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Biological response to experimental damage of the phragmocone and siphuncle in *Nautilus pompilius* Linnaeus

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Three adult specimens of *Nautilus pomplilius* Linnaeus from the Philippines were experimented on to estimate the biological response to damage of the phragmocone and siphuncle in this cephalopod mollusc. In addition, the data obtained from the experiments were used for discussion of shell damage in ammonoids and in other extinct cephalopods. Specimen's phragmocone and siphuncle were perforated and severed artificially, followed by observations in the laboratory tank during periods of 75 and 132 days. For at least 2 or 3 months, all individuals survived after damage to the phragmocone and siphuncle despite loss of neutral buoyancy. Based on our observations after completion of the experiments, the severed adoral remaining part of siphuncle healed by the siphunclar epithelium. In addition, perforation of the phragmocone was partly repaired by shell secretion from the dorsally extending mantle due to subsequent volution of shell growth. Our experiments revealed that damage to the phragmocone and siphuncle in *Nautilus* was not necessarily a lethal injury. It may be possible that such biological response also applies to extinct ammonoids and nautiloids. In a similar case of extinct ammonoids and nautiloids, damage to their phragmocone and siphuncle may also not have been a lethal injury as with *Nautilus*. However, some factors leading to death are likely to be dependent on the degree of damage to the phragmocone and siphuncle and influence of hydraulic pressure. ☐ *Ammonoids, injury, nautiloids,* Nautilus, *phragmocone, repair, siphuncle.*

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Recent *Nautilus* maintains neutral buoyancy by removing the cameral liquid from the phragmocone via the siphuncle (Denton & Gilpin-Brown 1966; Greenwald & Ward 1987; Ward 1987). The siphuncle is a strand of tissue composed of an inner blood circulatory region and outer epithelium (Willey 1902; Stenzel 1964; Fukuda *et al.* 1981; Ward 1987; Tanabe *et al.* 2000). The siphuncle is housed in the siphunclar tube consisting of layers of calcareous and conchiolin hard tissue, and completely extends from the base of the body chamber to the apex of shell within the phragmocone. Ammonoids of the extinct chambered cephalopods also had phragmocone and siphuncle essentially similar to *Nautilus* (Tanabe *et al.* 2000), although differences in chemical composition of the connecting ring structure between *Nautilus* and ammonoids have been recognized (Mutvei *et al.* 2010). The phragmocone and siphuncle in extinct chambered cephalopods also served an important role in buoyancy control.

Many observed shell injuries in fossil cephalopods were apparently caused by predators (Kauffman & Kesling 1960; Saunders *et al.* 1987; Doguzhaeva 2002; Kauffman 2004; Keupp 2006; Turek & Manda 2010). Apart from this, some other kind of accident may be responsible for shell damage. Owing to such attacks by predators and accidents, it is highly probable that the phragmocone and siphuncle may also be injured. Particularly, the phragmocone and siphuncle of thin-shelled early growth portion are prone to damage (Maeda 1987; Maeda & Seilacher 1996). In normally coiled ammonoids and nautiloids, damage of the inner whorl, including juvenile portions of the shell, is comparatively rare during their life, because successive whorls overlap and the inner whorl is protected. On the other hand, in the case of heteromorph ammonoids and loosely coiled and orthoconic nautiloids, there is a high risk that the juvenile portions of the shell may suffer serious damage, because the successive whorls do not protect it. Many heteromorph ammonids (e.g. *Polyptychoceras* and *Baculites*) from the Cretaceous Yezo Group of Hokkaido, northern Japan suffered from breakages of the phragmocone and siphuncle in juvenile portions of the shell (Okamoto &

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Shibata 1997; Tsujino *et al.* 2003). Although there may be several examples that shells of heteromorph ammonoids show damage after death, it is also possible that individuals survive after damage to the phragmocone and siphuncle.

Hitherto, there has been little research related to the biological response in chambered cephalopods after the phragmocone and siphuncle have been damaged. To estimate the biological response to damage of the phragmocone and siphuncle in *Nautilus*, we performed laboratory experiments with *Nautilus* whose phragmocone and siphuncle were partially perforated and severed artificially. In this article, we describe the biological response of *Nautilus* in detail after damage to the phragmocone and siphuncle. We also discuss the palaeontological significance of extinct chambered cephalopods following our experimental results.

Materials and methods

Three adults (two specimens, 1, 2, ~200 mm shell diameter; one specimen, 3, ~120 mm shell diameter) of *Nautilus pompilius* from the Philippines, which were obtained from an aquatic animal import trader, were used for laboratory experiments. Initially, three specimens of *Nautilus* were kept at a mean water temperature of 18°C in the laboratory tank (120 × 120 × 60 cm) for several months. Then, following our experimental objectives, using an electric drill, we bored a hole measuring 8–10 mm in diameter into the ventral part of the phragmocone directly above the hood, and cut off the siphuncle with scissors (Fig. 1). Specimens were immediately returned to the laboratory tank after completion of the procedure. Subsequently, we observed the progress following perforation of the phragmocone and severance of the siphuncle for 75 days in specimens 1 and 2 and for 132 days in specimen 3.

Fig. 1. Using an electric drill, a perforation of 8–10 mm diameter was bored on ventral part of the phragmocone directly above the hood of *Nautilus pompilius* from Philippines. The siphuncle was cut off with scissors.

For optical and scanning electron microscope observations, tissue of the siphuncle surrounding the severed part in all specimens was carefully removed from the shell in viable condition after 75 and 132 days respectively. They were then fixed with 10% formalin. For preparation of tissue sections, the sectioned piece of the siphuncle in specimen 1 was dehydrated with an ethanol series, cleared with xylene and embedded in paraffin. Thin longitudinal sections were made at intervals of 8μm thickness using a microtome. Selected sections were mounted on glass slides and were stained using the Haematoxylin and Eosin staining method. Next, they were observed by means of an optical microscope (Nikon model UFX-IIA). In specimen 2, the sectioned piece of siphuncle was also embedded in paraffin following the same method described above. The longitudinal median section was prepared with a microtome. It was dewaxed using xylene and freeze-dried by t-butylalcohol, and then observed with SEM (Jeol model JSM-5300). In specimen 3, kept in captivity for 132 days, we observed not only the siphuncle but also the perforated part on the phragmocone.

Terminology concerning the microstructural elements of siphuncle in this article is that used by Greenwald *et al.* (1982) and Tanabe *et al.* (2000).

Results

Three specimens of *Nautilus* experimented upon in the laboratory tank survived after damage to the phragmocone and siphuncle for 75 and 132 days respectively without enervation. Immediately after damage to the phragmocone and siphuncle, they lost neutral buoyancy due to waterlogging of the broken chamber and temporarily sank to the bottom of the tank (Fig. 2). Their ability to maintain neutral buoyancy had suddenly become mitigated, although they could manage to swim actively by expelling seawater out with the hyponome. It was difficult for them to keep afloat in the tank for long periods of time.

Severed part of the siphuncle and the healing

From our observations inside the chambers of all specimens after completion of the experiment, we observed that a small amount of seawater had flowed to the phragmocone at the apical first and second chambers from the perforated chamber (Fig. 2).

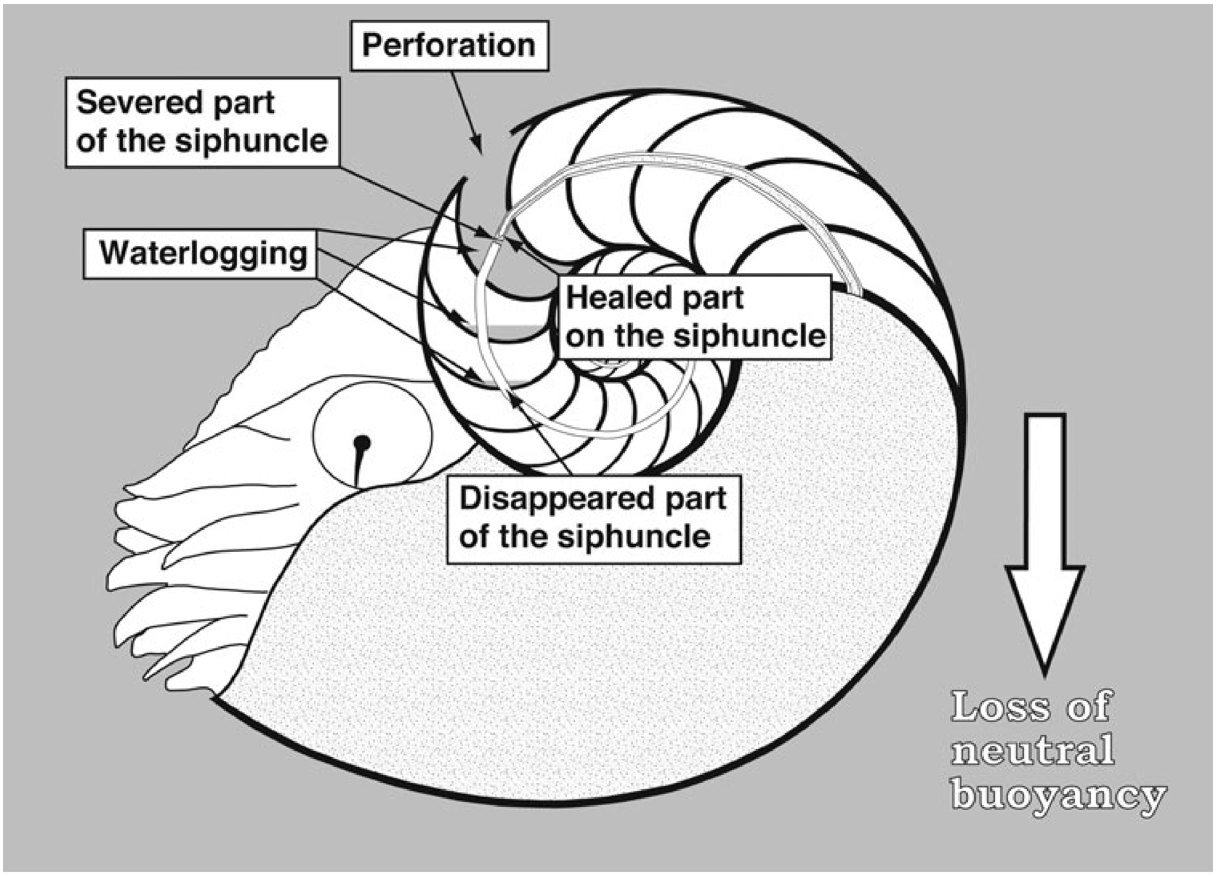

Fig. 2. Schematic illustration of an adult individual of *Nautilus pompilius*, with a perforation on the phragmocone and severed siphuncle.

The siphunclar tube enclosing the siphuncle remained as it had been when it was severed, and regeneration was not observable (Figs 2, 3A). There were some productions such as the mucous membranes between the severed parts of the siphunclar tube (Fig. 3A). The siphuncle served on both apical and adoral sides was also unattached as in the original condition (Figs 2, 3B). The adoral portion of the siphuncle remained within siphunclar tube (Figs 2, 3B). In contrast, the apical portion of the siphuncle from the severed part had already disappeared (Fig. 2). The apical portion of the siphuncle is thought to decompose because of separation from the living body.

We noticed that the severed part of remaining siphuncle healed and was closed up as scar (Fig. 3C–F). The scar on the severed part of the siphuncle in specimen 2 was rounded resembling the top of a pestle (Fig. 3C). Judging from SEM observations of the scar on the siphuncle sectioned medially after being freeze-dried, it was found that the scar was completely covered by the epithelium (Fig. 3D). Based on optical observations of the stained tissue sections of the siphuncle in specimen 1, the severed part of the siphuncle was also regenerated by the siphunclar epithelium, although the severed part had a dimple in the centre unlike the rounded scar as shown in Fig. 3C, D (Fig. 3E, F). A large central vein and an artery were observable as the inner structure of the siphuncle in longitudinal section (Fig. 3E). In comparison with the general area possessing siphunclar epithelium, the scar on the siphuncle was covered by thick siphuncular epithelium on which a large number of nuclei were concentrated (Fig. 3F).

Perforated part on the phragmocone and the regeneration

In specimen 3 of *Nautilus* at 132 days after drilling the phragmocone, we observed both the external and interior portion of the shell wall surrounding the damaged area. The injured area on the ventral part of the phragmocone was mostly covered over and hidden by the hood and dorsally extending mantle (supracephalic mantle fold), and the perforation was undetectable (Fig. 4A). The perforated part on the phragmocone was convoluted a few centimetres towards the apical side by the shell accretionary growing during

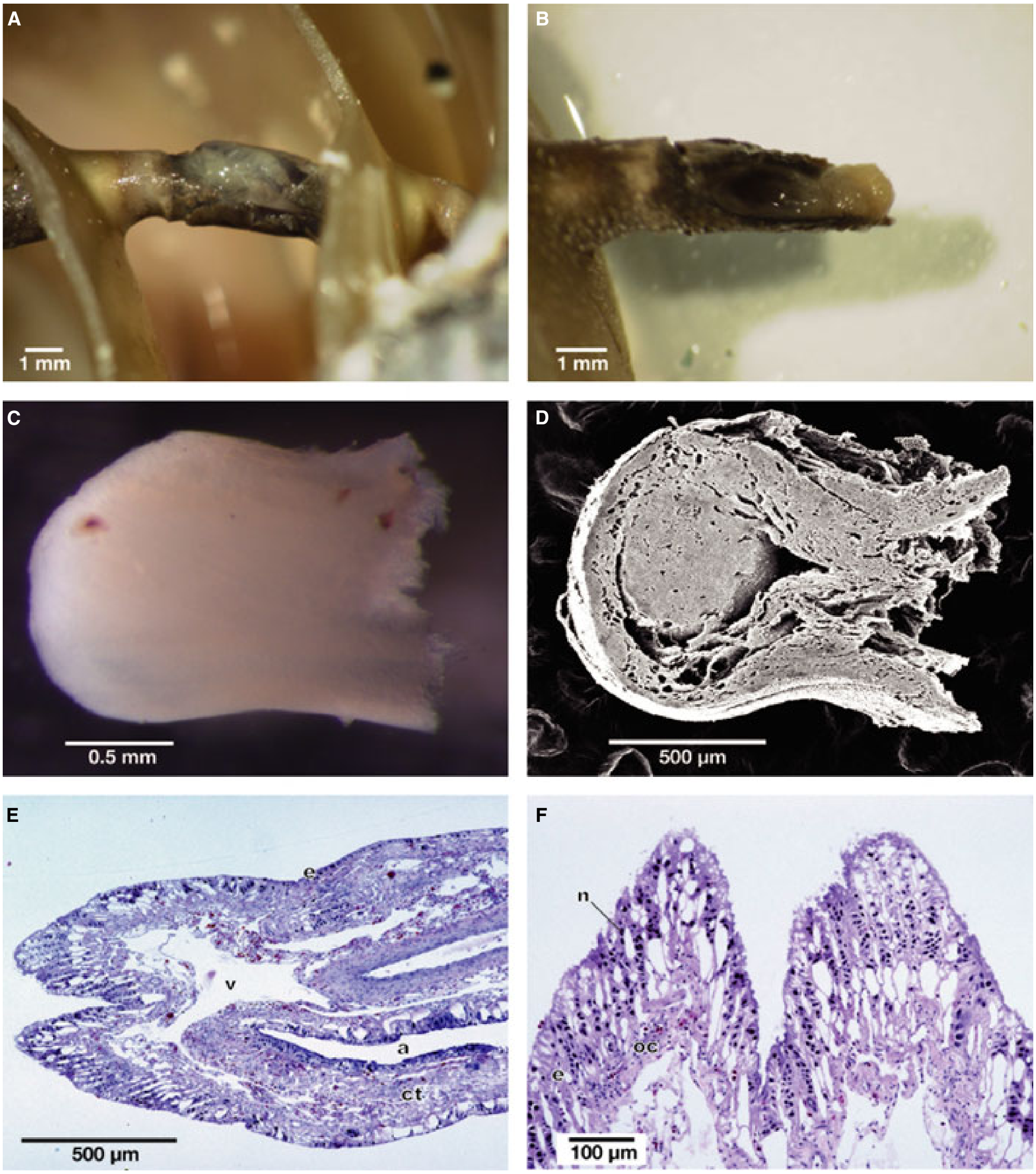

Fig. 3. Photographs of the severed part of siphuncle in *Nautilus pompilius.* A, B, overview of the severed part of siphuncle immediately after completion of the experiment in specimen 3. A, there is no healing of the siphuncular tube. There are some productions such as the mucous membranes between the severed parts of siphuncular tube. B, the siphuncle only remains within the adoral siphuncular tube. The left-side of the photographs reaches to adoral chambers. C, optical micrograph of the severed part of siphuncle in specimen 2. The severed portion of siphuncle on the left-side of the photograph heals with a rounded surface. D, SEM micrograph of the longitudinal section of C. E, optical micrograph of the longitudinal section of the severed part of siphuncle in specimen 1. F, close-up of the severed portion of E. The siphuncle was stained using a Haematoxylin and Eosin staining method. The scar of siphuncle was covered by thick siphuncular epithelium on which a large number of nuclei were concentrated. e, epithelium; a, artery; v, vein; ct, connective tissue; oc, outer connective tissue layer; n, nucleus.

132 days. In normal conditions of specimen 3, however, the adoral edge of the perforated part on the phragmocone was slightly observable above the hood (Fig. 4A). As the hood and supracephalic mantle fold of *Nautilus* are somewhat movable in an anteroposterior direction, the whole perforated part on the phragmocone appears when the living body, including the hood and the supracephalic mantle fold, retracted to the aperture.

We found that the thin shell layer, which had an irregular surface resembling a bowl-shaped depression, expanded to seal up the perforated part on the

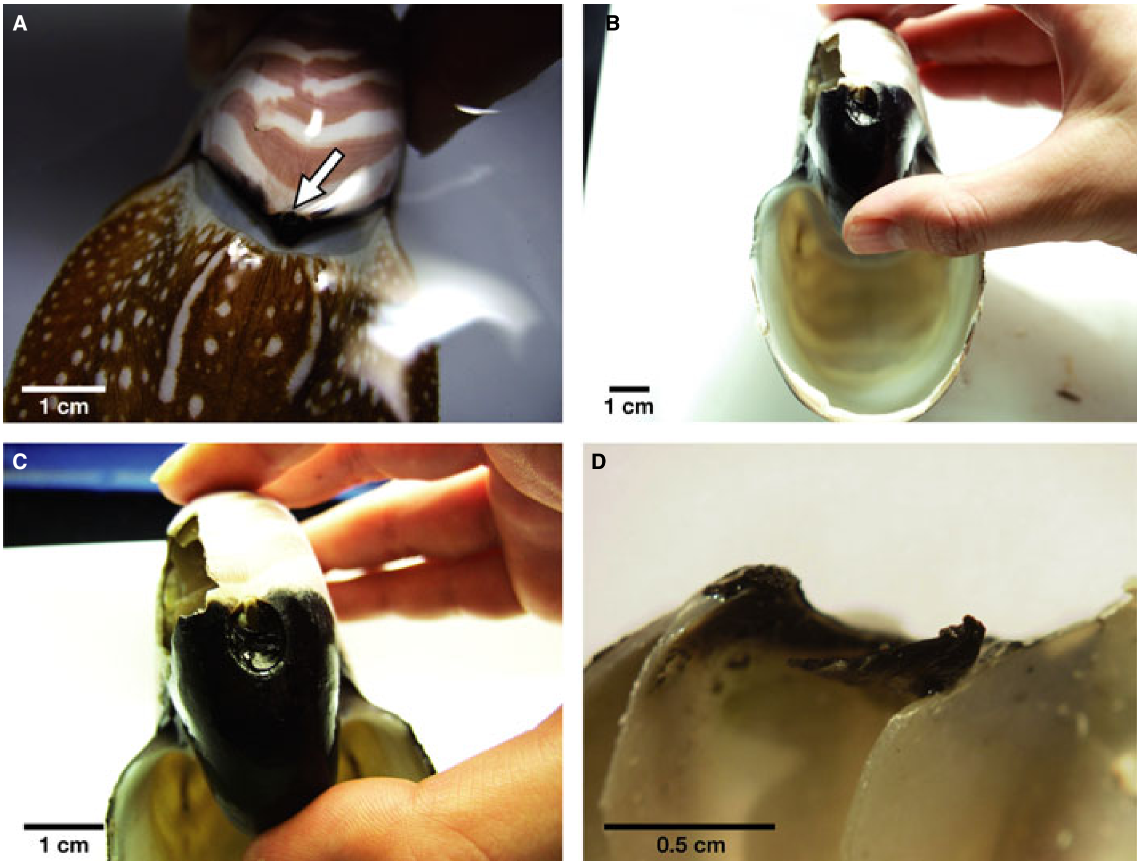

Fig. 4. Photographs of shell repair on the phragmocone in specimen 3 of *Nautilus pompilius*. A, most of the injured area of phragmocone is covered over by the hood and the dorsally extending mantle. The position of perforation is indicated by the arrow. B, overview of shell repair on the phragmocone after removing the soft body. C, close-up of shell repair. D, lateral view of shell repair on the phragmocone. The thin shell layer formed a bowl-shaped depression which was acting to seal on the perforated part of the phragmocone.

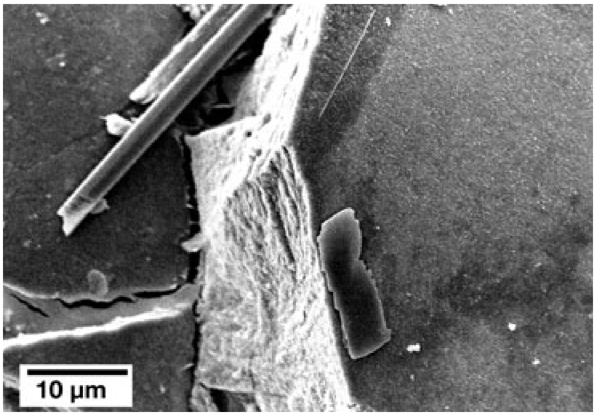

Fig. 5. SEM micrograph of the surface of the shell layer sealing the perforated part of the phragmocone in specimen 3. The shell layer is thought to be composed of organic material.

phragmocone in specimen 3 (Fig. 4B, C). The bowl-shaped thin shell layer sealing the perforated part of the phragmocone is thought to be composed of organic material, because the calcareous shell microstructure such as nacreous and prismatic layers is not observable (Fig. 5). The thin shell layer on both external and interior surface is covered by a thin, blackened coating called 'black film' for the attachment of the supracephalic mantle fold as well as the hood (Fig. 4D). In addition, the black film was present not only on the surface of thin shell layer but also partly on the ventral shell wall and the adoral septum wall in the interior chamber (Fig. 4D). Shells of *Nautilus* have the black film as soft-tissue attachment structure on the dorsum in front of the aperture (Ward 1987). Attachment of black film on the shell around the injured area of the phragmocone is evidence that the repaired thin shell layer was made by the supracephalic mantle fold.

The perforated part of the phragomocone is not completely sealed up by the thin shell layer. Apical edge of the thin shell layer is still unattached to the ventral shell wall (Fig. 4D), and a small elliptical perforation (3.3 mm in major axis and 1.4 mm in minor axis) is observed on the upper-centre part of the thin shell layer (Fig. 4B, C). Even though the external surface of the thin shell layer is smooth, on the interior surface a lot of round-arched lirae such as growth lines are visible (Fig. 4D).

Discussion

Palaeontological significance of the experimental data

Throughout the experiments, *Nautilus* showed healing of the severed part of remaining siphuncle by the epithelium, although neutral buoyancy was significantly affected. Furthermore, we revealed that the perforation on the phragmocone located directly above the head of *Nautilus* had been partly repaired by shell secretion from the dorsally extending mantle (supracephalic mantle fold) due to volute under the hood by shell accretionary growth. These results indicate that damage to the siphuncle and phragmocone in *Nautilus* as with our experimental case is not necessarily a fatal injury.

It may be possible that such biological response also applies to extinct ammonoids and nautiloids having similar phragmocone and siphuncle to *Nautilus*. Unfortunately, discovery of a healed scar on fossilized remains of siphuncular soft-tissues in fossil cephalopods still is not known. On the other hand, an example has been noted with a repaired perforation of the phragmocone in fossil cephalopods (Kröger & Keupp 2004).

Kröger & Keupp (2004) were first to report that the nautiloid *Trocholites depress* (Eichwald) from the Middle Ordovician Lasnamägi Limestones of North Estonia had a healed scar on the phragmocone. The shell breakage on the phragmocone was sealed by a complex sequence of calcitic and ?conchiolinic layers. The healed scar is located on the ventral part of phragmocone within the body chamber at the rear, and the siphuncle in the perforated chambers remains completely intact. They assumed that the perforation on the phragmocone had been located on the venter of the shell directly above the peristomal opening of the body chamber. They also reported that the perforation had been promptly repaired by the posterior mantle during the following volution of growth of the phragmocone (Kröger & Keupp 2004). Shell repair sealed by a complex sequence of calcitic and ?conchiolinic layers results in a concave depression on the surface of the phragmocone (Kröger & Keupp 2004). Such shape of shell repair on the phragmocone in *T. depress* is similar to that of *Nautilus* described in this article. Furthermore, the innermost layer which initially sealed the shell breakage on the phragmocone of *T. depress* was a sheet enriched with conchiolin (Kröger & Keupp 2004). This is concurrent with the experimental data that the perforation of *Nautilus* healed by organic layer with black film.

Our study using animal experiments succeeded to roughly recreate comparable phenomenon which was likely to have taken place to *T. depress* under the Middle Ordovician sea. Kröger & Keupp (2004) showed that the Middle Ordovician nautiloid *T. depress* survived after major damage to the phragmocone, and repaired the perforation on the phragmocone under natural conditions of marine environment. They also describe that the requirements for survival and repair in *T. depress* were dependent on the position of the damage on the head of the animal and lack of damage to the siphuncle. According to our experiments, however, it was found that lack of damage to the siphuncle may not be important. The experimental results suggest that biological response after shell breakage on the phragmocone do not differ between extinct and extant nautiloids.

Waterlogging of the chambers and additional factors

In all experimental individuals of *Nautilus*, waterlogging was not recognized in adoral chambers from the perforated chamber (Fig. 2). On the other hand, a small amount of seawater entered the apical first and second chambers from the perforated chamber (Fig. 2). The porous siphunclar tube has natural permeability (Maeda 1999). Seawater is presumed to penetrate into the underpressurized apical chambers via the siphunclar tube because the cameral gas pressure within the phragmocone of *Nautilus* is somewhat lower than 1 atm (0.6–0.8 atm: Denton & Gilpin-Brown 1966; 0.8–0.9 atm: Ward 1987). As a result, such a difference between adoral and apical chambers may indicate that due to the siphuncle remaining, this prevented waterlogging to adoral chambers by using some mechanism.

This study carried out animal experiments inside a laboratory tank under an ambient hydrostatic pressure of approximately 1 atm near water surface. Therefore, the hydraulic environment within the laboratory tank is not the same as that of native habitat of *Nautilus*, which usually lives at a depth of between 150 and 300 m, which is its optimal habitat (Saunders & Ward 1987). Most ammonoids are also assumed to live in natural surroundings with a higher hydrostatic pressure of more than 1 atm (Moriya *et al.* 2003). If *Nautilus* and ammonoids suffer damage to the phragmocone and siphuncle in their natural habitat of high hydrostatic pressure, a large amount of seawater might flow into apical chambers as well as adoral chambers.

In future research, we aim to address the issue to clarify biological response to damage of the phragmocone and siphuncle in chambered cephalopods under high hydrostatic pressure similar to that of their natural habitat.

Conclusions

Our experiments revealed the following:

1. Damage to the phragmocone and siphuncle in *Nautilus* is not necessarily a lethal injury, although neutral buoyancy is significantly affected by shell breakage and waterlogging to the phragmocone;
2. It may be possible that a similar biological response also applies to extinct ammonoids and nautiloids having similar phragmocone and siphuncle to *Nautilus*; and,
3. The perforation on the phragmocone located directly above the head of *Nautilus* is partly healed by shell secretion from the dorsally extending mantle during subsequent volution of growth of the phragmocone. Here, characteristics of shell repair on the phragmocone in *Nautilus* closely resemble those of the Ordovician nautiloid *Trocholites depress* reported by Kröger & Keupp (2004). We assume that biological response after shell breakage on the phragmocone did not differ between extinct and extant nautiloids.

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