

## The Breeding Behavior of *Nautilus belauensis*

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### Abstract

Experiments on the reproductive behavior of *Nautilus belauensis* brought from Palau were carried out in the laboratory, and newly-devised tank was used for this purpose. It was seen that on most occasions, copulation took place successfully, and eggs were laid by the three *Nautili*. From the observations carried out, copulation lasted between 15 minutes to one hour at the most. Also, copulation took place mostly at night and early morning. The time span of egg spawning was about one hour, and the egg size ranged from 3-4 cm in length. It was also noticed that most of the eggs were laid inside of the vessels.

### Introduction

In 1986, the Japanese Consultation Experts published a report on the copulation and spawning of *Nautilus macrophalus*. Furthermore, a short note on breeding behavior and a report on the generative organs regarding *Nautilus pompilius* was documented in 1988. A record of detailed data on the observation of its breeding activity, however, has not been reported. It was recently reported in newspapers that the Toba Aquarium succeeded in the spawning and hatching of *Nautilus pompilius*, however, there was no scientific report on this subject. On the copulation behavior of *Nautilus pompilius* Mikami and Okutani (1987, 1981) have described in detail. Regarding the spawning behavior of *Nautilus pompilius* Willey (1897b) described it but no detail process given.

In the present study, we report on the copulation and spawning aspects of the reproductive behavior of *Nautilus belauensis*. For this study, *Nautilus belauensis* were caught in the seas off the Palau Islands and transported to the Kagoshima Marine Park. A series of observations were carried out regarding their activity in a newly devised tank for their breeding.

### Materials and Methods

For our experiment, we used four *Nautilus belauensis* brought to the Marine Park on the 16 and 17 September, 1988, and five brought on the 12 and 13 January, 1989. We used three water tanks A, B, and C. As spawning was expected, and for easy observation of their behaviour, vessels for spawning were designed and placed inside tank A.

Five cylindrical transparent steel vessels (7cm in diameter, 10cm in depth)

were used as the spawning apparatus for *Nautilus*. The vessels were firmly attached to a black vinyl plate (30×15cm) by steel screws. 7mm diameter holes were made in these vessels to allow for the free flow of sea water. The vessels were fixed vertically to the wall of tank A (Fig. 1). We avoided placing these vessels around the inlet and outlet of sea water and chose the place where few residues gathered.

## Observations and Results

### A. Copulation Behavior

The copulations behavior of *nautilus* was as follows: the male and female *Nautili* faced each other, stretching their long tentacles. The male drew the female towards its side and inserted its spadix, located at the left-side of the opponent's mouth, into a gap in the soft part of the female's ventral (Tsukahara 1986).

Copulations were often observed throughout the year. In particular, we had an opportunity to observe a series of copulations from beginning to end, totally six times, during May and October, 1990. The date and time of copulation, combinations of coupling, and the duration of copulation are shown in Table 1. The coupling pairs were No. 5 (male) and No. 1 (female), and No. 32 (male) and No. 43 (female). These combinations were not exchanged. Five out of six copulations took place between evening and night time; one copulation took place in the morning. The duration of the copulations was from about 15 minutes to one hour.

The position of the copulation pair was that the male faced the female not along a straight line, but, on a dogleg line as seen from the top at an angle of about 140° and 170°.

Table 1: Copulation of *Nautilus belauensis* observed in aquarium of Kagoshima Marine Park.

Date	Male × Female	Copulation time (minutes)
5-May-89	No. 5 × No. 1	19 : 08 ~ 19 : 26 (18)
11-May	No. 5 × No. 1	20 : 40 ~ 20 : 56 (16)
1-Jun	No. 5 × No. 1	18 : 07 ~ 18 : 22 (15)
7-Jul	No. 5 × No. 1	16 : 55 ~ 17 : 37 (42)
22-Aug	No.32 × No.43	17 : 31 ~ 17 : 50 (19)
7-Oct	No.32 × No.43	9 : 20 ~ 10 : 25 (65)

Copulation behavior observed as follows:

a) No.1 (female) × No.5 (male), May 1st.

At 6:58 p.m. the female adhered to the upper part of the wall of the tank A. The male attached itself to the lower part of the female shell with two tentacles; both stood still. At 7:08 p.m. when the female left the wall, the male separated from the female. Then the male and the female immediately faced each other. The male extended its tentacles to the female's shell; the male held the female by the bottom of her shell with three left and right tentacles, respectively. The male gradually

retracted its tentacles and the two came close together. Pushing by the male's hood, the female's hood was depressed; consequently, the female's pinhole eyes were pressed into its shell so far they seemed to disappear. At 7:11p.m. The male floated near the surface of the water still holding the female. The male was slowly floating in the stream for a while. Then, the male extended its two tentacles to the wall and attached itself. At 7:19 p.m both Nautili suddenly started to sway strongly from side to side. The hood of the female was pressed further to the extreme place. At 7:21 p.m. the swaying became weaker and finally the male tentacles were detached and it left the female. Thus, her pressed pin-hole eyes appeared again outside the shell. The hood gradually returned to its original place. At 7:25 p.m. the female Nautilus ascended to the surface, tentacles inside the case, and then began to swim along the wall. The male, on the other hand, descended to the bottom and swam around with its tentacles extended. This was the end of the copulation.

b) No.1(female) x No.5 (male), May 11th

At 8:40 p.m. while the female was eating shrimp, the male came close and held the bottom of the female shell; therefore, the female shell became inclined. The male's tentacles extended to more than 10cm and got attached to the female's shell. In particular, the two top-left tentacles of the male extended to 15cm and stuck to the upper part of the female's hood, as if pressing on the hood. At 8:48 p.m. both Nautili swayed strongly as the male rotated vertically around. The male attached itself to the left side of the female, forming a dogleg line. Since the female rigidly lowered her hood, the black and blue-white edge of the shell top, which is usually covered by the hood, was disclosed. After rotating around seven or eight times, the female stopped moving. At 9:04 p.m. the female gradually lifted her hood and seemed to leave the male, so that the male detached its long tentacles and ejected mucus from around its mouth. Before the male came close, the female had been eating shrimp and still held them. At 9:13 p.m. the female spit out the half engulfed shrimp which were seen stuck with the mucus.

c) No. 32 (male) x No. 43 (female), August 22nd.

At 5:30 p.m. No. 5 (male) adhered to the back of the shell of No. 32. No. 43 then faced No. 43 so close that its tentacles slightly touched the female. At 5:31 p.m. No.5 was going to leave No. 43; at the same time, No. 32 started copulating by entwining the female. Although the female's hood was lowered, its mouth was seen a little bit from a gap in the male's left tentacles. At 5:51 p.m. the male separated from the female. At 6:01 p.m. when we gave them small fish, the female did not eat; rather, it spit out the fish entangled with a lump of mucous; whereas the male ate the fish completely.

d) No.32 (male) x No. 43 (female), October 7th.

At 9:20 a.m. the male began to copulate with the female swimming around the surface by holding the bottom of the female shell. The male extended its tentacles, one from above, and more than six from the right and left sides, respectively, and attached itself to the female shell. At 9:30 a.m. the male lowered itself to 10 cm below the surface and stuck to the wall with two right-side tentacles. At that time, the male's breathing rate was 35 times per minute. We could not determine the breathing rate of the female because it seemed that the female was depressed by the male. At 10:02 a.m. the male left for the female and swam attached to the female. A little while later, the female totally lowered its hood and the whole soft part, except for the hood, was withdrawn into the shell. Since the female covered its mouth with its hood, the male spadix was caught between the shell and the hood. The male shook and rotated itself strongly every seven to nine seconds to free itself from the female. The female, however, kept itself inside the shell and no further action was observed. The male swam around so as to pull out its spadix, keeping its tentacles stuck to the female shell. After several minutes, the female returned its hood to the original place. When the female's eyes were readily visible, the motion of the male gradually decreased. However, the female again withdrew itself into the shell and her hood alone was seen on the shell; then the male started shaking vigorously. The duration of this second "hiding" was shorter than the first. About one hour later, the two *Nautili* were off to the side. The male detached its tentacles from the female shell and slowly pulled out its spadix. Then, the female started swimming around the water surface, while the male descended directly to the bottom of the tank. We observed that the male did not immediately withdraw its spadix but left it extended for about three minutes. Because the female hood was strongly pressed down by the male's tentacles, her hood was disfigured and became shorter. The female's tentacles also shrank, but later recovered within five minutes.

Copulation behaviour was almost the same on all occasions, except for minor differences depending upon the couplings. At the beginning of copulation, the male was more active than the female. The female gave precedence to eating. After copulation, the male ate food but the female did not.

## B. Spawning behavior.

From January to October, 1989, three female *Nautili*, Nos.1, 33, and 43, laid 33 eggs in total (26 in tank A, 3 in tank B, 4 in tank C). 25 out of 26 eggs were laid in the spawning vessels; one egg was laid in the gap between the vessels and the tank wall. The eggs were laid at the bottom of the spawning vessels and part of the egg shells were stuck to the bottom. Because no space was available, egg No. 10 was laid on the side wall of the vessels. The largest number of eggs was 11, in vessels B; the next was six, in vessel C. In vessels A and D, the spawning number was three; in vessel E was two eggs. One was outside of the vessels (Table



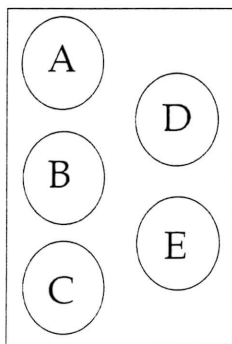


Fig. 1: Egg laying position in vessel inside of aquarium.

2, Fig 1). The spawning date and the number of eggs are given in Table 3. Since three females were kept in the same tank A, we were not able to identify the mothers of eggs No. 16 - 26. We also could not identify the mothers of eggs No. a - d because two females were kept in tank C. It was obvious that there was an interval of spawning from 4 to 20 days in the case of No. 1, and from 6 to 25 days for No. 43.

The size of 24 eggs ( which were not used for other experiments) ranged from 3.05 to 4.04 cm in height and 2.51 to 4.59 cm in the major axis.

Female No. 1 laid 8 eggs in vessels B, 4 eggs in C, and one each in vessels A, D, and E. They selected their spawning site intentionally. However, when the three females were kept in the same water tank, there was no difference found between their spawning sites: three eggs in vessels B; two in vessels A, C, and E, respectively; one was outside of vessels E. In tank B, female No. 43 laid three eggs in a vessels placed near the water surface. This was one of the four vessels set along the center of the tank. No egg was found outside of the vessels. In water tanks A and B, newly-laid eggs were found near the old eggs, but not on the old ones. When there was no space for new spawnings, like egg of No. 10 cited above, new eggs were laid on the side wall of the same vessels but slightly to a more remote place.

In tank C, all four eggs a, b, c, and d, were laid in a hollow place of the tank wall. The eggs a,c, and d were laid on the bottom and the side wall where they stuck. Egg b, on the other hand, was laid and stuck to the wall very close to the surface. Spawning behavior observed at intervals during *Nautilus* living activity was as follows:

a) No. 1 (female), March 28th

At 8:15 a.m. No.1 female held the vessel B with six tentacles and protruded its tentacles to the inside of the vessel. It was also seen that the oral tentacles were protruded in and No. 6th egg was laid. At 8:22 a.m. it left the spawning area.

Table 2: The number of eggs laid in each tube indicating No. 1 *N. belauensis* of eggs laid in each area.

The numbers in circle denotes the eggs laid by *N. belauensis* No. 33 and No. 34. The circled number were those of the other No. 1 *N. belauensis*. Egg No. 21 was found to be laid outside of vessel.

Vessel	No. of egg layed	Total No. of eggs
A	15,16,17	3
B	1/2/5,6,9/12,13,14/18,19,22	11
C	3,7,8,10/23,26	6
D	11/20,25	3
E	4/24	2
Without cylindrical vessel	21	1

Table 3: The eggs laid by 3 different *Nautilus* were used for developmental experimant.

S.X; Soft-Xray carried out on various days that did not show any further development of eggs.

0 ; Not used in experiment.

The dates indicate the experimental day or when the specimens were terminated from the experiment.

No. of Eggs Laid	Date of Spawning	Spawning Nautilus No.	remarks
1	1-Jan-89	1	Used for experiment 4th April 1989
2	Jan	1	Kept in formalin on 25th April 1989.
3	Feb	1	0
4	Feb	1	Used for experiment 4th April 1989
5	21-Mar	1	0
6	28-Mar	1	S.x(9th March 1990)
7	2-Apr	1	0
8	15-Apr	1	0
9	19-Apr	1	0
10	23-Apr	1	S.x(9th March 1990)
11	29-Apr	1	0
12	10-May	1	0
13	17-May	1	0
14	25-May	1	S.x(9th March 1990).Died on 12th November 1989 after the experiment.
15	30-May		S.x(9th March 1990)
16	27-Jun		S.x(9th March 1990)
17	29-Jun		0
18	4-Jul		0 Described from experiment 1993.
19	6-Jul		0
20	9-Jul		0
21	9-Jul		0
22	13-Jul		0
23	15-Jul		0
24	17-Jul	33	S.x(8th March 1990)
25	18-Jul		S.x(9th March 1990). Used for developmental experiment (11th November 1989).
26	20-Jul		S.x(9th March 1990). Died on 11th November 1989 after the experiment.
27	11-Sep	43	
28	17-Sep	43	Used for protein analysis experiment on 9th March 1990.
29	12-Oct	43	Died - rotten smell.

## b) No. 1 (female), April 7th

At 7:41 a.m. No.1 female was seen swimming along the surface and came close to the spawning tank. One minute later, it protruded its right tentacle towards the vessel D. It searched inside of the vessel by protruding and retracting its tentacles, and later left the vessel. Later it got attached to the aquarium wall. At that time searching behavior was recorded.

## c) No. 1 (female), May 7th

At 6:15 p.m. No.1 female was seen moving towards the vessel A and got attached. It was also seen inside the vessel protruding its hyponome 4cm and searching movement of the inner part of the vessel. From the outside of the vessel, it held its body by one tentacle. After 7 minutes the body slightly vibrated, and 8 minutes later there was no movement of the mouth or oral tentacle seen. Twenty-seven minutes later the soft part including the hood and other soft part made two up and down movement and moved upward. After 40 minutes later, in vessel D, one of the tentacle under the hood got attached to the outside of the vessel with slightly protruded tentacle. The above two right tentacles got attached from inside the vessel. Under vessel E the bottom right tentacle got attached and controlled itself. After 57 min. later the hood moved up and down two times and later became still. Again after 58 min. later the body moved up and down two times. After 65 min., oral tentacle protruded inside of the vessel towards the inner surface. After 67 min. it left the vessel and swam away. After leaving the vessel for 3 min. and from the front opposite wall got attached. After 1 min. in vessel A the 4 right tentacles and the mouth part moved towards the opening and rested side ways. After 27 min. later the protruding and retraction of the tentacle took place. After 45 min. the tip of the oral tentacle was fully extended and later it left that area. At this time probably spawning behavior did not take place.

## d) No. 1 (female), May 17th

At 5:12 a.m. No.1 female was seen extending its tentacles in spawning vessel B. At that time the breathing rate of the Nautilus was 44 times per minute. There was a slight vibration of the body, but no movement seen. The hood part was towards the vessel side. At that time 3-4 mm pale white part of the shell was seen. At 5:13 a.m. the tentacle which was seen attached to the inner side of the vessel slowly got detached. After this time it was seen surfacing and moving around. However, after one minute it was again seen near the vessel B, but later left the vicinity of the vessel. Again it was seen approaching the vessel B and protruding its tentacles. Once again after one minute it got attached to the right entrance of the vessel. At this time the two right tentacles were seen inside of the spawning container, but not attached. Two minutes later it left the wall and swam away. At that time no recording was taken.

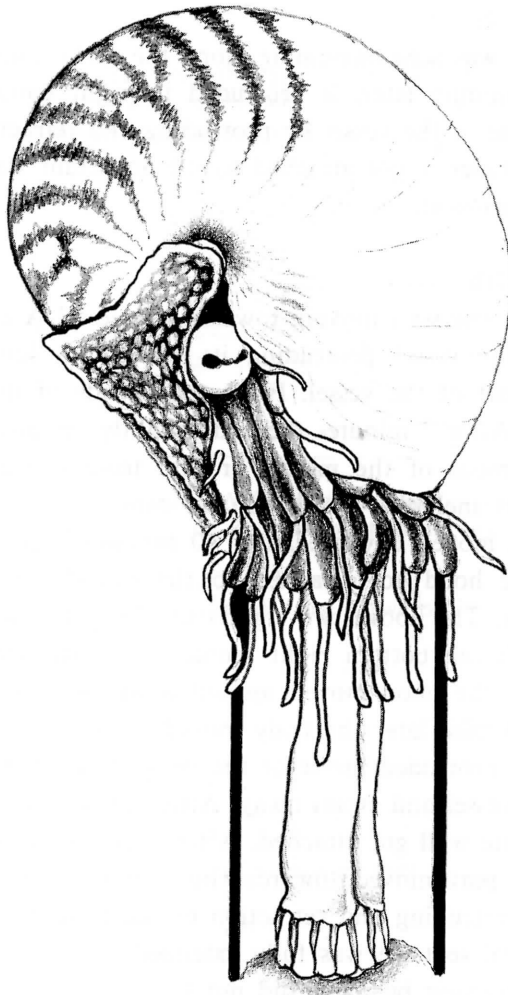


Fig. 2: An illustration of egg laying in *Nautilus belauensis* inside the vessel. Protrusion of oral tentacles of hand shaped lobes engaged in the laying of egg. The oral tentacles are with drawn.

e) No. 1 (female), July 16th

At 6:07 p.m. No.1 female was seen attached to vessel B. In vessel B it was protruding its tentacles as well as in vessels A, C, D, E the tentacle were attached and controlled its body. After 8 min. oral tentacle was seen protruding straight and later retracted. After 10 min., it protruded again and one minute later retracted. After 13 min. it protruded its right tentacle to the inner end of the vessel. At that time the shape of the tentacle was widely expanded and from below and above. After 25 min. the left tentacle got detached and the body became one sided. At the inner end of the vessel B, 3 eggs were laid. After 28 min. it got slanted left side and protruded its oral tentacle and later contracted its tentacle. Once again after 33 min., it tried to get to the original position and 35 min. later extended its oral



Fig. 3-a. The laid egg: when the oral tentacles are withdrawn, small elongated holes remain on both side of the egg capsule, and several slits that allow sea water to circulate freely between the two parts of the egg capsule.

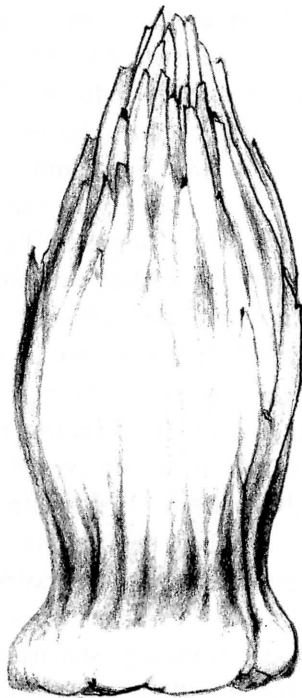


Fig. 3-b. The side view of egg structure (about 3cm in length).

tentacle and retracted. Again after 36 min. later, it extended its tentacles and retracted. After 38 min. the whole body vibrated and went under the inner base of the aquarium and no spawning took place. However, on July 17th at 4:20 a.m. No.1 female swam around the spawning vessel. At 4:17 a.m. No.33 female was seen attached to vessel E. So female No.1 did not approach near it. At 4:26 a.m. the female No. 3 tried to enter between inner aquarium wall and No. 33 but could not proceed further. After 10 min. it again tried to enter back way but failed. After 16 min. the third attempt was successful in attaching to vessel A and tried to go above the female No.33 but could not proceed further. The forth attempt by its back. After 26 min., 5th trial was made from the front of the vessel but again failed. At that time No. 33 was seen laying eggs and hence did not go close to No.33. At 5:55 a.m. it moved towards the vessel but did not get attached. When the female No. 33 was spawning the male did not show any response in going closer to this particular female.

f) No. 33 (female), July 17th.

No. 33 was circling around a spawning vessel. At 4:17 a.m., No. 33 held vessel E with several tentacles. Then, it extended its oral tentacles to the bottom of the vessel, but shrunk them within one minute. After extending its two tentacles right beneath its hood and examining the vessel with them, shrunk these tentacles and then adhered itself to the lower part of the outside of vessel D (located above vessel E). Four of the right-side lower tentacles were stuck to the inside bottom of vessel E. No. 33 attached to the vessel with its tentacles. Its tentacle was extended to the spawning site in a forward direction. Its hood emerged from its shell towards the bottom of the vessel; consequently, the black and blue-white part of the shell head appeared in a gap of 3-4 mm. Six minutes later, No. 33 again extended its mouth tentacle to the bottom of the vessel; the top of the oral tentacle appeared to be swelled. This tentacle shrank within minutes. Twelve minutes later, the whole of the oral tentacle had not completely withdrawn into its shell and the end of the tentacle, 3 cm in length, remained outside. After 20 minutes, the tip of the oral tentacle gradually widened and split like a broom at the tip. Thirty minutes later, No. 33 started to expand its oral tentacle to the bottom of the vessel (the breathing rate was 56 times per min.). After 36 minutes, the soft part was completely extended from the shell to the bottom of the vessel. The oral tentacle was seen extended to the left side of the bottom (the breathing rate increased to 58 per min.). The oral tentacle finally reached the bottom. Five tentacle cases were inserted 3cm in length to the vessel. The tentacle extended 5cm in length from the cases. The tips of the lower part of the oral tentacles were seen well stretched, but the tips of the upper parts were gradually shortened. Then, a semi-transparent milky-white lump was seen at the end of the oral tentacle. After 45 minutes, it was observed that an egg rolled down the column of the oral tentacle to the bottom of the vessel. Forty-seven minutes later, the tip of the oral tentacle became thinner,

and the egg looked like a table-tennis ball laid and stuck to the bottom of the vessel (the breathing rate was then sixty-one times per minutes). After fifty minutes, the tentacle shrank into the shell and the soft part came close to the shell. Fifty-five minutes later, the oral tentacle remained stretched. After sixty minutes, it was gradually withdrawn into the shell. Sixty-one minutes later the *Nautilus* left the vessel. After spawning, these *Nautili* never approached the same vessel. During spawning, the male never came near the female.

An illustration of a *Nautilus* laying eggs is shown in Fig. 2 Fig. 3 female laying eggs in-between the cylindrical tube. Fig. 3a The egg capsule structure seen when the oral tentacles withdrawn. Fig. 3-b Several slits seen from the side. These holes provide free flow of sea water inside the capsule.

In the laboratory experiment a series of photos were taken regarding the development of *Nautilus* eggs. The shape of the *Nautilus* egg in a cylindrical vessel is shown in Fig. 2. Fig. 3. Microscopic observatoions were made and pictures taken. Further details of the egg at various stages are given in Plate.

Further details of the histology of *Nautilus belauensis* eggs have been well described by Tanabe et. al. (1991). Prior to Tanabe's findings, Willey 1897 described the *Nautilus belauensis* eggs and detail structures.

## Summary

### A. Copulation Behavior

- 1) copulation behavior suggests the recognition of sex difference, copulation occurred six times between the same pairs.
- 2) The male is active in copulation, whereas it was often observed that the female gave preference to eating.
- 3) The copulating position is that the male and the female face each other forming a dogleg line at an angle of  $150^{\circ}$  -  $170^{\circ}$
- 4) In the case of *Nautilus belauensis*, the male held the female at the lower side of its shell, not embracing the female like *Nautilus macrophalus*.
- 5) Copulation took place through all seasons. The duration of the copulation lasted from 15 minutes to one hour at the most. Copulation was mostly observed at night and in the morning.

### B. Spawning Behavior

- 1) Spawning was observed from January to October, implying that spawning is independent of season.
- 2) The spawning sites were mostly hollow places in the tank wall and spawning containers. This indicates that favorable environmental conditions are required for spawning.
- 3) It takes about one hour for spawning with the help of oral tentacles
- 4) The number of eggs laid is one for each spawning. The size of the eggs is about 3-4cm on the major axis.

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## Plate 1

### Explanation of Plates

Eggs spawned by *Nautilus belauensis* inside a cylindrical vessel.

- Fig. 1. Side view: plastic vessel set inside Nb spawning tank A (spawned eggs) where spawned eggs are seen inside the plastic vessel.
- Fig. 2. Top view: spawned egg by Nb inside the plastic vessel. When the outer shell of the egg fixed in alcohol was opened, two layers of inner shell were seen similar to a bivalve shell.
- Fig. 3. The side view of Nb egg. The fertilized egg developed partially and later it was found that it ceased to develop. When the shell was opened, it was decayed. The inner two layers of the shell were like the shape of a hand.
- Fig. 4. Unfertilized egg. When the outer shell of the egg was opened, the yolk part was hard and in fresh condition.
- Fig. 5. Side view of the inner shell with one layer of outer shell. Fertilized egg. Inside the inner shell it was seen that the development of the egg was visible after three months of spawning.
- Fig. 6. *Nautilus belauensis* egg in its early development stage. The tip white part of the egg is the embryonic shell. The lower black part is the yolk. When the shell was opened, the yolk part became slightly elongated; however, the eggs of Nb are usually spherical.
- Fig. 7. Top view of a developed egg of Nb under the microscope. The upper bright part is the embryonic shell, and the lower amber color is the yolk part. This black color is due to the microscope field used in the microscope.
- Fig. 8. Side view of Nb egg indicated in Fig. 7.

Breeding Behavior of *N. belauensis*

