

## MORPHOLOGICAL STUDY OF THE GONAD OF *NAUTILUS POMPILIUS* IN PAPUA NEW GUINEA

Junzo TSUKAHARA, Kazushige TANABE, Akihiko SHINOMIYA  
and Kimihiko ŌKI

### Introduction

We have been performing morphological studies on the reproduction of the chambered *Nautilus* in the Philippines (HAYASAKA *et al.*, 1982), Fiji (TSUKAHARA and ONO, 1983, TANABE *et al.*, 1985, TSUKAHARA, 1985, 1988) and Palau (TANABE and TSUKAHARA, unpublished, TSUKAHARA, unpublished). To clarify of these studies, we collected the living *Nautilus pompilius* from Papua New Guinea, and dissected the reproductive organs. These were immediately weighed and fixed for histological studies.

### Materials and Methods

Thirty-four specimens of *Nautilus pompilius* captured off Port Moresby City, Papua New Guinea, late in November, 1990, were used for these studies. The soft parts of these specimens were dissected and the gonads and other reproductive organs were taken out and weighed. For the histological studies, small pieces of the testis and spermatophore were pre-fixed with 2% glutaraldehyde in 0.1 M  $K_2Cr_2O_7$ -NaOH buffer solution (pH 7.4) and 0.6M NaCl for 3 hours at room temperature, followed by post fixation with 1%  $OsO_4$  in the buffered NaCl solution for 1 hour at 0°C. After dehydration, the specimens were embedded in Spurr resin. Semi-thin sections for the light microscopic observation were stained with 0.01% toluidine blue in 0.1 M phosphate buffer (pH 7.2). Ultrathin sections were stained with uranyl acetate and lead citrate. These sections were observed with a Hitachi H-600 transmission electron microscope.

### Results and Discussions

#### *Sex ratio, gonad weight and index*

The 34 captured specimens were dissected, sexed, and weighed shell and soft part, and then 32 of their reproductive organs such as testis, accessory organs, spermatophore sac and penis were weighed (Table 1). Surprisingly all of trapped specimens were male. Sexual ratios of trapped *Nautilus pompilius* in the Philippines, Fiji, Papua New Guinea and *Nautilus belauensis* in Palau are shown in Table 2. From these results trapped females were never abundant except in the Philippines (TSUKAHARA, 1985), which, however, only obtained small numbers of specimens. In the Philippines, HAVEN (1972) showed that the captured males averaged 92% during eight months. She noted that the range of abundance of males during a year varied markedly. We also had the result that there was a seasonal variety of sex ratio in Palau (Table 2, Palau: summer and winter). In Papua New Guinea, we didn't capture individual females, while SAUNDERS (1984-1985) reported that he captured 22 males and 2 females from off Port Moresby, Papua New Guinea, in 250m depth. We should further

Table 1. Biological data of *Nautilus pompilius* in Papua New Guinea  
 Acc. O : accessory organ, Sp. Sac : spermatophore sac, W. : weight

Sp.No	Sex	Total W.(g)	Shell W.(g)	Soft Part W.(g)	Testis W.(g)	Acc.O.W.(g)	Sp.Sac W.(g)	Penis W.(g)	Gonad Index(%)
PM-1	Male	499.1	141.3	511.4	19.7	2.3	1.2	0.2	3.85
PM-2	Male	665.2	153.8	450.1	10.2	2.1	0.4	0.2	2.27
PM-3	Male	531.95	146.1	408.2	17.2	4	0.6	0.5	4.21
PM-4	Male	537.75	172.7	480.05	16.85	3.1	1.6	0.55	3.51
PM-5	Male	622.45	142.4	418.7	—	—	—	—	—
PM-6	Male	550.45	136.8	362.3	14.1	2.8	1.1	0.3	3.89
PM-7	Male	503.2	140.9	420.9	12.5	3	1.35	0.4	2.97
PM-8	Male	555.7	134.8	426.2	—	—	—	—	—
PM-9	Male	587.7	161.5	388.95	13.45	1.8	0.85	0.7	3.46
PM-10	Male	561	144.05	387.9	18.3	3.2	1.05	0.35	4.72
PM-11	Male	561.1	157.05	634.3	16.3	2.6	1.3	1	2.57
PM-12	Male	565.45	179.3	519.4	17.1	3.8	1.3	0.6	3.29
PM-13	Male	782.8	148.5	474.8	16.4	3.5	1.2	1	3.45
PM-14	Male	639.9	165.1	496.9	14.6	2	0.6	0.5	2.94
PM-15	Male	668.3	171.4	463.7	17	3.1	1.5	0.7	3.67
PM-16	Male	596.7	159.9	436.8	14.4	3.8	1	0.6	3.30
PM-17	Male	583.8	147	402.7	17.2	2.1	0.5	0.4	4.27
PM-18	Male	572.4	169.7	456.3	15.5	2.1	0.7	0.5	3.40
PM-19	Male	620.3	141.9	478.4	13.3	2.2	0.8	0.8	2.78
PM-20	Male	655.4	177	462.2	18.9	4.1	0.8	0.6	4.09
PM-21	Male	607.2	145	501.9	20.6	3.1	1	0.8	4.10
PM-22	Male	664.7	162.8	459.2	16	3.2	1.3	0.7	3.48
PM-23	Male	621.9	162.7	486.7	18.1	3.8	1.1	0.4	3.72
PM-24	Male	571.2	160.8	410.4	22.4	3.4	1.1	0.6	5.46
PM-25	Male	552.2	141.8	524.4	15.2	2.8	1	0.5	2.90
PM-26	Male	681.7	157.3	484.5	19.8	3.6	0.8	0.8	4.09
PM-27	Male	671.9	187.4	389.5	16.3	3.1	1	0.9	4.18
PM-28	Male	543.2	153.7	451.1	15.8	2.1	1.1	0.5	3.50
PM-29	Male	660.75	169	420.9	19.1	3.4	0.6	0.4	4.54
PM-30	Male	573	152.1	393.4	15.6	3.4	0.8	0.5	3.97
PM-31	Male	540.8	147.4	548.4	9.3	2.4	0.6	0.6	1.70
PM-32	Male	715.2	166.8	477.9	19	3.5	0.9	0.6	3.98
PM-33	Male	628.2	150.3	405.9	20.9	3.9	1.2	0.7	5.15
PM-34	Male	548	142.1	441.9	15.5	2.9	0.7	0.5	3.51
average		601.19	155.6	455.19	16.46	3.01	0.97	0.58	3.44
std dev		63.57	13.34	55.31	2.92	0.67	0.3	0.2	1.6

Table 2. Sex ratio of *Nautilus*

Specimen collected from:	Male No.	Female No.	Total	% of Male
Philippines(Haven, 1977)	2940	255	3195	92%
Philippines(Tsukahara, 1985)	4	14	18	22%
Fiji(Tsukahara & Ono, 1983)	10	2	12	83%
Fiji(Tsukahara, 1985)	23	13	36	64%
Palau(1988, Aug-Sept.)	20	17	37	54%
Palau(1989, Jan.)	28	15	43	65%
Papua New Guinea(Saunders, 1984-1985)	22	2	24	92%
Papua New Guinea(1990)	34	0	34	100%

investigate to capture the specimen on other seasons and by other methods except trapping.

Gonad Index (GI) is calculated as the ratio of wet weight of gonad to soft part of *Nautilus* body. The results are shown in Table 1. GIs of *Nautilus* in Papua New Guinea are compared with that of male specimens in the Philippines, Fiji, and Palau (Fig. 1). The result suggested that the distribution pattern of GI in Papua New Guinea is very similar to that in Fiji.

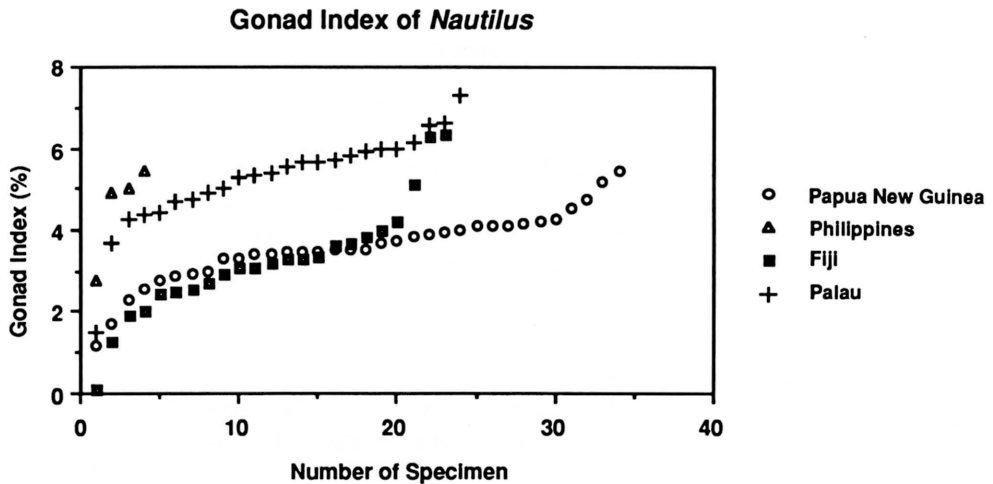


Fig. 1. Gonad Indexes of male *Nautilus* in Papua New Guinea, Philippines, Fiji and Palau. Gonad Index is calculated as the ratio of wet weight of testis to soft part of body (percentage).

Almost all the males, which GIs are over 2.5%, have a developing spermatophore in a spermatophore sac. Full grown spermatophore were found in the lumen of the penis heavier than 0.6g in weight. From these results it is suggested that the greater number of *Nautilus* captured off Port Moresby may have already been prepared for the reproduction in summer.

#### *Histological observations*

The thick section of the testis of the specimen (PM-1) was observed by light microscope (Fig. 2). Many spermatogonia can be seen near the basal lamina, while spermatogenetic and spermiogenetic figures are found on the inner side of tubules. At the central area of the tubule many semi-matured spermatozoa are crowded.

Electron microscopic observations of the testis are shown in Fig. 3 and 4. Many mitochondria are crowded near one side of the nucleus of younger spermatocyte and fused with one another to make two larger mitochondria (Fig. 3, arrow). The spherical nucleus elongates gradually to a pear shape as subsequent development progress and later changes to a spindle shape (Fig. 4). Near the top of the pear shaped nucleus, dense amorphous materials formed in the Golgi apparatus are crowded (Fig. 3, arrow head), and later may be set on the top of the nucleus as the acrosomal granule (Fig. 4, arrow, Fig. 6, arrow).

Numberless mature spermatozoa are arranged with their head toward the outer jelly-like substance of spermatophore tubules of specimen TP-3 (Fig. 4). Their nucleus is reached about  $36\mu\text{m}$  in length and about  $0.5 \times 0.3\mu\text{m}$  in diameter (Fig. 5, 6). The area near the

top of the nucleus is more flat and  $0.8 \times 0.15\mu\text{m}$  in diameter (Fig. 6 arrow head). The mature spermatozoa in the spermatophore of *Nautilus pompilius* captured in the Philippines has a nucleus with a length about  $35\mu\text{m}$  and a diameter about  $0.3\mu\text{m}$  (ARNOLD and ARNOLD, 1978) and of *Nautilus pompilius* captured in Fiji has a nucleus with a length about  $37\mu\text{m}$  and a diameter about  $0.3 \times 0.5\mu\text{m}$  (TSUKAHARA, 1985). The morphological changes during sperm formation and the ultrastructural features of mature spermatozoon in these three groups are very similar.

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### Explanation of Figs. 2-7

- Fig. 2. Transverse section of testis. sg : spermatogonium, arrow : semi-matured spermatozoon. bar :  $50\mu\text{m}$
- Fig. 3. Young spermatid in which the mitochondria crowded at one side of the nucleus (arrow). N : nucleus of spermatid, arrow head : dense amorphous materials formed in the Golgi apparatus. bar :  $2\mu\text{m}$
- Fig. 4. Cylindrical nucleus (N) of semi-matured spermatozoon with condensed chromatin. Arrow : acrosomal granule. bar :  $2\mu\text{m}$
- Fig. 5. Cross section of mature spermatophore. arrow : sperm rod. bar :  $50\mu\text{m}$
- Fig. 6. Cross section of mature spermatozoa. N : nucleus, F : flagellum. bar :  $1\mu\text{m}$
- Fig. 7. Longitudinal section of mature nucleus. arrow : acrosomal granule. bar :  $1\mu\text{m}$

