Stealth Effect of Red Shell Coloration in ~~Laqueus rubelus~~ *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. ~~The~~ We examined the se~~r~~lective advantage ~~of~~ of red ~~coloration in the~~ shell coloration ~~of~~ of the terebratulid brachiopod *Laqueus rubellus* ~~(a terebratulid brachiopod) was checked in terms of interactions of prey and~~ in predator evasion. ~~The study was based on comparison of~~ We studied benthic suspension feeders ~~seen~~ 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, ~~A~~almost all species ~~exhibited~~ appeared red ~~coloration under visible light,~~ and resembled rocks and bioclasts; ~~while~~ in infrared light, only ~~the shell of~~ *L.* ~~*aqueus*~~ *rubellus* ~~was~~ showed this stealth effect~~dark under infrared light, similar to rocks and bioclasts~~. ~~Provided~~ ~~t~~The functional eyes of some macropredators ~~such as fishes and coleoids, which~~ are specialized ~~as~~ for detecting light in the blue-to-green region of the ~~visible~~ spectrum;~~, and~~ some have ~~even the~~ long-wavelength photoreceptors.~~ of malacosteids,~~ The unique coloration of *L.* ~~*aqueus*~~ *rubellus* confers ~~should avoid~~ an ability ~~both visible and infrared detection by~~ to evade both these predator~~s~~ types ~~living~~ at ~~in~~ the bottom of the sublittoral ~~bottom~~ zone under both visible and infrared light. This ~~fact~~ suggests that ~~that~~ terebratulids have evolved ~~have evolved ability~~ to remain ~~more or less~~ essentially invisible ~~with~~ even as the ~~improvements of optic~~ visual detection abilities of predators have improved.

Comment [A1]: The phrase "stealth effect" has not been used anywhere in the text, although it nicely describes the camouflage technique.

I've used it at a couple of instances so that key words from the title are consistently used in the rest of the paper.

Please make sure you use the revised title where required.

Comment [A2]: I have added this sentence to serve as a background to put your study in context.

Comment [A3]: "Sublittoral bottom zone" may sounds slightly non-standard. I have revised this term assuming that you are referring to the sea bottom in the sublittoral zone, here and at subsequent instances.

If, however, you are referring to "the deeper sublittoral zone," please use this phrase instead everywhere.

1. Introduction

Competiti~~ve~~on ~~framework exists in~~ for resources and survival is characteristic ~~in~~ of the natural ~~settings~~ environments of most organisms, and this reciprocal interaction ~~is~~ has been the driving force ~~of~~ in evolutionary arms races ~~in evolution~~ [1]. Predator-prey ~~I~~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~~ cannot 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 ~~have been exposed to~~ predation ~~for~~ by macropredators throughout the Phanerozoic~~.~~ ~~They have developed~~ by developing several strategies ~~to~~for warding off- potential predators. For example, some bivalves ~~exhibit~~ have thickened valves that physically ~~prevent~~ protect them against predator attacks ~~physically~~ [3–5], while others ~~exhibit~~ have ~~magnified~~ enhanced burrowing or swimming ability [6–8]. Crinoids and ophiuroids have evolved the ability to ~~automize~~ autotomize and regenerate their tentacles ~~that~~ when they are bitten off by predators [9–11].

~~On the contrary~~In contrast, rhynchonelliformean brachiopods ~~represent~~ — immobile, sessile organisms with thin shells [12, 13]— ~~in which neither~~ do not appear to have evolved physical, physiological, ~~nor~~ or behavioral defenses ~~have not evolved~~ against predators and yet have flourished.

~~Of the rhynchonelliformean brachiopods, t~~Terebratulids are known to be the most successful group among these organisms, having ~~lived~~ survived from the Devonian to the modern era~~s~~.

Comment [A4]: I have revised and connected these sentences so as to explain why the focus moves specifically to this group of organisms. This improves the flow between the previous paragraph and what comes next.

Comment [A5]: I have moved this sentence from the previous paragraph to the next one because it starts narrowing the focus down to terebratulids.

~~They possess semi-circular valves and a pedicle for attachment to a hard substratum.~~ ~~As against the~~Unlike ~~simple look of~~ other rhynchonelliformean brachiopods that have a dull appearance, ~~the shells of~~ many living terebratulids have shells ~~exhibit~~ with distinctive ~~colors~~ 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.* ~~*aqueus*~~ *rubellus*, were collected ~~with~~ using a dredge (wideth, 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

Comment [A6]: This information does not seem relevant in the context of color/appearance that you go on to discuss. Hence, I have omitted this sentence altogether.

Comment [A7]: I have added this sentence to clarify the reason you chose to study this specific terebratulid. This motivation was originally mentioned late in the discussion but is more relevant here.

Comment [A8]: You have studied other benthic organisms as well for comparison, even if your focus was *L. rubellus*.

So I suggest that you revise this sentence as follows:

"Therefore, we studied the optical properties of the shell of *L. rubellus*, in comparison with that of other benthic organisms, under visible and infrared light."

Comment [A9]: Please check if you need to mention the model and manufacturer details.

Comment [A10]: The width of what are you referring to? The mouth? Please clarify.

Comment [A11]: Please check if you should provide the geographic coordinates of the sampling location.

Comment [A12]: I'm slightly unsure what "outermost" shelf bottom refers to since this is an unconventional term. Please check if this can be revised as "bottom of the outer shelf."

a wide area, but *L.~~aqueus~~ rubellus* ~~is abound~~flourishes only around the sublittoral shelf edge [16, 17].

> **Comment [A13]:** As written, this sentence seems out of place and doesn't explain why it's significant that *L. rubellus* flourishes in this area.
>
> If you added this information intending to explain why you chose a specific sampling location, I suggest that you move this sentence to the beginning of the paragraph.
>
> Further, "sublittoral shelf edge" does not appear to be a standard term in this field. Did you instead mean "edge of the outer shelf"?
>
> Use consistent terminology to refer to the same geographical feature if you're justifying your choice of location.

2.2. Materials

> **Comment [A14]:** Please check if you need to briefly specify how you performed taxonomic identification of the species sampled.

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

> **Comment [A15]:** The relevance of this information here in the methods section is unclear. Please consider removing it.

2.3. Observation Methods

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

> **Comment [A16]:** Please mention the city and country of all the manufacturing companies mentioned in the Materials and Methods section.
>
> If the manufacturer is US-based, the city and state generally suffice.

2.4. Quantitative Analysis of Grayscale Images

For the quantitative ~~examination~~ determination of visibility ~~for~~ as recorded in infrared images, we obtained ~~the~~ a grayscale histogram ~~of grayscale color~~ using the image~~-analysing~~analysis ~~software~~ program ~~called~~ ImageJ. The image of each animal was taken with a distance of 1 m~~etre~~ ~~distant~~ between the animal and ~~from~~ the video-scope. Animal outlines in the grayscale images were drawn ~~by~~ using the polygon-selection tool ~~of polygon selections~~ in ImageJ, and then the area inside the outline was analy~~s~~zed to obtain a 256~~-~~shade~~s~~ ~~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 that were observed under visible light conditions ~~are~~ were red-~~-~~colored (Figures 3(a) and 3(b)), except ~~the crinoid~~ *M.~~etacrinus~~ rotundus* (Figure 3(e)), which ~~is~~ was white to ivory ~~in color~~. *L.~~aqueus~~ rubellus* ~~has~~ had a thin shell that ~~is~~ was ~~colored~~ orange to empire red and ~~is~~ transparent enough to ~~see~~ reveal the organism inside (Figures 3(a) and 3(b)). The color of ~~L~~larger shells tended to be darker ~~in color~~. The shells of *C.~~ryptopecten~~ vesiculosus* and *N.~~emocardium~~ samarangae* ~~are ornamented with~~ had a ~~mosaics of~~ red-~~-~~and-~~-~~white ~~colors~~mosaic pattern. The coloration patterns ~~of coloration exhibit~~ showed interspecific variation (Figure 3(a), Figure 3(b). The shell of ~~Cryptopecten~~ *C. vesiculosus* ~~is~~ has a patchy ~~colored by~~ wine-~~-~~red ~~pigment in a patchy fashion~~pattern, while that of *N.~~emocardium~~ samarangae* ~~is ornamented with~~ has several radial orange bands. ~~The s~~Scleractinian corals ~~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 (~~u~~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~~the images taken in visible light, infrared images ~~displayed~~ revealed a difference in color intensity among taxa. ~~As was apparent from the infrared images,~~ They showed that the shells of *L.~~aqueus~~ rubellus* were the darkest and ~~were similar in~~ their coloration resembled that of ~~to the attached~~ bioclasts and rock fragments (Figures 3(c) and 3(d)). ~~The s~~Shell darkness tended to increase with shell length. ~~Meanwhile~~In contrast, the shells of ophiuroids and ~~the crinoid~~ *M.~~etacrinus~~ rotundus* were the brightest~~, contrasting sharply with the coloration of *Laqueus*~~ (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 ~~C~~counts of each grayscale plot among the individuals are significantly different (Figure 4; *P* < 0-.-0-0-1, pairwise ANOVA). The ~~M~~mean values ~~in the case of~~ for *L.~~aqueus~~ rubellus* were around 40, ~~that~~ which ~~was~~ were the lowest (darkest) among the animals. The mean values observed for ~~B~~bivalves, ophiuroids, and scleractinian corals ~~exhibit~~ were 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~~ obtained for two crinoid *Metacrinus* show a gentle convex shape, with the peak occurring at around 90 ~~in~~ for ~~Metacrinus 1~~ one individual and around 160 ~~in~~ for ~~Metacrinus 2~~ the other.

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*."

Moreover, once again, this result has not been discussed in the discussion section. If the color intensity of molluscan shells is lower, are they better or as good as *L. rubellus* at evading predators?

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 means 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.

Discussion

~~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~~ How this 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 a~~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 wavelength~~s~~ in the visible spectrum~~,~~ and ~~its~~ 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,~~ ~~would~~ will therefore appear black ~~in color~~ at the bottom of the sublittoral ~~bottom~~zone. *L.* ~~*aqueus*~~ *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 ~~go~~ remain ~~unrecognised~~ undetected by ~~the eyes of~~ macropredators such as fish and squid [20–24].

Comment [A28]: As commented in the results section, several of your findings have not been elaborated on or even mentioned in the discussion.

This may prove a roadblock during the peer review stage. Please go through your results and discussion critically to ensure there are no gaps.

Comment [A29]: You have used only two subheadings in the discussion and that too only for two specific topics. The rest of your discussion is unstructured. I've therefore deleted these subheadings.

Comment [A30]: When you say organisms associated with *L. rubellus,* are you referring to other brachiopods or other organisms found in the sublittoral zone? Please clarify.

Comment [A31]: This sounds like an inference you've made from your results, but you have cited five papers. Has this observation already been made by other authors and your study is corroborating the finding? Please clarify.

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 Since this species especially flourishes at the bottom of the sublittoral zone, the low color intensity 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 deep 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 *L. aqueus 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 of because it resembles dark rocks and skeletal fragments. The appearance of *L. aqueus rubellus* shells under infrared light suggests that Laqueus it has evolved a survival strategy in which its shell behaves optically like a nonliving object on at the bottom of the sublittoral bottomzone.

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 suggests 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.

L. aqueus 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.

Comment [A32]: A simple restatement of results before the inference here may help readers comprehend the text better, since they may not remember which specific result you are talking about.

Further, your original sentence implied that all the organisms studied showed the same color intensity and hence may share the same strategy under infrared light. But your results specifically mention *L. rubellus* as being particularly dark.

I've therefore revised your sentence accordingly.

Comment [A33]: You have referred to such predators *in general* both before this sentence and in relation to *L. rubellus*.

So it is not clear why *Photostomias guernei* is significant. I would advise you to either explain its relevance or remove this specific mention altogether.

Comment [A34]: You have focused on the infrared visibility of only *L. rubellus*, whereas provided data for all the other organisms in the results.

I would recommend that you offer insights comparing the success of all organisms studied, which you've said have varying infrared visibilities.

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~~ at the sea bottom is also affected by competition among benthic organisms, which belong to a similar guild [30–32]. ~~As a consequence~~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, h~~Highly efficient vision systems are ~~evident~~ seen in teleost fishes and coleoid cephalopods, both of which originated in the early Mesozoic and drastically diversified during the Jurassic [33–35]. Spirifer~~in~~ids, which were one of the most ~~thrived~~ successful brachiopod groups and showed no indications of color [36], became extinct soon after the diversification of these macropredators, 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 macropredators [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 ~~Because~~ *L.* ~~*aqueus*~~ *rubellus* individuals ~~shells examined here~~ tended to ~~exhibit~~ have more vivid red coloration ~~in larger~~

Comment [A35]: It’s not clear what the species of the benthic community are involved in. Did you perhaps mean that this predator-prey coevolution is not restricted to *L. rubellus* but also occurs in other species of the benthic community from the same guild since they are competitors exposed to the same pressures?

Comment [A36]: Since your paper focuses chiefly on predator-prey coevolution, it is not immediately clear how diffuse coevolution is relevant in this context. Please elaborate on this.

Comment [A37]: This section of the sentence is not very clear. Did you mean that their coloration did not evolve over time?

Comment [A38]: Statements about motivation behind your study should not appear so late in a paper. I have already added this motivation in the introduction, where it’s more relevant.

Also, “etiology” is the study of causes of diseases and does not appear relevant in this context.

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 thisOur 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.

Comment [A39]: It may not be clear to readers how the description of the protein responsible for the coloration is relevant here, or what is significant about the pigment being deposited gradually during the growth phase.

Please add some comments on why these topics are noteworthy in the context of evolution.

Comment [A40]: As written, the discussion seems to end rather abruptly. Please review if you need to mention any study limitations first.

Also, while the future research direction you have mentioned is valid, what peer reviewers may like to know is what broad implications your findings have in this field of study.

I would advise you to review the aims and scope of your target journal and see if you can add specific insights on any study implications that will be of interest and value to the journal's readers.

References

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