Paleobiology, 7(4), 1981, pp. 481–493

The chamber formation cycle in *Nautilus macromphalus*

Peter Ward, Lewis Greenwald, and Yves Magnier

Abstract.—The chamber formation cycle in externally shelled, chambered cephalopods consists of mural ridge formation, secretion of the siphuncular connecting ring, septal calcification, and cameral liquid removal. Radiographic observation of the chamber formation cycle in specimens of *Nautilus macromphalus* allows direct observation of the various processes of the chamber formation cycle in a chambered cephalopod, and gives direct measures of rates. New chamber formation in *N. macromphalus* initiates when slightly more than half of the cameral liquid has been removed from the last formed chamber. At this volume, the liquid within the chamber drops from direct contact with the permeable connecting ring to a level where it is no longer in direct contact and must move onto the connecting ring due to wettable properties of the septal face and septal neck. This change from "coupled" to "decoupled" emptying coincides with the formation of a mural ridge at the rear of the body chamber, in front of the last formed septum. With completion of the mural ridge, the septal mantle moves forward from its position against the face of the last formed septum and attaches to the new mural ridge, where it begins calcifying a new septum in front of the newly created, liquid-filled space. Emptying of the new cameral liquid from this space commences when the calcifying septum has reached from one-third to two-thirds of its final thickness. The cessation of calcification of the septum coincides with a liquid volume in the new chamber of approximately 50%, at which point the cycle begins anew. During the chamber formation cycle apertural shell growth appears to be continuous. Since apertural shell growth is the prime factor leading to increased density in seawater, and hence decreased buoyancy, the period in the chamber formation cycle between the onset of septal calcification and the onset of emptying would be a time of greatly decreasing buoyancy. This is avoided by the removal of decoupled liquid from previously produced chambers. In this way constant neutral buoyancy is maintained. The time between chamber formation events in aquarium maintained *N. macromphalus* appears to be between 70 and 120 d.

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Accepted: July 8, 1981

Introduction

The late Cambrian-early Ordovician nautiloid radiation is a classic example of an adaptive radiation: the rapid diversification of new taxa from limited ancestry due to the achievement of an evolutionary breakthrough, or expansion into newly formed or newly vacated ecospace (Simpson 1953). Both aspects probably played a part in the nautiloid adaptive radiation. The early nautiloids have been interpreted as organisms which were to some degree nektonic: their rapid radiation in the lower Paleozoic may reflect colonization of an underexploited, neritic habitat. To achieve this colonization, however, the earliest cepalopods first had to free themselves from the bottom and negate the penalty of carrying a dense, carbonate shell. Their key evolutionary breakthrough must have been the attainment and maintenance of neutral buoyancy. As shown by Yochelson et al. (1973), this involved two steps: producing a closed space (chambers) in the apex of the shell and removing liquid from this space.

The post-Ordovician history of the chambered cephalopods is marked by a great richness of shell and septal form. In two respects, however, the chambered cephalopods have been conservative: all have a siphuncle running through each chamber, and all have shown the same growth pattern of chamber placement behind a forward-moving body. Thus it seems likely that all chambers originated as fluid-filled spaces created by forward movement of the body within the body chamber of the shell. These spaces were then sealed off by secretion of a calcareous septum and emptied of liquid by the siphuncle.

The two-step process of chamber formation and chamber emptying can be observed today in species of *Nautilus, Sepia,* and *Spirula.* Of

0094-8373/81/0704–0009/$1.00

these, only *Nautilus* retains an external shell and thus may be our best analogue for understanding the chamber formation and chamber emptying processes in ancient shelled cephalopods.

Analysis of the chamber formation process in living *Nautilus* has been approached in two ways: through study of shell architecture and microstructure (i.e. Gregoire 1962; Erben et al. 1969; Mutvei 1972; Bandel and Boletzky 1979; Blind 1976, 1980), or through studies of the mechanisms of liquid removal (Denton and Gilpin-Brown 1966; Collins and Minton 1967; Ward and Martin 1978; Ward 1979; Collins et al. 1980; Greenwald et al. 1980). There is now a clear picture of how new septa are laid down and how the resultant cameral space is emptied of cameral liquid and ultimately filled with gas. In contrast, the relative interaction among these processes and apertural shell and tissue growth, rates of the various aspects of the chamber formation process, and controls of chamber formation are unclear or unknown. We have approached these latter aspects of the chamber formation process by posing the following questions: 1) What are the relationships between body chamber length, cameral liquid volume, and stage of chamber formation? 2) How thick is a newly forming septum when cameral liquid emptying is initiated in a newly formed chamber? 3) How long does each of the distinct processes of the chamber formation cycle take? 4) What are controls on the chamber formation process?

Materials and Methods

Nautilus macromphalus were captured in baited traps at 300 to 400 m depths on the forereef slope near Noumea, New Caledonia. After capture, the experimental animals were maintained in large, opaque fiberglass tanks connected to a running seawater system. Fresh seawater was refrigerated to 16°C before entry into the tanks. Aeration was provided by air bubblers.

Cameral liquid volumes were determined by comparing the liquid levels in radiographs to levels in *Nautilus* shells with known cameral liquid volumes or by direct volume measurement of cameral liquid removed through holes drilled in the shell. Radiography was conducted with a Min-X-Ray 110 (Mikasa Company). All radiography was on Kodak No-Screen film (type NS-2T) at exposures of 0.78 sec, 68 kV, 13 mA.

To make sure that successive radiographs taken over several weeks time would always have the nautilus in the same orientation (living position), the external shell of each nautilus in the radiograph program was marked with a vertical line in waterproof ink. A special cradle of styrofoam was fashioned so that the nautilus could be photographed in their living position. The cradle was marked with a vertical line on its base and sides, so that the lines on the box and the shell of the nautilus being radiographed were aligned. In this way, successive radiographs were taken at the same orientation each time.

To assess the effect of radiography on the experimental specimens, two nautilus were maintained without radiography for 105 and 135 d respectively, showing apertural growth rates of .15 and .152 mm/d. Although the radiographed specimens showed a slightly slower mean growth rate (.09 mm/d), several grew at faster rates than the non-radiographed specimens (see section below on apertural rates). We found water temperature to have far greater effect on growth and health of the animals than did the treatment with radiographs.

The relationship between cameral liquid volume and liquid level observed in the radiographs can be demonstrated by Fig. 1. Since all of the observed *Nautilus* were radiographed in the same position (living position), the same percent cameral liquid volume resulted in a similar liquid level, regardless of absolute chamber volume, *in non-approximated chambers*. Closely spaced, or approximated chambers, show somewhat different volume-level relationships. Septal thicknesses were measured directly from radiographs, using a Wild-M5 Stereomicroscope with 20× measuring occular and transmitted light.

Forty-four immature *Nautilus* were radiographed and observed over periods between 12 and 77 d. Observational periods for specimens 79-2, 3, 5, 22, 26, 27, 30, 31, and 34 were terminated by a chance incursion of fresh water during a large rainstorm, killing all of these animals. Up to this event, however, all animals

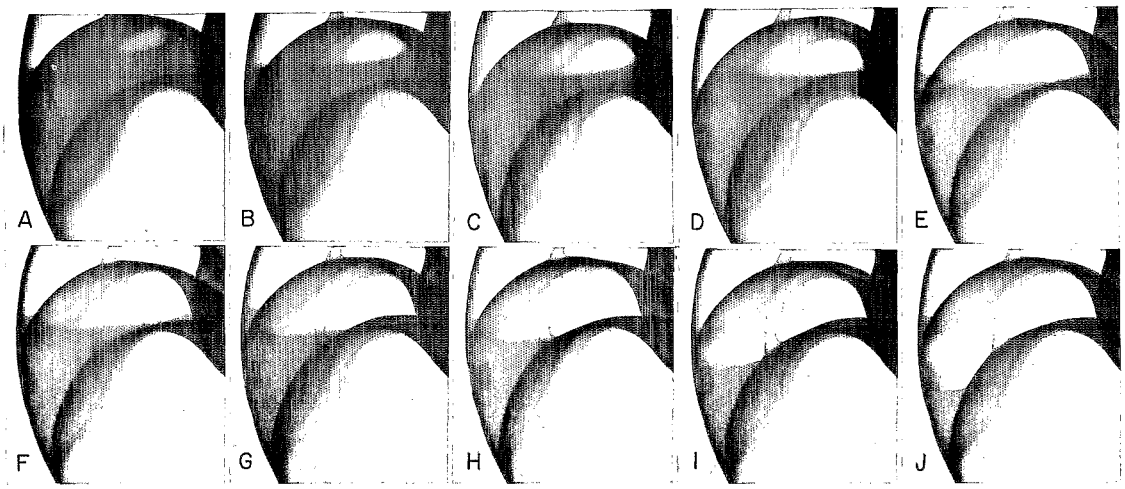

FIGURE 1. The relationship between cameral liquid volume and liquid level is demonstrated in the above radiographs. The total volume of the chamber examined was 18 ml; the chamber was then injected with water volumes in 10% increments. This relationship will hold for all but approximated chambers, regardless of volume. Volumes: A = 90%, B = 80%, C = 70%, D = 60%, E = 50%, F = 50%, G = 40%, H = 30%, I = 20%, J = 10%.

were in an apparently healthy condition. All other observations were terminated by our departure.

As has been noted in a previous aquarium study with *N. macromphalus,* apertural shell growth occurring in the aquarium is abnormal in its roughened and thickened appearance. It is not known if the growth rates are also abnormal. It is our assumption that the roughened edges of shells grown in surface aquaria are due to constant breakage against the sides of the tanks, thus producing the scarred appearance noted by Martin et al. (1978). It is not our intention in this paper to make definitive statements about the rates of chamber formation in nature. Instead, we hope to draw attention to the relative sequence of events in the chamber formation process. We have included the observed rates of events that occurred in our experimental programs, since these rates represent the best information to date about the chamber formation process.

Stages in the chamber formation cycle.—The chamber formation cycle as observed with radiography can be differentiated into distinct periods, or stages, which are useful for defining rates of the various processes which make up the formation of a new chamber. We can arbitrarily define the commencement of the chamber formation cycle as the moment when secretion of the mural ridge begins. The mural ridge is a thin, annular band of calcium carbonate which is secreted on the interior of the body chamber, in front of the last-formed septum. Calcification of the mural ridge can be called the stage of *mural ridge formation* (MRF). In radiographs the mural ridge is visible as a faint, thin line, which becomes progressively denser through time. Our dissection of *N. macromphalus* undergoing mural ridge formation showed that the septal mantle is still pressed against the last formed septum during this stage. The stage of mural ridge formation ends with the forward movement of the septal mantle in the body chamber, followed by its re-attachment on the new mural ridge. Once attached, the septal mantle begins calcifying a new septum, commencing the second stage in the chamber formation cycle. We call this stage *Pre-emptying septal secretion* (SS). Coincident with septal secretion, the siphuncle begins calcifying a new connecting ring within the new chamber space, which is completely filled with cameral liquid. During this stage, which in radiographs is marked by a gradual thickening of the new septum and connecting ring, no cameral liquid emptying occurs. Chambers previously constructed, however, can be seen to be emptied of liquid. The third stage, which we call *coupled emptying* (CE), initiates with the first removal

of cameral liquid from the new chamber. As we will show below, coupled emptying begins prior to completion of septal calcification. As the new chamber is drained of liquid, the new septum continues to thicken. Emptying by the siphuncle causes the liquid level in the chamber to descend until it is at a level where it is no longer in direct contact with the connecting ring. At this point the liquid is termed *decoupled* (Denton and Gilpin-Brown 1966). Coincident with the transition between coupled and decoupled emptying, radiographs show that new mural ridge formation begins, signalling the start of a new cycle of chamber formation. Even though new chamber formation has started, the observed chamber continues to empty, until it is completely drained of liquid. We can term this final period the stage of *decoupled emptying* (DE).

Results

1.) *Distribution of cameral formation stages.*—Immature *Nautilus macromphalus,* captured at depths between 300 and 400 m on July 11, August 8, and August 17, 1979, were radiographed immediately after capture. From these radiographs we determined the stage of chamber formation present for the last-formed chamber, as well as cameral liquid volume (as a percentage of the total chamber volume), body chamber angle, and total number of septa (Table 1).

Of the 43 specimens captured on these three dates, 18 (42%) were engaged in mural ridge formation or pre-emptying septal secretion. The other 25 specimens were engaged in either coupled or decoupled emptying of the most recently formed chamber. There appears to be no suggestion of synchrony in any stage of septal formation; in other words, we can detect no evidence suggesting that a striking majority of specimens undergoes similar stages of septal formation at the same time.

2.) *Cameral liquid volume and body chamber angle.*—Collins et al. (1980) first demonstrated that an inverse, linear relationship exists between body chamber angle and cameral liquid volume of the latest formed chamber in specimens of *N. pompilius* from the Fiji Islands. We have observed a similar relationship to hold in our sample of newly captured *N. macromphalus.* Cameral liquid volumes in the most recently formed chamber, computed as a percentage of chamber volume rather than as absolute volume as in Collins et al. (1980), have been plotted against angular body chamber length in degrees (Fig. 2). As in *N. pompilius,*

TABLE 1. Cameral formation stages in *Nautilus macromphalus.*

No.	Stage	$^{v}ch_1$	$^{v}ch_2$	$Angle_t$	A_2	# Chambers
		Captured 7/11/80				
79-1	CE	50	5	110		26
79-2	MRF	40	10	106	132	27
79-3	MRF	40	10	109		25
79-4	CE	90	20	103	126	28
79-5	MRF	50	20	102	128	26
79-8	CE	80	10	96	122	29
79-20	CE	90	20	100	126	30
79-22	SS	100	40	85	109	31
79-25	CE	40	10	108	130	26
79-26	SS	100	30	88	116	29
79-27	SS	100	30	94	116	29
79-29	CE	95	20	94	118	25
79-30	CE	70	<10	108		29
79-31	CE	>95	30	91	116	28
79-32	CE	80	<5	108		29
79-33	MRF	40	<5	117		26
79-34	MRF	50	<5	112		24
		Captured 8/10/80				
79-36	SS	100	30	95	120	26
79-37	SS	100	<10	91		28
79-38	CE	>95	<5	94	119	27
79-39	SS	100	30	97	120	30
79-40	MRF	40	<5	114		25
79-41	CE	90	<10	95		27
79-42	CE	90	<5	102		28
79-43	SS	100	<10	92		30
79-44	CE	90	10	95	118	29
79-45	CE	>95	<5	100	125	30
		Captured 8/17/80				
79-59	MRF	40	<5	112		27
79-60	CE	>95	20	95	122	25
79-61	CE	50	<5	106		27
79-62	MRF	50	<10	110	136	28
79-70 (B_1)	CE	80	<10	98		27
79-71 (B_2)	CE	>90	<10			29
79-72 (B_4)	SS	100		88		25
79-73 (B_5)	CE	60		103		
79-81 (C2)	CE	90		100		
79-82 (C4)	SS	100		88		
79-83 (C5)	CE	85		99		
79-84 (C7)	CE	57		116		
79-85 (C8)	SS	100		97		
79-86 (C9)	CE	88		113		
79-87 (C10)	CE	70		108		
79-88 (C11)	CE	75		112		

Totals for 3 samples, n = 43

MR	8 = 19%,	CE	24 = 56%
SS	10 = 23%,	DE	1 = 02%
	18 = 42%,		25 = 58%

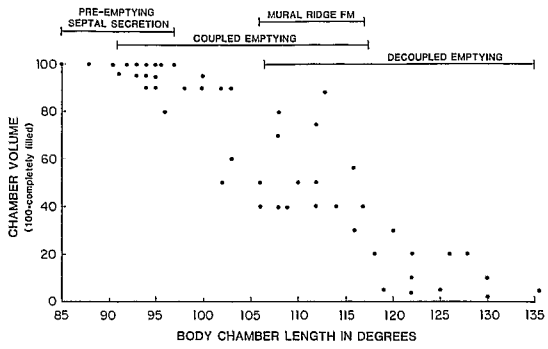

FIGURE 2. Chamber liquid volume (as a percentage of total chamber volume) plotted against body chamber length, in degrees. The body chamber lengths (or body chamber lengths plus angular length of last chamber) of the various emptying stages are shown in brackets above diagram. Each datum point is from one chamber.

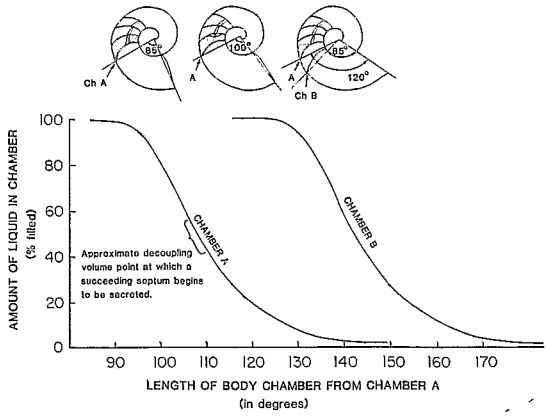

FIGURE 3. The relationship between body chamber length and liquid volume can be demonstrated in the above figure. An organic membrane placed on the mural ridge closes off the new chamber. The septal mantle uses this organic membrane as a template and begins secreting layers of nacreous calcium carbonate onto the organic membrane. This newly-forming septum closes off a body chamber of approximately 85° in angular length. Meanwhile, apertural shell growth continues, enlarging the body chamber length. The enlarging body chamber will increase density of the *Nautilus*, thereby decreasing buoyancy. This density increase is offset by liquid removal from previously produced chambers, which have liquid still being emptied, even though a new chamber is being produced. The thickening septum of the newly-built chamber will not be emptied until the new septum is of sufficient thickness to withstand the pressure differential between ambient pressure and chamber pressure (nearly a vacuum when liquid emptying begins). This point coincides with a body chamber angular length of about 90°. Buoyancy decrease from further body chamber size increase is offset by removal of liquid from the new chamber, plus continued removal of decoupled liquid from the previous chambers. When the chamber is slightly more than half emptied of liquid (with a body chamber angle of about 110°), a new mural ridge is secreted. Once completed, the *Nautilus* moves forward in the body chamber, and renews the cycle by secreting a new septum at the site of the mural ridge.

there appears to be strong negative correlation between cameral liquid volume and body chamber length: chambers completely filled were found in specimens with the shortest body chambers, while chambers that had undergone increasing percentages of chamber emptying were associated with relatively longer body chambers. The distribution of points in Fig. 2 is horizontal at the 100% volume mark (the chamber is completely filled), drops in roughly linear fashion to volumes of 5 to 10%, and then appears to level out again, indicating that at both very high and very low cameral liquid volumes of a given chamber, apertural growth is accompanied by little or no emptying. The shortest body chamber angle in a specimen observed to have begun chamber emptying was 91°, and the mean body chamber length for five specimens observed to have just begun emptying (discerned by the presence of a small bubble at the top of the chamber) was 95°.

In contrast, the mean body chamber angles of seven specimens either having extremely thin, new septa when freshly caught or showing the first evidence of new septal secretion while in the aquarium was 88°. Since the body chamber is shorter in these animals, it appears that apertural growth must be continuous during the initial septal secretion process, resulting in apertural increase of approximately 5° between the onset of septal calcification and the initiation of emptying.

The right side of the graph, representing chambers which have nearly been emptied (never the latest formed chambers in our specimens), is also suggestive of a break in slope from the main body of points. It may indicate that the rate of cameral liquid removal slows when 90 to 95% of the chamber has been emptied, assuming that the rates of apertural shell growth have remained constant throughout. A schematic view of emptying curves for two ideal chambers is shown in Fig. 3. Further observations will be necessary to show whether or not the emptying curve is better described by a linear or polynomial function.

3.) *Relationship between liquid volume and stage.*—Figure 2 shows little overlap in cameral

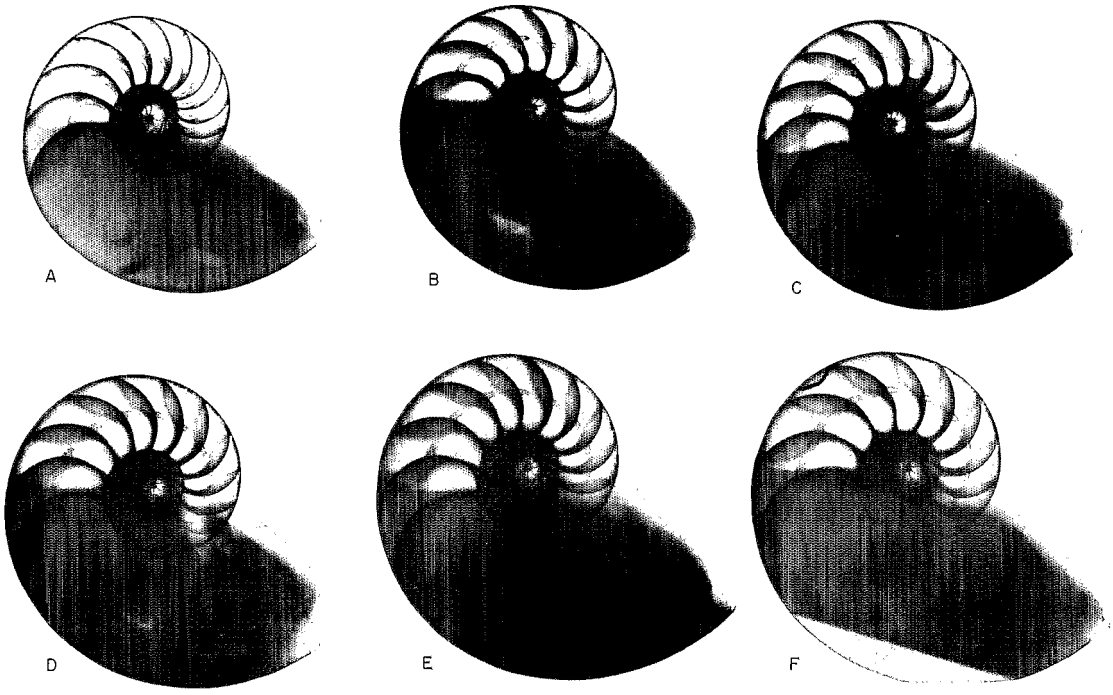

FIGURE 4. Mural ridge formation in six different *Nautilus macromphalus*. Note the liquid level in the latest formed chamber; in all specimens, liquid level is at, or near the decoupling point (where cameral liquid is no longer in direct contact with permeable parts of the siphuncle). Note also the great similarity in angular body chamber lengths. A. 79-33, B. 79-34, C. 79-61, D. 79-59, E. 79-62, F. 79-40.

liquid volumes between the most recently formed chambers sampled (Ch. 1) and those of the second most recent chambers (Ch. 2), illustrating an important relationship between liquid volume and stage of emptying. Cameral liquid volumes of the most recent chambers of *Nautilus* with chambers yet to be produced are rarely less than 40% filled with liquid; most range from 100% to 50% filled. Conversely, we have never observed a specimen of *N. macromphalus* with a volume in Chamber 2 of more than 40%.

As first noted by Ward (1979), chambers that have had about 50 to 60% of their cameral liquid removed have liquid levels at, or very near the coupled-decoupled transition zone, where liquid no longer is in direct contact with the permeable connecting ring. Considerable importance has previously been ascribed to this transition (Denton and Gilpin-Brown 1966, 1971; Ward 1978, 1979). The reason for the complete absence in our sample of specimens with volumes less than 30% in the most recent chamber is that *the coupled-decoupled transition coincides with initation of a new chamber* (Ward et al. 1980). We consider this observation to be extremely important, because there is no a priori reason why this must occur. In our sample, ten *N. macromphalus* were captured with, or underwent formation of, mural ridges while in our radiographic observation program (Fig. 4). At the time of mural ridge formation all showed cameral liquid levels in their last completed chamber either exactly at, or very near, the transition point that appears to be at the intersection of the septal neck with the connecting ring.

4.) *Septal calcification and liquid emptying.*— Denton and Gilpin-Brown (1966) have suggested that gas pressure within the chambers of *Nautilus* never exceeds one atmosphere, regardless of depth. If true, this suggests that one of the primary functions of cameral liquid is to support calcifying septa against implosion by ambient pressure. Cameral liquid removal would then be initiated only after the septum is

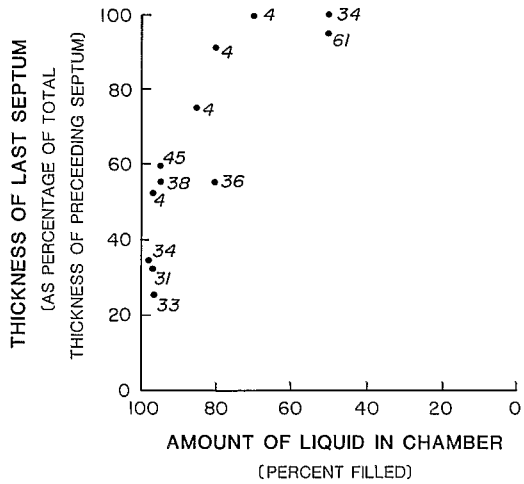

FIGURE 5. Septal thickness (as a percentage of the total thickness of the preceeding septum) plotted against liquid volume (percent chamber volume filled with cameral liquid). Specimens undergoing first emptying can be seen to have septa ranging from 25 to 60% of their final, expected thickness. Specimens having concluded septal thickening can be seen to have emptied between one-quarter and one-half of their cameral liquid.

of sufficient thickness to withstand the great pressure differential between the two sides of the septum. Most workers agree that ambient (high) pressure is directed against the concave surface of the calcifying septum, while the initiation of emptying produces a near-vacuum within the chamber (Ward et al. 1980; Collins et al. 1980).

At what point is the calcifying chamber thick enough to withstand the pressure differential? The only information concerning this question is an observation by Denton and Gilpin-Brown (1966), of a newly forming septum, approximately one third its final expected thickness, which closed off a still completely filled chamber. We have gathered new information about this aspect of the chamber formation cycle in *Nautilus macromphalus,* by measuring the septal thickness of newly forming chambers, either in newly caught specimens, or in animals maintained in the aquarium. We have transposed the data into relative septal thickness by dividing the measured thickness of the calcifying septum by the thickness of the immediately preceeding septum; this allows comparison of septa of differently sized *Nautilus*. In reality, each septum in the immature shell is slightly thicker than the last (see Hamada 1963). For our purpose, however, this difference is probably less than the experimental error in measuring.

It would be of interest to know the relative septal thickness at the commencement of liquid emptying and the volume still left in the chamber when the septum has reached its final thickness. To this end we have plotted relative septal thickness against chamber volume from 27 radiographic observations of 12 *N. macromphalus* (Fig. 5).

It is evident that first emptying occurs long before the calcifying septum reaches its maximum thickness, for septal thicknesses in front of newly emptying chambers ranged between 25 and 60% of the expected final value in six specimens, with a mean of 44%. The additional thickening that occurs after first emptying may be safety factor. An additional observation may be important concerning the six *N. macromphalus* from our sample which were in the first stages of emptying. The three specimens with the smallest septal thickness values (25, 32, and 34%) at first emptying were all animals that underwent septal formation while in the aquarium. The other three animals, all with the higher values (55, 57, and 60%), were specimens that were undergoing the first phases of liquid removal when newly captured from 300 to 400 m. Although few in number, the data allow the inference that ambient pressure of chamber formation somehow controls the initiation of emptying, as the three animals from 300 to 400 m depth all show significantly thicker septa at the start of emptying than did the three that underwent emptying in the very low ambient pressures of the aquarium.

The attainment of maximal septal thickness occurred in animals with chambers between 30 and 50% emptied of liquid. Maximal thickness must be reached by the decoupling point (approximately 50% emptied), since this point coincides with the forward movement of the body and hence removal of the secretory posterior (septal) mantle away from the face of the last formed septum.

5.) *Duration of the stages of chamber formation.*—Because of the long time involved in the complete chamber formation process, we were unable to follow any single animal completely through the entire cycle. We have, however, portions of the complete cycle recorded in ra-

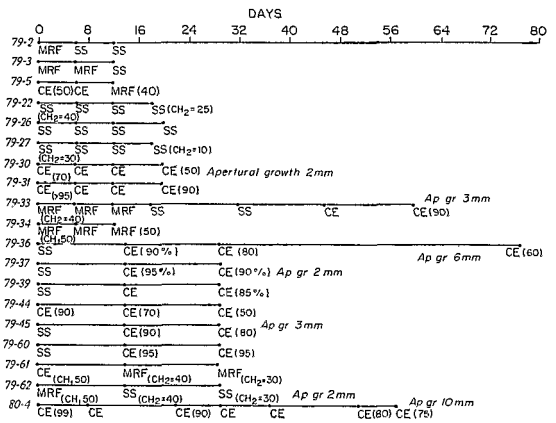

FIGURE 6. Times of radiographs for 19 *N. macromphalus* used in the study. Each dot indicates that a radiograph was taken. The stage observed in each radiograph is indicated beneath each dot. MRF stands for mural ridge formation, SS = pre-emptying septal secretion, CE = coupled emptying. The volumes of either the first or second chamber are shown in brackets, where 100% = chamber completely filled with cameral liquid, 0 = emptied of cameral liquid.

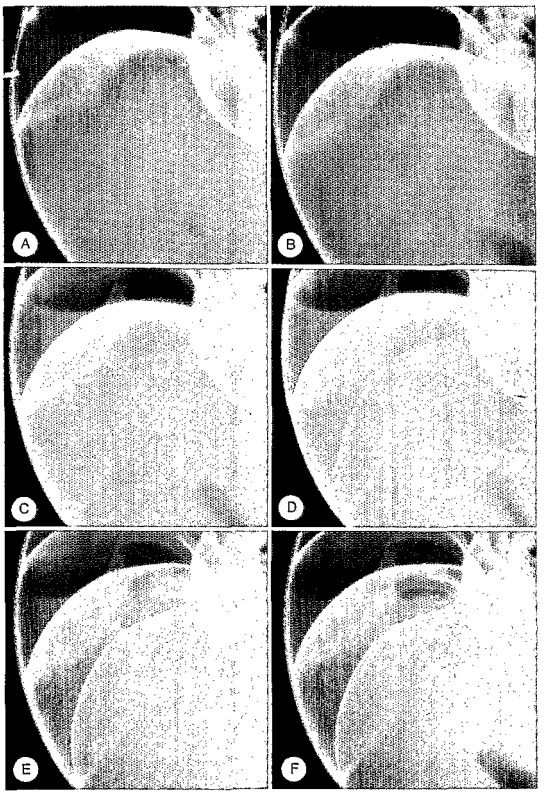

FIGURE 7. Six radiographs of a newly forming chamber in specimen 79-33. Radiograph A coincides with the radiograph taken at day 6 in Fig. 6. Radiograph B was taken 6 d after A; C = 12 d after A; D = 26 d after A; E = 40 d after A and F = 54 d after A. Radiographs A and B show mural ridge formation; radiographs C and D show pre-emptying septal secretion; while radiographs E and F show coupled emptying.

diographs of 19 *N. macromphalus,* followed for periods of 12 to 77 d. From these radiographs, we can arrive at estimates of average duration for the various stages of the chamber formation cycle for *N. macromphalus* grown in surface aquaria.

The times of radiographs, stage of chamber formation at the time of each radiograph, relative chamber filling, and amount of apertural shell growth for long term series are shown in Fig. 6. The durations we are interested in are: 1) average amount of time necessary for the secretion of the mural ridge; 2) the time between onset of septal secretion and the onset of emptying; and 3) the time between onset of emptying and the coupled-decoupled transition.

a. *Mural ridge formation.* The secretion of the mural ridge on the inside of the body chamber can be viewed in specimens 79-33 and 79-61. In the former the first radiograph shows no evidence of mural ridge, while the second shows the first, faint evidence of the mural ridge as a very fine line on the inside of the body chamber (Fig. 7). The first evidence of septal secretion, as shown by a thin outline of new septum and siphuncle, is found in a radiograph taken 18 d after the initial radiograph. Since there is evidence of the mural ridge, but not the new septum, at day 12, we can assume that the duration of mural ridge formation in this specimen was a minimum 12 and maximum of 18 d. In specimen 79-61 a mural ridge is first visible on a radiograph taken at day 14 and is still visible at day 28, indicating the existence of a minimum of about 14 d. In specimen 79-34 a mural ridge was present at first capture and was still present 12 d later. From these observations we conclude that the mural ridge is secreted in a minimum of 14 d and that the succeeding stage of septal secretion follows immediately after completion of the mural ridge. We estimate that septal secretion itself may begin from two to three weeks after initiation of mural ridge formation.

b. *Septal secretion.* Septal secretion occurs in two phases: secretion before and after the initiation of chamber emptying. Pre-emptying septal secretion can be observed in specimens 79-

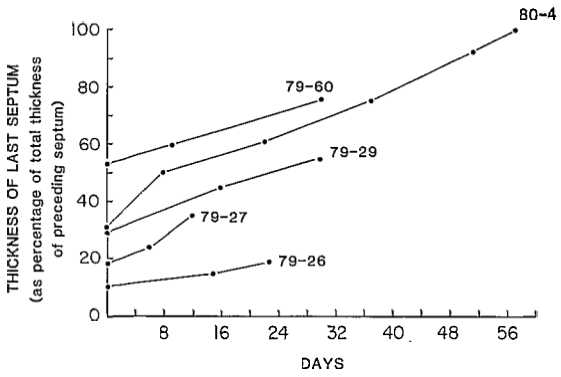

FIGURE 8. Septal thickening in 5 specimens of *N. macromphalus*.

22 and 79-27, which both showed this stage in the 18 d they were observed and in 79-26, which remained in the pre-emptying septal calcification stage for the entire 29 d of its observation. In 79-33, radiographs showing mural ridge formation and coupled emptying were taken 34 d apart; the duration of pre-emptying septal secretion is therefore less than this. From these observations we conclude that the pre-emptying septal secretion stage lasts between 18 and 34 days.

c. *Coupled cameral liquid emptying rates.* The rate of cameral liquid removal from the radiographically observed animals can be derived by comparing the relative chamber contents at the start and end of the observational period. As can be seen from Fig. 6, emptying rates varied widely. The most rapid chamber emptying was seen in 79-44, which started the observation series with a chamber 90% filled and ended 30 d later with a volume of 50%, a reduction rate of 1.3% of the total cameral liquid volume per day. At this rate the removal of half of the chamber volume, the minimum amount of cameral liquid necessary to bring about cameral liquid decoupling, could occur in 38 d. The mean emptying rate for nine specimens was 0.75% of total volume per day, leading to 50% emptying in 67 d. The mean rate for four animals with decoupled chambers was found to be nearly identical: 0.73% per day. Combining these rates, we arrive at an estimate of complete cameral liquid removal (coupled and decoupled) in approximately 135 d for animals at the surface. It is perhaps worth noting that observed emptying rates at depths have always been lower than emptying rates observed in surface animals (Ward and Martin 1978).

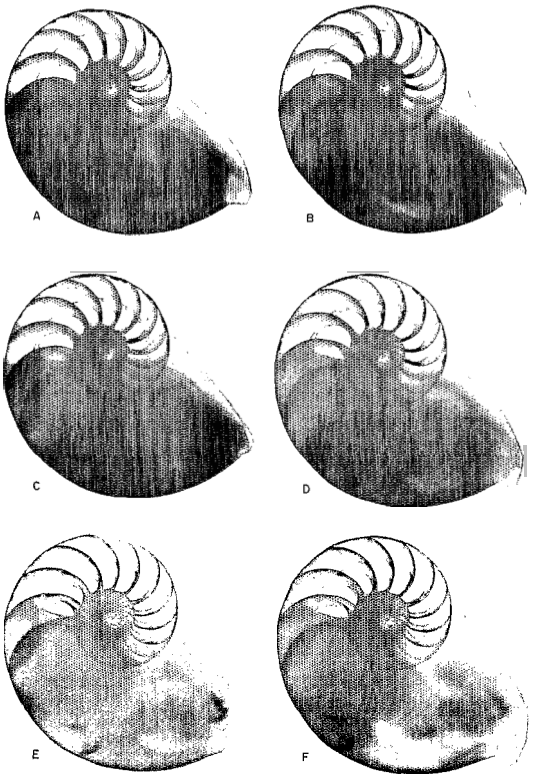

FIGURE 9. Coupled emptying in specimen 80-4. In radiograph A, coupled emptying has just commenced. The enlarging bubble in the ensuing radiographs takes the place of emptied cameral liquid. Note that the new septum continues to thicken during emptying. All previous researchers have maintained that emptying occurs only *after* the septum reaches its ultimate thickness. Note elongation of body chamber, due to apertural shell secretion, in sequence of radiographs. Radiograph A = day 0; radiograph B = day 8; C = day 22; D = 37; E = day 51; F = day 57.

d. *Septal calcification rates.* The absolute rates of relative septal thickening can be derived by plotting relative septal thickness against days of the observation period, as shown in Fig. 8. We have plotted increases in relative septal thickness of five animals observed radiographically over periods between 12 and 57 d. If the data of specimen 80-4 are averaged back through time at the same slope, it allows an estimate of 80 to 100 d for complete septal secretion in this specimen.

e. *Rates of apertural growth.* Like cameral liquid removal, rates of apertural shell growth during the observational period were highly

variable. The maximal rate of shell growth was observed in specimen 80-4 (Fig. 9), which showed 10 mm of apertural shell growth in 57 d, or .175 mm per day. At the diameter of specimen 80-4, with 28 chambers, the spacing between chambers is slightly more than 20 mm. With the apertural shell growth rate shown, this specimen would have needed slightly less than 120 d to grow sufficient apertural shell to make room for the new chamber space at the rear of the body chamber. In other specimens apertural shell rates were much slower, showing a mean rate of .09 mm/d. Two specimens were maintained without radiography in the same tanks at 16° for periods of 105 and 135 d, showing, respectively, rates of .150 and .152 mm/d. In contrast, *N. macromphalus* kept under identical conditions, but at 22–25°C, showed a mean growth rate of 0.25 mm/d (Martin et al. 1978). At this latter rate 20 mm of apertural shell growth would take 80 d. Finally, Kanie et al. (1979) observed a maximum growth rate in *N. macromphalus* of 0.14 mm/d (temp. 14–19°C).

By averaging minimum estimates for durations of the following stages we can arrive at a minimum estimate for the length of time needed between successive septal secretion events in *N. macromphalus* of the size range we have studied. If we take minimum estimates for mural ridge formation (14 d), pre-emptying calcification (18 d), and coupled cameral liquid emptying (38 d), we arrive at a figure of 70 d between any two identical stages of the chamber formation process. This compares with minimum estimates of septal calcification to full thickness (80 d) and apertural shell growth (arbitrarily chosen as 20 mm) of 120 d for our fastest growing specimens, and 80 d for growth rates of .25 mm/d, the mean growth rate reported for this species by Martin et al. (1978). All of these estimates are for growth under aquarium conditions. As has been demonstrated by Martin et al. (1978), apertural shell growth at surface conditions results in abnormal shell appearance. Our feeling is that apertural growth in aquaria may be slowed relative to that in nature, in part due to the constant breakage of the delicate apertural shell against the sides of the aquaria.

6.) *The relationship between chamber liquid volume and liquid osmolality.*—Denton and Gilpin-Brown (1966) first noted that cameral liquid emptying occurs after marked reduction of cameral liquid osmolality relative to blood. They pointed out the possibility of an osmotic model for cameral liquid removal, in which salt ions are removed by the siphuncular epithelium, making the cameral liquid hypotonic to blood within the siphuncle. In this view, the cameral liquid then diffuses across the siphuncular tube into the lumen, thus emptying the chamber. A consequence of this model is that it could not account for emptying at depths greater than about 250 m, the depth at which hydrostatic pressure balances osmotic pressure created between the blood and cameral liquid. This led Wells (1980) to suggest that *all chambered cephalopods have been confined to depths less than 250 m.* Since *Nautilus* with partially or completely emptied chambers are commonly caught at depths far in excess of 250 m (Ward et al. 1977; Ward and Martin 1980; and this study), the "simple osmosis" model seems unlikely for deep animals. Denton and Gilpin-Brown (1973) referred to a "local osmosis" model, after the discoveries of Diamond and Bossert (1967), that could account for deeper emptying. Greenwald et al. (1980) recently showed ultrastructural siphuncular architecture consistent with the local osmosis model and demonstrated experimentally that emptying deeper than 250 m was likely to occur. In either case (i.e. local or simple osmosis) emptying would require less osmotic work if the concentration of the cameral liquid were reduced before or during emptying.

Ward (1979) showed that osmolality values in emptying chambers show a regular relationship, starting with high osmolalites (equal to seawater) that descend to minima (coinciding with the coupling-decoupling transition point) and then increase until final emptying is achieved. The two data sets presented, however, were very different at the low volume sides of the curve. In specimens of *N. macromphalus* from less than 100 m, the low volume osmolalities rose to, or near, their original seawater values. In specimens of *N. pompilius* from Fiji, however, the low volume samples rose only to about 30 to 40% of their original (seawater) value. This difference was attributed to the very different depth of capture of the two populations (less than 100 m for *N. macromphalus,* greater than 200 m for *N. pompilius*).

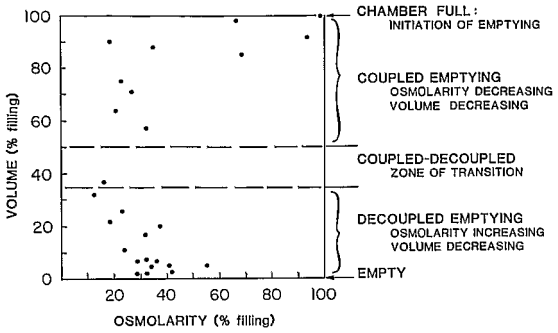

FIGURE 10. Chamber volume (100 = filled with liquid, 0 = emptied of liquid) plotted against cameral liquid osmolality, where 100 = osmolality of seawater, 0 = fresh water.

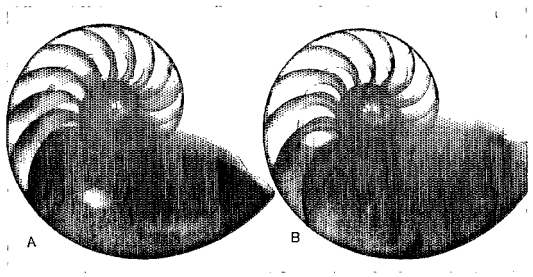

FIGURE 11. Radiographs of specimens 79-4 and 79-5 immediately after capture from 450 m. These two specimens represent the smallest *N. macromphalus* captured alive to our knowledge. Note the strong approximation of the final two septa in the specimen on the left. The thinness of the latest formed septum and filled chamber behind this septum indicates that the specimen on the left is in the stage of pre-emptying septal secretion. The volume of liquid in the chamber immediately behind the last-formed chamber is anomalously low.

We have produced a similar graph for our sample of *N. macromphalus* captured from 300 to 400 m (Fig. 10). The graph is very similar to the distribution of points for *N. pompilius* from similar depths (Ward 1979, fig. 3). This appears to confirm the suggestion by Ward (1979) that cameral liquid osmolality in decoupled chambers reflects overall depth of habitat before capture.

One further observation from our data points is worth reporting. We have observed that depression of osmolality precedes initiation of emptying. This indicates that some period of ionic removal from the entire volume of cameral liquid precedes the actual emptying of the cameral liquid (Greenwald and Ward, submitted).

Discussion

1.) *The control of chamber formation.*—The chamber formation process involves the following systems: 1) apertural shell growth, to enlarge the body chamber, 2) forward movement and growth of the body within the shell, 3) septal secretion, and 4) removal of cameral liquid, which itself can be divided into coupled and decoupled emptying. We would like to know if these systems are independent, or if they involve some degree of interdependence, so that rate changes or perturbations in one system can be compensated for by changes in some other system. Secondly, we would like to know if physical attributes of these systems, such as body chamber length, attitude of the aperture in the water, or cameral liquid level or osmolality serve as signals or triggers for new chamber formation.

We can offer our intuitive feeling and some slight evidence about these larger questions.

First, we know that *Nautilus* will change the rate of cameral liquid removal in response to insufficient or excess buoyancy (Greenwald et al. 1980; Ward et al. 1980). Secondly, we now know from recent research that the removal of shell aperture, thereby greatly increasing buoyancy, will produce partial refilling of previously emptied chambers (Ward and Greenwald, unpubl.). These two observations suggest that the cameral liquid system allows great flexibility in the overall buoyancy equation. We suspect that a slowdown or stoppage of apertural shell growth results in appropriate changes in the liquid removal system.

Can similar sorts of buoyancy perturbations result in changes in the other buoyancy systems? For example, what would happen if an immature *Nautilus* had a large portion of the body chamber broken off at the aperture, or if the removal of cameral liquid in the most recent chamber were stopped for some reason? Would new chamber formation proceed as scheduled? There are certainly examples of irregularity in septal spacing in our observed population, showing that the incrementation of septation is susceptible to changes (Fig. 11). We would like to know if the spacing of chambers provides a

further means of buoyancy flexibility. We suspect this is the case.

Finally, what controls the actual timing of chamber formation? We strongly suspect that either body chamber length, or liquid level in the last-formed chamber, or both, tell the *Nautilus* when to start new chamber formation. We have an observation which may be relevant. In the course of other research, we made two *Nautilus* strongly buoyant by the removal of all cameral liquid from the most recently formed chamber. In both of these animals, the chambers emptied were engaged in coupled emptying. In both cases new chambers were formed approximately two weeks earlier than we would have predicted (based on the average rates of chamber formation in untampered, aquarium maintained specimens). Also, in both cases, the new septa formed were highly approximated. We conclude that in these two cases, the premature removal of cameral liquid resulted in initiation of a new chamber formation cycle.

2.) *The rates of chamber formation.*—The rate at which a *Nautilus* can make new chambers had been widely debated. Denton and Gilpin-Brown (1966) proposed that chambers are secreted every 14 d on the average. Martin et al. (1978) made three estimates. Based on weight fluctuations, they proposed that chambers may be secreted each month leading to full growth in three years. Growth curves based on weight increase gave a different estimate, of full growth in four to five years. If we assume that 30 chambers are secreted at regular intervals, this leads to an estimate of 48 to 60 d per chamber. Finally, based on growth line counts, Martin et al. (1978) estimated full growth to take place in five to six years, resulting in new chamber formation each 60 to 72 d. These latter two estimates approximately agree with our observations.

In spite of these independent estimates suggesting periods greater than one month per chamber, the 30 d estimate is becoming fixed in the literature as the average duration between septa, because of the work of Kahn and Pompea (1979). These workers used the 30 d estimate of Martin et al. (1978) as a basis for an assumption that secretion of septa in *Nautilus* is tied to the lunar month. In spite of criticism (Saunders and Ward 1979), the Kahn-Pompea hypothesis is already entering textbooks as general knowledge (i.e., Dott and Batten 1980). However, most evidence to date, including rates of septal calcification, apertural shell growth, and of cameral liquid removal argues strongly against a septal secretion cycle in one month or less. The resolution of this question will have to await the recapture of tagged, immature *Nautilus* that have undergone known amounts of growth in the natural habitat.

Since the initiation of study of the *Nautilus* buoyancy system, it appears that investigators have been obsessed with rapid rates: rapid rates of apertural growth, rapid rates of septal secretion, and especially rapid buoyancy change. This is due in large part to the longstanding belief (never confirmed by observation) that *Nautilus* undergoes nightly migration (Willey 1902) and to do so it would need the ability to rapidly pump water into and out of chambers (Heptonstall 1970). We hope that the recent documentation of the buoyancies and cameral liquid volumes in *Nautilus,* showing that cameral liquid is of use only to the growing animal (Ward et al. 1977; Ward 1979; Collins et al. 1980; Ward et al. 1980), has finally laid to rest this erroneous assumption. The present evidence on growth and buoyancy control suggest *slow* rates. With slow growth rates and slow removal of cameral liquid, there is a much better chance of avoiding dangerous fluctuation or imbalance in buoyancy, and indeed such fluctuations are not seen in nature (Ward and Martin 1978). Our view of *Nautilus* is of a slowly growing, slowly foraging, long-lived organism of the deep nektobenthos, rather than an active, deep to shallow water, rapidly growing submarine.

Acknowledgments

This work was supported by NSF grant PCM 7823624 to P. W. and L. G. We thank Dr. P. de Boissezon, Director of the O.R.S.T.O.M. Laboratory in Noumea where this work was done and M. and Mme. Auguste Soulard, Noumea, for logistical assistance.

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