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Studies on the egg quality of *Penaeus monodon* Fabricius based on morphology and hatching rates

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Eggs of *Penaeus monodon* are classified into five different types on the basis of morphological criteria and hatching rates as follows:

A_1 eggs undergo normal development with 58% hatching rate; A_2 eggs show delayed and/or abnormal development with 32% hatching rate; B eggs are unfertilized and characterized by irregular cytoplasmic formations; C eggs are unfertilized and show no change in appearance; and D eggs are unfertilized and show extensive bacterial presence. The various morphological types are described in Table 1.

The single most important feature that distinguishes the good (A_1) from the not-so-good (A_2) and the bad (B, C, and D) eggs is the development of the cytoplasm or the embryo. Approximately 30 minutes after spawning, the eggs in a given batch can be classified into those that undergo cleavage and those that do not. The embryo in A_1 eggs undergoes normal development through the two-cell, blastula and morula stages and hatch out as healthy nauplii after 12-15 hours. On the other hand, A_2 eggs exhibit delayed and/or abnormal development with asymmetrical limb buds, broken setae and a consequently lower hatching rate.

Among the eggs that do not undergo cleavage, as early as three hours after spawning some develop cracks or breaks in the cell or plasma membrane through which the cytoplasm flows, creating irregular formations typical of type B eggs. Type C eggs retain the single cytoplasmic mass more or less unchanged. Rarely, bacterial invasion leads to the appearance of D eggs compose mainly of the external membrane with very little cytoplasm and plenty of bacteria inside.

Around 10 minutes after spawning, the cytoplasm starts to separate from the external membrane and within another 20 minutes the separation is complete. Within this external membrane, the developing embryo (A_1 and A_2) is completely ensheated in a thinner and more flexible embryonic membrane, earlier observed by Villaluz et al. (1972). This inner embryonic membrane is the equivalent of the fertilization membrane in other invertebrates and is not found in B, C and D eggs. During the process of hatching, the nauplius may free itself completely from the embryonic membrane and consequently leave it inside the external egg membrane. The nauplius may still be partially or completely ensheated in the embryonic membrane upon emergence from the egg.

The mean hatching rate of the five egg types (Table 2) further provides data to support the validity of these types, in addition to the morphological criteria in Table 1. The t-test shows a highly significant difference in mean hatching rates between A_1 and A_2 eggs. Data on the relative proportions of good versus bag eggs from different kinds of spawners reveal interesting

Table 1. Morphological criteria of various egg types of *Penaeus monodon* , as observed in the morning after spawning (between 9:00 to 10:00 a.m.)

	Development	Derivation	Symmetry	Shape	External membrane	Embryonic membrane	Remarks
A_1	Normal with limb & setae formation	Fertilized egg	Nauplius inside bilaterally symmetrical	Spherical	Continuous, generally free of bacterial & other growth	Distinct	58% hatching rate; healthy nauplii strongly photo-tactic
A_2	Delayed in comparison to A_1 eggs; nauplius generally abnormal with broken setae, unequal limbs, etc.	Fertilized egg	Nauplius inside, may or may not be bilaterally symmetrical	Usually spherical	Continuous, rarely with breaks; may have some bacteria	Distinct	32% H.R.; nauplii may be weak, and show abnormalities
B	No development; cytoplasm with large & small irregular formations	Unfertilized egg; also from C eggs	Asymmetrical	Spherical or elongated	Continuous or broken; may have bacteria and other growth	None	0% H.R.
C	No development; cytoplasm one single, often undifferentiated mass	Unfertilized egg	No symmetry	Usually spherical; rarely oblong	May be continuous or broken with bacteria and/or protozoans	None	0% H.R.
D	No development ; very little cytoplasm left because of bacterial invasion	From B and C (unfertilized eggs)	No symmetry	Irregular	Broken and discontinuous in many parts	None	0% H.R.

trends. Clearly, eggs from wild spawners have the highest proportion (49.3%) of A_1 eggs as well as hatching rates (35.0%) followed by ablated wild stock with 38.9% A_1 eggs and 30.4% hatching rate (Table 3). In contrast, ablated pond females have only 23.5% A_1 eggs and the lowest hatching rate of 19%. The implication is that although the quality of eggs from ablated stock is close to par with those from wild spawners, eggs from ablated pond stock remain inferior.

Table 2. Hatching rate of different morphological egg types of ablated pond and wild stock *Penaeus monodon* (average of 14 runs, 1 spawning per run).

Egg type	A_1	A_2	B	C	D
Mean no. eggs sampled	63.4	62.8	58.6	60.1	43.5
Mean % hatching rate**	58.0	31.8	0.0	0.0	0.0
Standard deviation of % HR	23.5	20.4	0.0	0.0	0.0

** t-test showed a significant difference between A_1 and A_2 at 1% level ($t = 7.53$).

Table 3. Proportion of different egg types and hatching rate of spawnings from ablated and wild *Penaeus monodon*.

	No. of spawnings	Average proportion egg types (%)						Hatching Rate (%)
		A_1	A_2	B	C	D	Total	
Ablated pond stock	61	23.5	1.81	43.45	25.08	6.16	100	18.95
Ablated wild stock	172	38.87	4.51	38.76	14.49	3.37	100	30.43
Wild (unablated)	7	49.3	2.6	17.7	25.08	4.8	100	35.03

Liao and Huang (1972) cite hatching rates of 82-95% for spawnings from four wild *P. monodon* females. In Aquacop (1977), the average proportion of normal eggs from ablated *P. monodon* is less than 50% with abnormal development more frequent. Although the mean hatching rates cited in this study may be low (19-35%), this is partly because the data come from hundreds of spawnings, including those with zero hatching.

The establishment of a highly linear relationship between percent A_1 eggs and hatching rate (Table 4) provides a very useful tool to aid the hatchery technician in determining which spawnings to reject outright and which to rear through the larval and postlarval stages. To obtain a 30% hatching rate, spawnings should have a minimum of 38% A_1 eggs for both pond ablated and wild ablated females.

Table 4. Relationship between % hatching rate (HR) and % A_1 and A_2 eggs of ablated *Penaeus monodon*.

	% A_1 eggs vs. % HR	% A_2 eggs vs. % HR
Ablated pond stock (n = 61)		
F – value	76.847**	1.403
r^2	0.646	0.023
Ablated wild stock (n = 172)		
F – value	343.113**	8.959**
r^2	0.665	0.050

** Significant at 1% level.

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