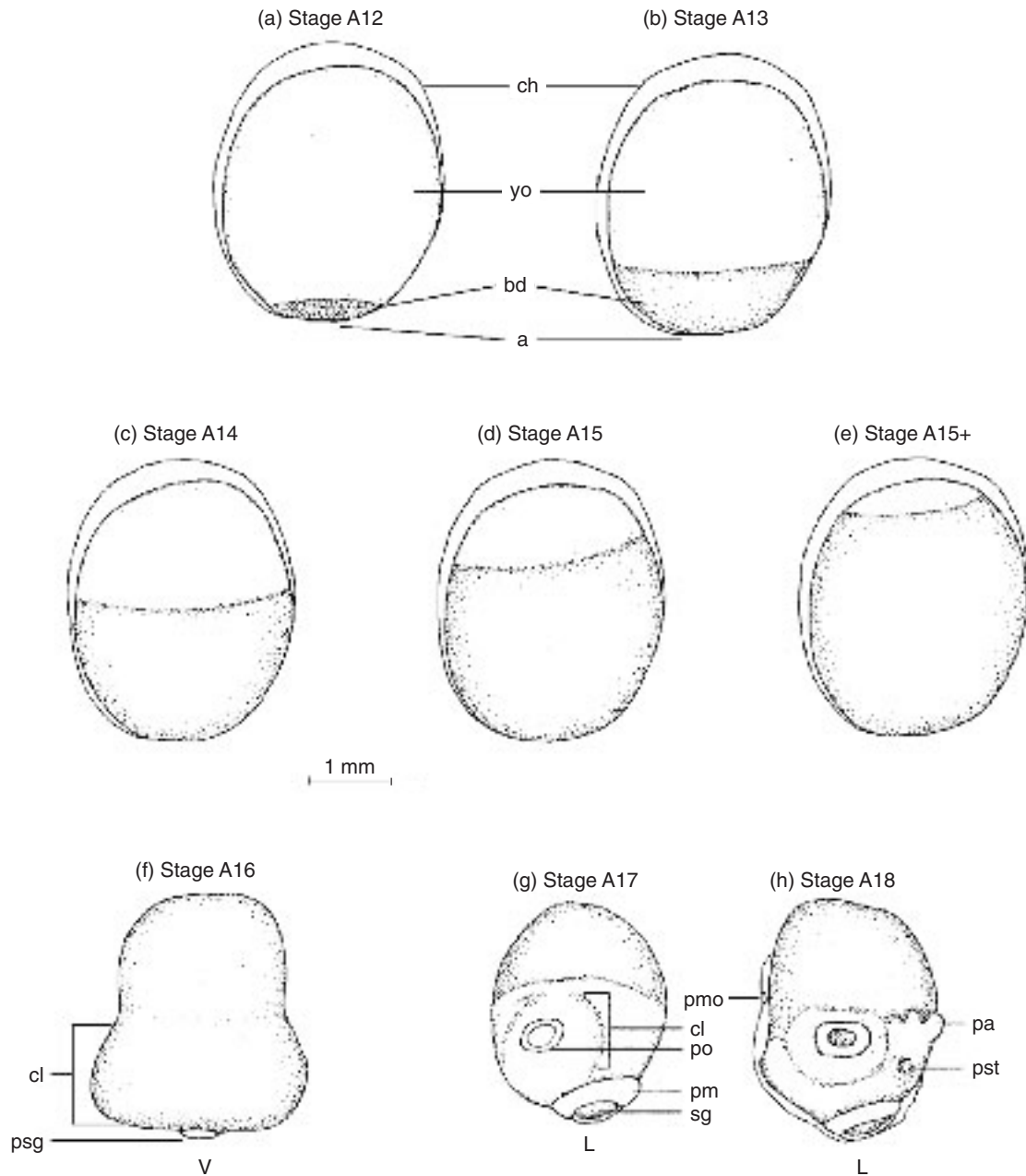The post-cleavage development pattern of chokka squid

Fig. 1: Newly distinguished embryonic developmental stages for chokka squid *Loligo vulgaris reynaudii*. Pre-organogenesis, i.e. germ layer formation – (a) A12: separation of the blastoderm into an ectodermal and mesodermal germ layer. Germ layer proliferation, i.e. blastoderm growing – (b) A13: blastoderm covers approximately one-third, (c) A14: one-half, (d) A15: two-thirds, and (e) A15+: four-fifths of the egg surface. Organogenesis – (f) A16: paired cephalic lobes (area from which the optical primordia will form) begin to protrude; ring-like structure (shell gland primordium) forms at the animal pole – (g) A17: mantle primordium forms around the shell gland and the optical vesicle primordia form as ring-like structures on the cephalic lobes; border of the shell gland becomes slightly elevated – (h) A18: arm and statocyst primordia first appear, optic vesicle primordia becomes distinctly thickened, and stomodeum appear anteriorly. The lateral (L) and ventral (V) views show the animal pole (a), blastoderm (bd), chorion (ch), cephalic lobe (cl), primordium of arms (pa), primordium of mantle (pm), primordium of mouth (pmo), primordia of optic vesicles (po), primordium of shell gland (psg), primordium of statocysts (pst), shell gland (s) and yolk (yo)

(a) Stage A29

Underdeveloped mantle

Yolk sac protruding from side of body

(b) Stage A29

(c) Stage A27

Enlarged buccal mass

Elongated body

Exposed gills, ink sac
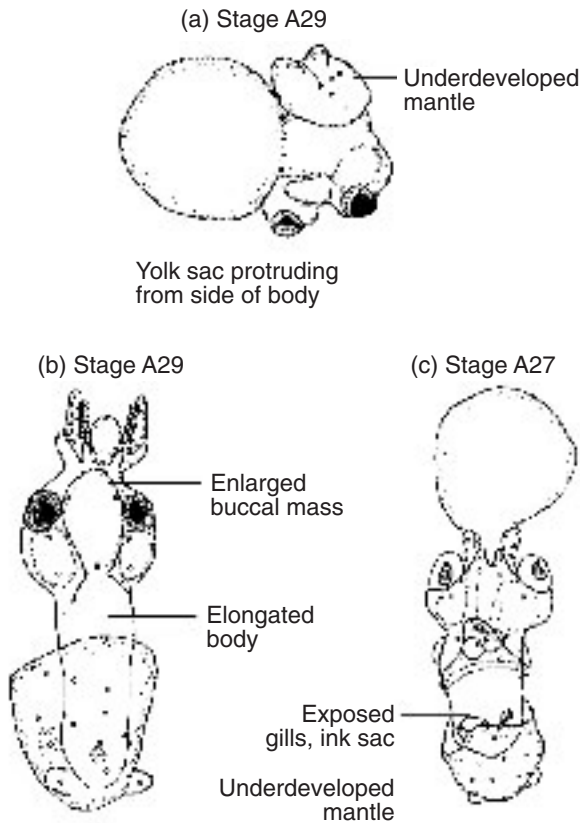
Underdeveloped mantle

Fig. 2: Gross morphological abnormalities were observed at temperatures <12°C and >15°C: (a) body completely deformed, (b) elongated body and enlarged buccal mass and (c) mantle deformed

Table I: Temperatures recorded for the stable temperature incubations

Target stable temperature (°C)	Observed average temperature (°C) and *SD*
7	7.10 (0.33)
9	8.87 (0.37)
12	12.20 (0.35)
15	14.60 (0.15)
18	18.03 (0.39)
21	21.19 (0.48)
24	24.22 (0.79)
28	27.54 (0.52)

described in Figure 1. The first and third stages described in Blackburn *et al.* (1998) were separated into four and three stages respectively; the former into Stages A12, A13, A14 and A15 and the latter into Stages A16, A17 and A18.

Morphological features such as the blastoderm, funnel folding, Hoyle's organ and chromatophores proved useful in separating stages throughout the development of chokka squid embryos. Eggs were not observed directly after spawning and therefore only one stage, A12, was observed during gastrulation. During stages A13–15+, growth of the blastoderm was clearly visible and was used to distinguish these early stages. Similar observations were made on *L. pealei* by Arnold (1965), on *L. forbesi* by Segawa *et al.* (1988) and on *L. bleekeri* by Beag *et al.* (1992). In this study, organogenesis started at A16. The stages immediately after organogenesis were separated and distinguished by the appearance of the cephalic lobes, shellgland primordium and mantle primordium. These stages were not identified by Blackburn *et al.* (1998).

Morphological abnormalities in embryonic development were observed at temperatures <12 and >15°C. These are classified into four types in Table II. Only gross morphological abnormalities are illustrated in Figure 2. At water temperatures ≤9 and ≥21°C, ab-

for 1–3 day old embryos (until hatching) was divided into 20 stages, i.e. between A12–30. The identified early stages observed in this study are illustrated and

Table II: Morphological abnormalities observed at temperatures ≤ 12 and ≥ 15°C

Type of deformity	Description
Mantle deformity	A variety of mantle deformations was observed — The mantle developed no further than approximately Stage A22, whereas the rest of the body developed normally, or the mantle separated or was torn, either ventral-dorsally or anterior-posteriorly. The former was most prominent and usually accompanied an elongated body. The viscera were exposed during all of these mantle deformities
Elongated body	Elongated bodies caused a normally formed mantle to cover only part of the body, with most of the viscera exposed
Enlarged buccal mass	The enlargement of the buccal mass was easily recognized by the subsequent enlarging of the head. In some cases, the enlarged buccal mass could attain one-third of the head size
Complete body deformity	Complete deformation of an embryo consisted of many aspects and usually occurred at an early stage. For instance, the yolk sac protruded from the side of the body, the eyes fused into one, there was a complete lack of mantle formation, or the head protruded from the side of the body

Table III: Occurrence of morphological abnormalities, shown as a percentage of the total abnormality found at each temperature

Abnormality type	% Abnormality by temperature							
	7°C	9°C	12°C	15°C	18°C	21°C	24°C	28°C
Mantle deformity	–	45	3.5	3.5	12	20	38	
Elongated body	–	2	0.5	0.5	0.5	25	39	50*
Enlarged buccal mass	–	2	0.5	0.5	1	4	2	
Complete body deformity	–	2	0.5	0.5	0.5	1	2	–
Opaque white, disintegration	95	–	–	–	–	–	–	50
Total % abnormality	95	51	5	5	14	50	81	100

*The elongated body was the main abnormality, but the condition occurred in combination with an enlarged buccal mass and mantle deformities

normalities in embryonic development increased from 50 to 100% (Table III). At high (28°C) and low (7°C) temperatures, the embryo turned opaque and began to disintegrate. The next most common form of abnormal development was deformity of the mantle (45% at 9°C), followed by an elongated body (39% at 24°C). Other forms, such as enlargement of the buccal mass and complete body deformity, contributed ≤4%.

Morphological abnormalities in *L. v. reynaudii* embryos have not previously been described. The deformed embryos that hatched during the present study struggled to jet and were unable to maintain their position in the water column. These embryos did not survive for longer than a few hours. Abnormalities have been noted in other squid species. For example, O'Dor *et al.* (1982) found abnormalities in *Illex icebrosus* and Sakurai *et al.* (1996) observed terminated development, inverted and deformed mantles, exposed viscera and death before hatching in *Todarodes pacificus*.

In conclusion, this work has expanded the embryonic development scheme for chokka squid eggs from 14 to 20 stages. Although these were sufficient to determine the influence of temperature on the egg growth, the development scheme for this species is still not complete, because the cleavage stages have not been observed or described. Only gross morphological abnormalities are described here. Abnormalities at the organ or cellular level need to be investigated.

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