Stealth Effect of Red Shell Coloration in Laqueus rubellus (Brachiopoda, Terebratulida) on at the Sea Bottom: An Evolutionary Insight into the Prey-Predator-Prey Interactions

Abstract

Predator-prey interactions among organisms that have flourished over time—like brachiopods—are important for studying evolutionary arms races. We examined the selective advantage of red coloration in the shell of Laqueus rubellus (a terebratulid brachiopod) was checked in terms of interactions of prey and predator evasion. The study was based on comparison of benthic suspension feeders found at about 130 m depth in Suruga Bay, Japan, with peculiar reference to focusing on their visibility under visible and near-infrared light conditions. In visible light, almost all species exhibited red coloration under visible light, and resembled rocks and bioclasts; while in infrared light, only the shell of Laqueus rubellus showed this stealth effect dark under infrared light, similar to rocks and bioclasts. Provided the functional eyes of some macropredators such as fishes and coleoids, which are specialized for detecting light in the blue-to-green region of the visible spectrum; and some have even the long-wavelength photoreceptors of malacostracans. The unique coloration of Laqueus rubellus confers an ability to evade both visible and infrared detection by these predator types living at the bottom of the sublittoral bottom zone under both visible and infrared light. This fact suggests that terebratulids have evolved ability to remain more or less essentially invisible with even as the improvements of optic-visual detection abilities of predators have improved.
1. Introduction

Competitive framework exists in for resources and survival is characteristic in of the natural settings of most organisms, and this reciprocal interaction is the driving force of arms races in evolution [1]. Predator-prey interactions of predator and prey are interesting for of interest in the research on evolutionary arms races because the corresponding adaptations of prey and predators demonstrate how organisms survive to enhance and/or modify their behavioral and functional performances within a biotic community for survival [2]. If either the predator or the prey can’t adapt to relevant changes in the other, extinction may occur.

Benthic suspension feeders, such as bivalves, brachiopods, and some echinoderms, are of special interest in such research because they have survived exposure to predation by macropredators throughout the Phanerozoic. They have developed several strategies to ward off potential predators. For example, some bivalves exhibit thickened valves that physically prevent them against predator attacks physically [3–5], while others exhibit magnified burrowing or swimming ability [6–8]. Crinoids and ophiuroids have evolved the ability to autotomize and regenerate their tentacles when they are bitten off by predators [9–11].

On the contrary, rhynchonelliformean brachiopods represent immobile, sessile organisms with thin shells [12, 13]—in which neither do not appear to have evolved physical, physiological, nor behavioral defenses have not evolved against predators and yet have flourished. Of the rhynchonelliformean brachiopods, Terebratulidae are known to be the most successful group among these organisms, having lived from the Devonian to the modern era.
They possess semi-circular valves and a pedicle for attachment to a hard substratum. As
against the simple look of other rhynchonelliformean brachiopods that have a dull
appearance, the shells of many living terebratulids have shells exhibit with distinctive coloration (pink, orange, red-, and red-brown pigments). It has been taken for granted that the
Such characteristic shell colors of living terebratulids have been believed to may exhibit have some a predator-deterrent effect [14, 15], but antipredator function of colors although no study has clarified how these colors serve this function has not been explained.

In our previous experiments in our laboratory [16], we have observed that the terebratulid
brachiopod *Laqueus rubellus*, which is empire red in color, is difficult to be seen by spot
using a video-scope under near-infrared illumination. This intriguing observation motivated
us to examine if this unique coloration contributed to the success of this animal’s survival at the bottom of the sublittoral zone. Based on subsequent observations using visible and infrared light, we describe Therefore, we studied the optical properties of the shell of this species *L. rubellus* under visible and infrared light and its ecological significance in order to explain why terebratulid brachiopods thrive on the sublittoral sea bottom.

2. Materials and methods

2.1 Sample Sampling location

Benthic organisms, including *L. rubellus*, were collected with using a dredge (width 90 cm) at a depth of 130–140 m off Osezaki in the Suruga Bay (Figure 1). Our sampling site was located on the outermost shelf bottom and contained mud and fine-grained sand with abundant debris, such as rounded gravel and bioclasts. The environmental conditions (e.g., water temperature, dissolved oxygen, pH, and the concentrations of chlorophyll a, dissolved oxygen, and nutrients concentrations) at the bottom of inner Suruga Bay are same-stable over...
a wide area, but *L. rubellus* is abound[flourishes] only around the sublittoral shelf edge [16, 17].

2.2. Materials

Figure 2 shows the A number of living benthic macroorganisms were obtained in the recovered dredge sample (Figure 2). Among the suspension feeders, *L. rubellus*, the stalked crinoid *Metacrinus rotundus*, and ophiuroids were the dominant species. In contrast to the free-living *Metacrinus rotundus* and ophiuroids, all living *L. rubellus* individuals were attached to bioclasts or rock debris using their attachment organ, the pedicle. Our samples had low numbers of two species of bivalves species,—*Cryptopecten vesiculosus* and *Nemocardium samarangae*,—and scleractinian corals occurred only in low numbers in our samples.

2.3. Observation Methods

We aimed to examine the differences in the visibility of among the recovered benthic organisms, so they were photographed in visible and infrared light while they were resting in a white seawater tray containing seawater. For photographs under visible light conditions, we used a digital camera (D70, Nikon) and an incandescent lighting system (PRF-500WB, National). To visualize photographs in infrared illumination, we the organisms were filmed with a video-scope (DCR-TRV20, SONY) under near-infrared light of around 800 nm wavelength of around 800 nm (DCR-TRV20, SONY), and the infrared images were captured as video frames. Hereafter, the results visibilities recorded from using these two methods are have been referred to as the natural and infrared visibilities, respectively.
2.4. Quantitative Analysis of Grayscale Images

For the quantitative examination of visibility as recorded in infrared images, we obtained grayscale histograms using the ImageJ software program. The image of each animal was taken with a distance of 1 metre between the animal and the video scope. Animal outlines in the grayscale images were drawn using the polygon-selection tool in ImageJ, and then the area inside the outline was analyzed to obtain a 256-shades-of-grayscale histogram.

3. Results

3.1 Natural Visibility (under Visible Light)

Figures 3(a), 3(b), and 3(e) show photographs under visible light conditions. All organisms observed under visible light conditions were red-colored (Figures 3(a) and 3(b)), except the crinoid _M.etacrinus rotundus_ (Figure 3(e)), which was white to ivory in color. _L.aqueus rubellus_ had a thin shell that was colored orange to empire red and is transparent enough to see the organism inside (Figures 3(a) and 3(b)). The color of larger shells tended to be darker in color. The shells of _Cryptopecten vesiculosus_ and _N.emocardium samarangae_ are ornamented with a mosaics of red-and-white color. The coloration patterns of coloration exhibit interspecific variation (Figure 3(a), Figure 3(b)). The shells of _Cryptopecten-C. vesiculosus_ is has a patchy color by wine-red pigment in a patchy fashion, while that of _N.emocardium samarangae_ is ornamented with has several radial orange bands. The _Scleractinian coral_ has have reddish soft parts within a white skeleton (Figure 3(a)). The upper sides of all ophiuroids show are red to reddish-brown colors, while the lower sides of their bodies are whitish (Figures 3(a) and 3(b)).

Comment [A17]: The results sections should have sentences stating what you found rather than what a figure contains.

Comment [A18]: You have reported not only the color of all these organisms but also their patterns in some detail. However, the discussion contains no comments on if or why these interspecific variations in pattern are significant. Please review if the mention of patterns is important. If yes, ensure that the discussion comments on this. If not, restrict your results to just the color.
3.2. Infrared Visibility (Under Near-Infrared Light)

Figures 3(c), 3(d), and 3(f) show photographs under infrared visibility, which are compared with Figures 3(a), 3(b), and 3(e), respectively. Unlike natural visibility, infrared images revealed a difference in color intensity among taxa. As was apparent from the infrared images, the shells of *L. aqueus rubellus* were the darkest and resembled those of attached bioclasts and rock fragments (Figures 3(c) and 3(d)). Meanwhile, in contrast, the shells of ophiuroids and the crinoid *Metacrinus rotundus* were the brightest, contrasting sharply with the coloration of *L. aqueus* (Figure 3(c); black arrowhead). Molluscan shells were gray in color but somewhat faint compared to *L. aqueus rubellus*. Sediment particles that were trapped in pectinid ribs were dark gray, as were resembling bioclasts and rock fragments (Figures 3(c) and 3(d); white arrowhead).

3.3. Grayscale Image Analysis

Figure 4 shows 256 shades of grayscale histogram for selected individuals. The counts of each grayscale plot among the individuals are significantly different (Figure 4; *P* < 0.001, pairwise ANOVA). The mean values in the case of *L. aqueus rubellus* were around 40, which were the lowest (darkest) among the animals. The mean values observed for bivalves, ophiuroids, and scleractinian corals exhibit similar mean values, the range of which were around 51–62, 52–77, and 58, respectively, but those of bivalves were slightly lower than those observed for the other two groups. The histograms in the case of *Metacrinus* show a gentle convex shape, with the peak occurring at around 90 for Metacrinus 1 and around 160 in for Metacrinus 2. The black arrowhead points to the peak of the histogram.

Comment [A19]: Once again, avoid writing sentences that only discuss what a figure shows.

Comment [A20]: Since you've reported this difference, I would advise you to include a comment in the discussion section on why this contrast is significant, especially in terms of ability to evade predators. Are ophiuroids and *M. rotundus* less successful in evading certain predators at the sea bottom than *L. rubellus*?

Comment [A21]: When you say "faint," do you mean that their color intensity was lower? If yes, please revise this sentence as follows: "Molluscan shells were gray but had lower color intensity than that observed for *L. rubellus.*"

Comment [A22]: This result too has no mention in the discussion.

Comment [A23]: This statement is slightly unclear. Could you specify which individuals you are referring to and what you mean by counts of plots?

Comment [A24]: The mean of which values are you referring to? Color intensity?

Comment [A25]: Once again, the discussion should clearly explain whether this is significant.

Comment [A26]: This is slightly unclear. Do you mean two individuals belonging to this species? If so, you may revise this as "two *M. rotundus* individuals," but it is rather unclear why you're referring to individual organisms specifically when you have not done so for any other species sampled. Do you need to justify this?

Comment [A27]: You have not used these designations (*Metacrinus 1* and *Metacrinus 2*) before. I've revised this sentence to avoid confusing readers. Please confirm that this is indeed what you meant.
4.1. Optical Evasion from Macropredators

Not being Remaining undetected by predators is an efficient survival strategy of decreasing the mortality rate of among sessile benthic organisms since they cannot employ escape strategies that mobile organisms do. Several have been believed to achieve a stealth effect through specific coloration [14, 15]. All the benthic organisms sampled in our study, except *M. rotundus*, had reddish coloration. The reddish coloration of the benthic organisms studied here may help them not be detected avoid detection by macropredators. This can be explained by the optical properties of visible light.

The reddish appearance of an object appears red means that if the red portion of the visible spectrum is reflected by its surface, while other wavelengths of visible light are absorbed. Red light has the longest wavelengths in the visible spectrum, and is the lowest energy is lower [18]. Such low-energy light is preferentially diffused under water, because of which resulting in a loss of the red optical element component of visible light is lost at the bottom of the sublittoral zone [18, 19]. Benthic organisms that appear reddish under visible light conditions therefore will therefore appear black in color at the bottom of the sublittoral zone. *L. aquaeus rubellus* and organisms associated with it on the outer shelf of Suruga Bay should must appear dark in color in their natural habitat, making it possible for them to remain undetected by the eyes of macropredators such as fish and squid [20–24].
When these organisms were studied under infrared light, they were found to vary in color intensity, with *L. rubellus* showing the darkest color. Unlike the natural visibility of benthic organisms, their contrasting infrared visibility suggests the possibility of another survival strategy against predators. Almost all deep-sea fishes have eyes that are sensitive to light in the blue-to-green visible spectrum because these wavelengths can penetrate deeply into the ocean [24]. Malacosteids, however, have retinal pigments that are particularly sensitive to red light, and these fishes have been compared to snipers armed with infrared “snooperscopes” at night [25, 26]. One such predator, the malacosteid *Photostomias guernei*, is reportedly has been reported to be present in the seas around Japan, as well as in Suruga Bay [27, 28]. However, it is unlikely that *Laqueus rubellus* is likely to remain undetected affected by even by deep-sea fishes with the long-wavelength sensitivity of deep-sea fishes, as it shows the similarly dark appearance because it resembles dark rocks and skeletal fragments. The appearance of *Laqueus rubellus* shells under infrared light suggests that *Laqueus* has evolved a survival strategy in which its shell behaves optically like a nonliving object on at the bottom of the sublittoral zone.

4.2. One Likely Possibility for the Evolutionary Arms Race between Sessile Benthic Organisms and Macropredators

The camouflage strategy of *Laqueus rubellus* to the detection abilities of macropredators Our findings suggest the presence of an intimate and evolutionary interplay or arms race between *L. rubellus* and its predators, which in turn suggests. This leads to several evolutionary scenarios, as discussed below.

*Laqueus rubellus* and the vision systems of its predators may have experienced selective pressure—the former for developing optical evasion ability and the latter for developing detection ability of the photoreceptor ability to detect long-wavelength light, respectively.
Each enhancement of in one group of organisms one exerts selection pressure for developing a compensating enhancement of in the other. This is a form of coevolution [1, 29]. In addition to this predator-prey interaction, brachiopod survival on the sea bottom is also affected by competition among benthic organisms, which belong to a similar guild [30–32]. Consequently, several species of the benthic community are involved, and their abundances are not independent. This corresponds to the concept of “diffuse (or guild) coevolution” [1].

In the modern sea, highly efficient vision systems are evident in teleost fishes and coleoid cephalopods, both of which originated in the early Mesozoic and drastically diversified during the Jurassic [33–35]. Spiriferinids, which were one of the most thrived brachiopod groups and showed no indications of color [36], became extinct soon after the diversification of these macro predators, even though they had possessed certain morphologies that are considered to be developed exquisite morphological adaptations for of the feeding system that are considered exquisite [37–41]. On the other hand, terebratulids did not become extinct but began to diversify and persisted to the modern era [42]. Considering the improvement over time in the predation abilities of macro predators [43], our results suggest that the red coloration and infrared opacity of terebratulids is an effective adaptation strategy to life for survival at the sublittoral bottom of the sublittoral zone, even though these organisms are immobile and seemingly defenseless.

The relationship between the coloration and the apparent evolutionary trend motivated us to consider the etiology of visibility and its evolution. Through biochemical analysis of intracrystalline proteins in the terebratulid shell, Cusack et al. [14] identified the N-terminal amino acid sequence of a 6.5-kDa protein that may whose function may be to embed a red carotenoprotein in the shell. In this study, the shells of larger L. aqueus rubellus individuals shells examined here tended to exhibit have more vivid red coloration in larger...
individuals; this indicates that the red pigment is probably deposited gradually during the growth of the secondary shell layer. Because the 6.5-kDa protein has been extracted from different shell layers in each species, it seems to represent a phylogenetic constraint [44].

Enigmatic problems remain in this hypothesis. Our hypothesis is yet to explain some problems, namely, the origin of infrared opacity and its evolution. Further studies will be needed to understand how terebratulids in the marine benthic community have evolved in response to increasing predation pressures.

References


