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Optical Properties of Body Mucus Secreted from Coral Reef Sea Slugs: Measurement of Refractive Indices and Relative Absorption Spectra

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Sea slugs are always covered in a mucus layer that has various functions including chemical defense that often involves aposematism and mimicry. Therefore, it is necessary for sea slugs to exhibit their body colors and patterns exactly, and the optical properties of mucus should support this requirement. We examined body mucus from heterobranch sea slugs collected in the Okinawan coral reefs. The refractive indices of mucus from 32 species ranged from 1.3371 to 1.3854 and were similar or slightly greater than the refractive index of seawater (ca. 1.34), indicating that light reflectance on the mucus layer is generally small. Moreover, dissolution of mucus into seawater would form a gradient of refractive indices and enhance the reduction of reflectance. We also obtained relative absorption spectra of the mucus from 32 species. In the range of visible light, absorption spectra of mucus suggest that the mucus layer is almost transparent and is not likely to interfere with the body colors. The presence of absorption peaks and/or shoulders in the UV (ultraviolet) range (280–400 nm) indicates that the mucus layer potentially serves as a sunscreen that absorbs UV radiation in 23 species, whereas prominent UV absorption was not found in the other 9 species. In a kleptoplasty sacoglossan *Plakobranchus ocellatus*, the refractive indices and presence or absence of UV-absorption showed that the optical properties of the mucus varied to some extent but did not show seasonal fluctuation. The UV-absorption in the mucus may also protect kleptoplasts in kleptoplasty sacoglossans. The present results support the importance of mucus as a functional optical layer for the shell-less life of sea slugs.

Key words: Mucus, Anti-reflection, UV protection, Sea slugs, Kleptoplasty

BACKGROUND

Sea slugs are shell-less or have a very small shell that is almost embedded in their soft bodies. Sea slugs are a polyphyletic group; Heterobranchia is the largest group that includes numerous families of the shell-less gastropods in addition to shelled gastropod taxa. In contrast to other gastropods that generally possess a shell that houses the whole body, heterobranch sea slugs always expose their soft bodies that often exhibit vivid colors and patterns. Since many secondary metabolites have been isolated from them (*e.g.*, Cimino et al. 2001; Benkendorff 2010; Avila and Angulo-Preckler 2020), chemical defense with aposematism is considered a representative adaptation for sea slugs to protect themselves from natural enemies (Faulkner and Ghisekin 1983; Giménez-Casalduero et al. 1999). It is therefore important for sea slugs to effectively exhibit the body colors and patterns.

In gastropods, the outermost tissue of the body

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is an epidermis bearing microvilli, and a mucus layer secreted from the epidermis overlys the epidermal surface (*e.g.*, Bubel 1984). Whereas production and secretion of mucus require a considerable energy cost (Davies et al. 1990, Davies and Hawkins 1998), the mucus layer serves various functions in gastropods (reviewed in Bubel 1984; Voltzow 1994; Davies and Hawkins 1998; Avila and Angulo-Preckler 2020), including slip resistance for locomotion (Denny and Gosline 1980) and suspension-feeding (Kappner et al. 2000), as well as chemical defense against predation and infection. The mucus trail has multiple functions such as homing, mate location, and communication among conspecifics (reviewed in Ng et al. 2013), and some nudibranch sea slugs follow the mucus trails of their prey nudibranchs for predation (Paine 1963; Nakano and Hirose 2011). Moreover, aeolid sea slugs produce mucus that inhibits the discharge of nematocysts of their cnidarian prey (Greenwood et al. 2004).

In the shell-less heterobranchs, the mucus layer may also contribute to reduction of glare caused by light reflection, since aposematism and mimicry need a clear presentation of the body colors and patterns. Light protection is also a potential function of mucus, as ultraviolet (UV) protective compounds have been isolated from various species (*e.g.*, Karentz 2001; Núñez-Pons et al. 2018). This function would be important particularly at shallow habitats in tropical and subtropical waters characterized by higher water transparency and lower incident angles of solar radiation than the areas in higher latitudes.

A wide variety of marine invertebrates have a mucus layer covering their body surfaces, and knowing the optical properties of this mucus would be an important key to better understanding its functions. Because the light reflection occurs at the border of different refractive indices, the refractive indices of the mucus layer are expected to be similar to those of the ambient seawater. However, the refractive indices of mucus have been rarely measured and compared in marine invertebrate taxa to date. Here, we measured the refractive indices of the body mucus from heterobranch sea slugs in Okinawan coral reefs. In this report, the mucus that always covers the entire body surface of an individual is referred to as "body mucus," to distinguish it from the secretions released during particular events such as attacks by natural enemies, like the purple ink released by sea hares (*e.g.*, DiMatteo 1982). Absorption spectra of the diluted mucus were also measured in the range of UV-visible light (wavelength: 250–800 nm) to elucidate the contribution on the coloration and light protection. Moreover, we verified the presence or absence of seasonal fluctuation in these optical properties in a kleptoplasty sacoglossan *Plakobranchus ocellatus* that retains photosynthetically active chloroplasts from the food algae in the digestive gland.

MATERIALS AND METHODS

Animals

Heterobranch sea slugs (108 individuals of 34 species) were collected by snorkeling or SCUBA diving on the coral reefs off Okinawajima Island, Japan from October 2021 to November 2022 (Table S1). The specimens were put in a 300-mL bottle filled with seawater and brought to the laboratory for collection of body mucus. After the mucus collection (see below), the specimens were brought back to their sampling sites and released. Among the specimens, the three individuals of *Chromodoris* sp. were considered to be an identical species, based on their color patterns (Fig. S1). The three individuals of *Elysia* sp. cf. *marginata* possibly included multiple cryptic species (see Krug et al. 2013). *Plakobranchus ocellatus* is often considered to be a species complex, and we collected the black-type and white-type *sensu* Krug et al. (2013) from the same site.

To investigate seasonal fluctuation of the optical properties of the body mucus, *P. ocellatus* individuals were collected from the sandy back reef at Toguchi Beach, Okinawajima Island, Japan (26°21'55"N, 127°44'10"E) in January, April, May, June, July, August, October, and November in 2022. In total, we collected 52 individuals (black type, 46; white type, 6).

Mucus collection

Excessive seawater on the body surface was gently removed from the animals with paper towels as much as possible. We lightly tapped the body with the pipette tip (20–200 μL) to stimulate mucus secretion. The body mucus secreted from the body was sucked up with the pipette tip and collected in a 1.5-mL microtube or directly applied to the refractometer. In collecting mucus for measurement of refractive indices, we carefully minimized dilution of the mucus with seawater seeping from the animal body as much as possible, but seawater contamination could not be completely prevented.

In collecting body mucus for absorption spectra, the amount of mucus was often too small and viscous to put in a microcuvette. In this case, we poured a small amount of seawater over the body surface to collect the body mucus, which was diluted and bulked up with the seawater. When the light absorption of the body mucus was too large to obtain absorption spectrum, it was diluted with appropriate amount of seawater. We cannot specify the dilution rate for each sample, because the collected mucus had been more or less diluted with seawater from the animal body.

Measurements

Refractive indices of the body mucus and seawater were measured using a pocket refractometer PAL-RI (Atago Co., Ltd.) with a small volume adapter. Because the seawater on the animal body was possibly mixed during the mucus collection, the measured values might be affected slightly by the small amount of seawater in the mucus sample.

The mucus sample diluted with seawater was dispensed into UV-compatible disposable microcuvettes, and the absorption spectra were obtained in the range of 250–800 nm at intervals of 1 nm using the spectrophotometer Genesis 10S UV-Vis (Thermo Scientific, Wisconsin, USA). The approximate wavelength of the absorption peaks and shoulders were visually read from the spectra of each specimen. Because the dilution rate for each sample was uncertain, the absorption values could not be compared among the samples directly. Therefore, we focused on the presence or absence of absorption peaks and/or shoulders in ranges of visible light (400–800 nm), UV-A (315–400 nm), and UV-B (280–315 nm). We ignored the absorption of UV radiation shorter than 280 nm, which is absorbed by the ozone layer and atmosphere and does not reach the ground and sea surface.

RESULTS

Refractive indices of mucus

Body mucus samples were collected from 34 species (19 genera, 14 families, 4 orders) of heterobranch sea slugs inhabiting coral reefs at 0–18 m deep (Table S1), and the undiluted mucus was applied to the refractometer. However, the refractive indices were not available for two species, *Sagaminopteron ornatum* and *Notodoris citrina*, because the quantity of the mucus samples was too low to measure. Except for *P. ocellatus*, the measured values of each specimen are listed in table 1 and plotted by species in figure 1. The measured values ranged from 1.3371 in *Hypselodoris whitei* to 1.3854 in *Phyllidia coelestis*. Overall, the refractive indices of the mucus were similar to or slightly greater than the refractive index of seawater (ca. 1.34). Some differences were found in the measured values from multiple individuals within a species, while we could measure only one specimen for 18 species. The maximum intraspecific difference in refractive indices was 0.039 among the three individuals in *Phyllidia coelestis*.

Relative absorption spectra of mucus

We obtained absorption spectra of mucus from 47 individuals of 31 heterobranch sea slug species and visually read prominent peaks and shoulders from the spectra (Table 1, Fig. S2). Spectra were also obtained from *P. ocellatus* individuals, which will be shown in the next section. In this study, the absorbance values could not be directly compared among the spectra, because dilution rates of the mucus with sea water varied among samples. Absorption spectra showed strong absorption around 280 nm and below for all samples. In the visible range (400–800 nm), the absorbances was small and spectra were almost flat in most species, indicating that the mucus is almost transparent in visible light (Table 1, Fig. 2). The exceptions were two of three specimens of *Chelidonura amoena* and two of two specimens of *Chelidonura hirundinina*, which had absorption peaks at around 425 nm and 444 nm, respectively (large arrow in Fig. 2B).

In the range of UVA–UVB (280–400 nm), absorption peaks or shoulders were found in the mucus from 23 of 32 species including *P. ocellatus*; mucus from some species had an absorption peak or shoulder (arrow and arrowhead in Fig. 2A, respectively), some had multiple peaks (arrows in Fig. 2B), and no prominent peaks or shoulders were found in 13 specimens of nine species (thick line in Fig. 2A). In ten species, the absorption spectra of mucus were examined in multiple individuals, and UV absorption was observed in nine of the species. Prominent absorptions were not found in the mucus from two of two *Dendrodoris nigra* individuals. In *Chelidonura hirundinina*, *Chromodoris* sp, *Hypselodoris bullockii*, *Asteronotus cespitosus* and *Sebadoris fragilis*, the mucus from conspecific individuals had absorption peaks/shoulders at approximately the same wavelength (Table 1). In *Chelidonura amoena*, *Phyllidia picta*, *Phyllidiella pustulosa* and *Plakobranchus ocellatus*, absorption peaks differed in wavelength among conspecific individuals, and in some specimens there were no prominent absorptions (Table 1, Table S1).

Seasonal fluctuation in *Plakobranchus ocellatus*

The refractive indices of the mucus were similar or slightly greater than the refractive index of seawater (ca. 1.34). While the measured values varied among individuals (black type: n = 46, average = 1.3437, SD = 0.00405; white type: n = 6, average = 1.34562, SD = 0.00415), we found no clear correlation between the refractive indices and the color type (black or white) or the seasons (Fig. 3, Table S1). The largest value was 1.3573 from a black type specimen collected on November 25, and the smallest was 1.3331 from a black type specimen collected on June 15 on which we had a heavy rain.

Among 52 specimens, absorption spectra of the mucus had no prominent peaks or shoulders in the visible-UVA-UVB range (280–800 nm) in 33 specimens, had an absorption shoulder at around 330 nm in six specimens, and had a shoulder at around 400 nm in 13 specimens (Fig. 4, Fig. S4, Table S1). A clear seasonal trend was not observed for the presence or absence of the shoulder, and none of the six white-type individuals had prominent shoulders.

Table 1. Refractive indices and light absorptions of mucus from heterobranch sea slugs (excluding Plakobranchus ocellatus)

Species	Refractive index	Absorption (nm)*	
		Peak (s)	Shoulder
Order Acteonida			
Family Aplustridae			
Aplustrum amplustre	1.3431	288	320
Order Cephalaspidea			
Family Aglajidae			
Chelidonura pallida	1.3459	292, 327	-
Chelidonura amoena	1.3405	323	-
Chelidonura amoena	1.3473	425	340
Chelidonura amoena	1.3454	311, 444	340
Chelidonura hirundinina	1.3505	286, 350, 426	
Chelidonura hirundinina	1.3658	286, 351, 426	
Family Gastropteridae			
Sagaminopteron ornatum	No data	315	-
Family Haminoeidae			
Phanerophthalmus luteus	1.3471	-	370
Order Nudibranchia			
Family Aegiridae			
Notodoris citrina	No data	388	-
Family Cadlinidae			
Aldisa albatrossae	1.3477	328	-
Family Chromodorididae			
Chromodoris colemani	1.3482	330	-
Chromodoris lineolata	1.3432	-	-
Chromodoris willani	1.354	No data	No data
Chromodoris sp.	1.3488	No data	No data
Chromodoris sp.	1.3397	318	-
Chromodoris sp.	1.3424	318	-
Goniobranchus coi	1.3426	No data	No data
Goniobranchus coi	1.3412	296, 307	-
Goniobranchus geometricus	1.3447	297	-
Goniobranchus kuniei	1.3413	-	370
Hypselodoris bullockii	1.3527	-	325
Hypselodoris bullockii	1.3461	-	325
Hypselodoris bullockii	1.344	321	-
Hypselodoris decorata	1.3812	327	-
Hypselodoris infucata	1.3572	-	-
Hypselodoris tryoni	1.3636	-	320
Hypselodoris whitei	1.3371	-	-
Order Nudibranchia (continues)			
Family Dendrodorididae			
Dendrodoris nigra	1.3406	-	-
Dendrodoris nigra	1.3468	-	-
Family Discodorididae			
Asteronotus cespitosus	1.3419	313, 362	-
Asteronotus cespitosus	1.3421	313, 362	-
Asteronotus cespitosus	1.3415	314, 361	-
Jorunna rubescens	1.3414	324	-
Sebadoris fragilis	1.3425	328	-
Sebadoris fragilis	1.3414	-	328
Sebadoris fragilis	1.3408	-	328
Family Flabellinidae			
Coryphellina lotos	1.3492	-	-
Family Phyllidiidae			
Phyllidia coelestis	1.3854	No data	No data
Phyllidia coelestis	1.3464	No data	No data
Phyllidia coelestis	1.3686	-	-
Phyllidia ocellata	1.3455	-	-
Phyllidia picta	1.349	283	-
Phyllidia picta	1.341	-	-
Phyllidia picta	1.3423	-	-
Phyllidia picta	1.348	288	-
Phyllidiella pustulosa	1.3406	No data	No data
Phyllidiella pustulosa	1.3444	No data	No data
Phyllidiella pustulosa	1.3464	-	-
Phyllidiella pustulosa	1.3419	288	-
Family Polyceridae			
Thecacera picta	1.3397	No data	No data
Order Pleurobranchida			
Family Berthella			
Berthella martensi	1.3457	-	-
Order Sacoglossa			
Family Plakobranchidae			
Elysia sp.cf. *marginata*	1.3428	No data	No data
Elysia sp.cf. *marginata*	1.3398	333	-
Elysia sp.cf. *marginata*	1.347	No data	No data
Thuridilla splendens	1.3461	-	-

*Approximate values were visually read from the spectra. "-" indicates no prominent peaks/shoulders in the spectra within the range of 280–800 nm.

DISCUSSION

Refractive indices and light reflection

The refractive indices of body mucus in 32 species were generally similar to or slightly greater than that of seawater, but there were some intraspecific variations as well as interspecific variations among the specimens (Figs. 1 and 3). The measured values might be slightly different from the refractive indices of the pure mucus, because the body mucus samples contained some amount of seawater seeping from the animal body. Moreover, since refractive indices will be smaller in the lower salinity sea water, the difference in refractive index should be variable between the mucus layer and the sea water to some extent due to rainfalls.

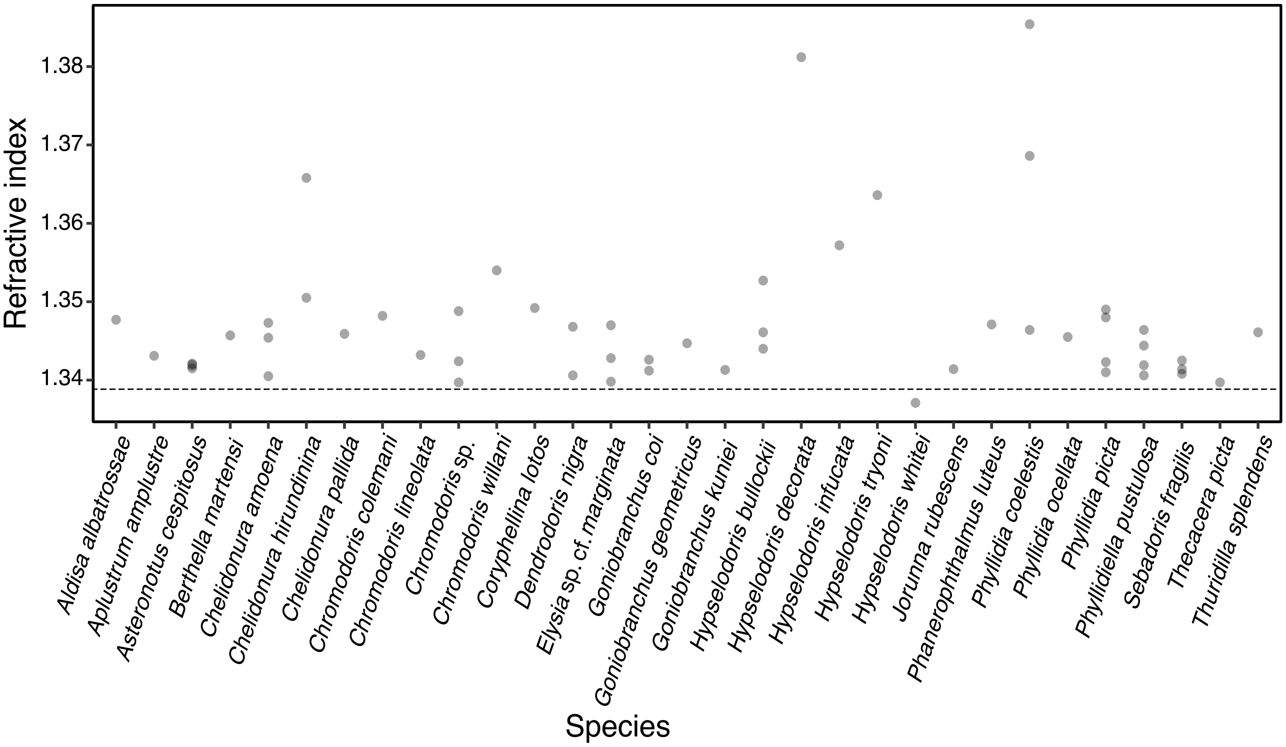

Fig. 1. Refractive indices of the body mucus from 31 species of heterobranch sea slugs. The dashed line indicates the approximate refractive index of seawater (1.339). This plot does not include the data of *Plakobranchus ocellatus*, which are shown in figure 3.

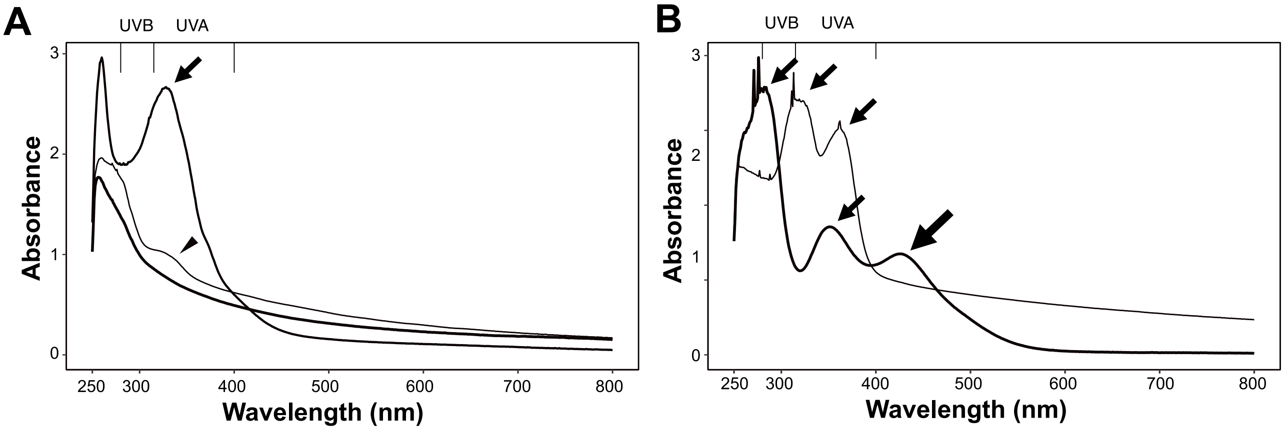

Fig. 2. Typical examples of absorption spectra of body mucus. (A), Spectrum with no prominent absorptions (thick line, *Phyllidia picta*), spectrum with one absorption peak (arrow: middle line, *Aldisa albatrossae*), and spectrum with one shoulder (arrowhead, thin line, *Sebadoris fragilis*) in the visible-UVA-UVB range (280–800 nm). (B), Spectra with a peak in visible range (large arrow) and/or multiple peaks in the UVA-UVB range (arrows). Thick line, *Chelidonura hirundinina*; thin line, *Asteronotus cespitosus*.

Accordingly, differences in the amounts of seawater contamination and salinity between samples might be the main cause of differences in refractive indices in the present survey. Whereas we cannot expect high accuracy in the measured values of refractive indices in mucus samples with the present method, it should be sufficient to grasp the approximate refractive indices of the body mucus.

Reduction of reflectance on the body surface should be adaptive for sea slugs to clearly exhibit the body colors and patterns that have aposematic and/or mimicry functions. Light reflection occurs at the boundaries between different refractive indices; a greater difference in refractive index results in an increase in light reflection. Thus, the small difference in refractive index between body mucus and seawater would be favorable in reducing light reflection on the body surface. In most cases, the measured values were slightly greater than the refractive index of seawater. Considering the potential contamination of seawater, the measured values are supposed to be slightly smaller than the refractive index of the original mucus just released from the body. The released body mucus is thought to swell in seawater and form a mucus layer on the body surface. As the mucus is water-soluble, the mucus layer gradually is dissolved in seawater, forming

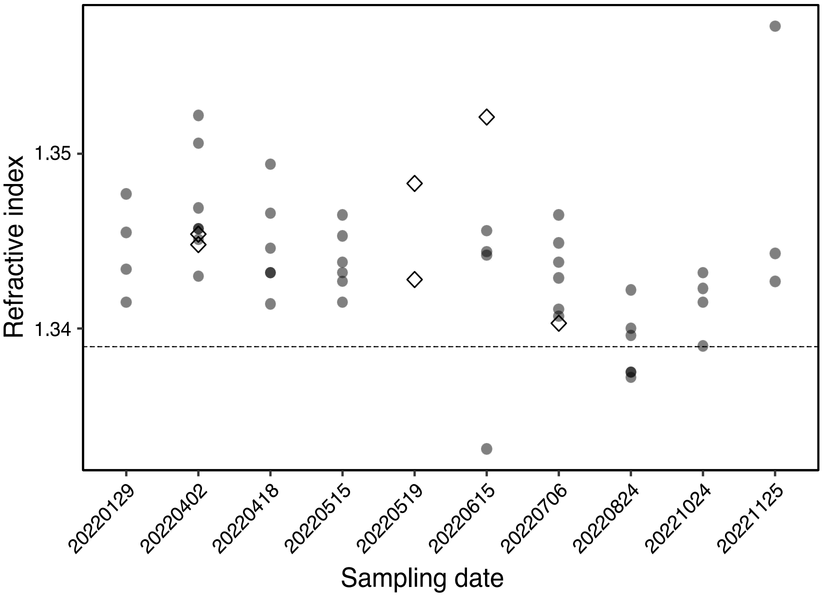

Fig. 3. Refractive indices of the body mucus from *Plakobranchus ocellatus* (black type, gray circles; white type, open diamonds). The dashed line indicates the approximate refractive index of seawater (1.339).

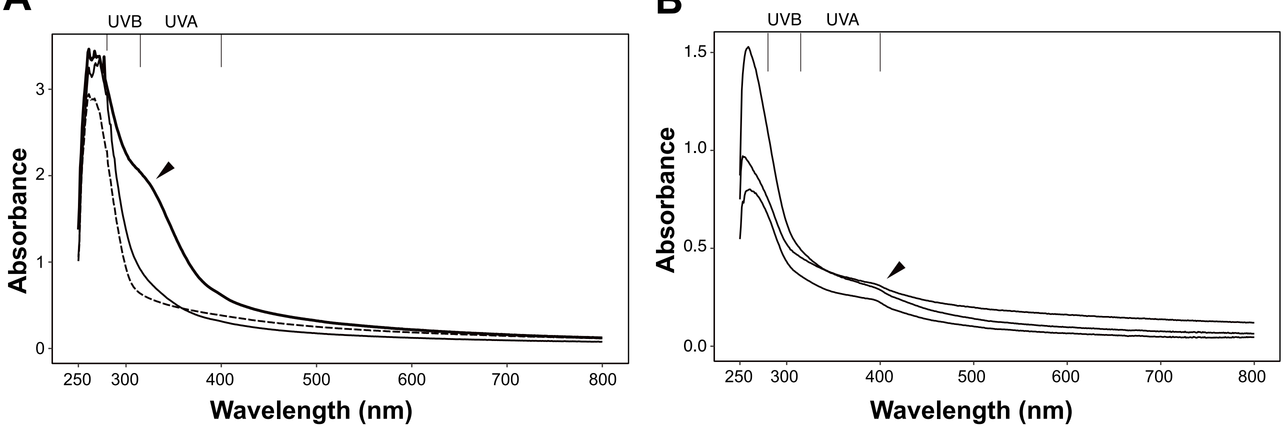

Fig. 4. Typical examples of absorption spectra of *Plakobranchus ocellatus* mucus. (A), Spectra with no prominent absorptions in the visible-UVA-UVB (black type, solid line; white type, broken line) and a spectrum with a shoulder at around 330 nm (arrowhead). (B) Spectra with a shoulder at around 400 nm.

a gradient of the refractive indices on the body surface. The reflectance of the body surface will be very small in sea slugs covered in a mucus layer, because light reflection will be further suppressed by the gradient of the refractive index at the boundary between the mucus layer and seawater (*e.g.*, Chattopadhyay et al. 2010).

Light absorption of mucus

Relative absorption spectra of body mucus diluted with seawater were obtained from 32 species including *P. ocellatus*. In the range of visible light (400–800 nm), nearly flat spectral curves indicate that the mucus is almost transparent and colorless in most of the species, and thus, the mucus layer is unlikely to interfere with the body color of the sea slugs. Although the mucus samples from *C. amoena* and *C. hirundinina* exceptionally had absorption peaks at around 425 nm and 444 nm respectively, these peaks are caused by the orange-colored secretions discharged upon the mucus collection (Fig. S3). The secretion of the colored fluid appears to be a protective response of the animals, and the individuals would usually wear transparent mucus in an unagitated condition to exhibit their original body colors. One of the three individuals of *C. amoena* did not secrete the colored fluid, and its body mucus had no absorption peaks in the range of visible light.

In the UV range (280–400 nm), body mucus from 23 of 32 species, including *P. ocellatus*, had absorption peaks or shoulders. Within ten species in which multiple individuals were examined, the conspecific specimens had no prominent absorption in one species and had absorption peaks/shoulders at approximately the same wavelength in five species. However, many more conspecific individuals should be examined to conclude whether the presence or absence of these UV-absorptions are species-specific. In four species, absorption spectra were not similar among conspecifics: the absorption peaks were different in wavelength and some specimens did not have prominent peaks. In these species, the UV-absorption in body mucus may vary among individuals depending on seasons, habitat, diet and other factors.

Since solar radiation contains harmful ultraviolet light, organisms, especially those living in shallow water, need protection from UV radiation that damages DNA, protein and other molecules. The mucus with a UV-absorption peak/shoulder probably protects the body from UV radiation. Since light attenuates in water, the threat of solar radiation varies greatly depending on habitat depth. However, in the present study, the specimens collected at deeper sites did not always lack UV-absorptions (Table S1). UV-absorption through the mucus layer is one of the countermeasures against UV radiation, but body color also protects the animal from solar radiation. Migration to shade may be a lower-cost strategy against harmful radiation for the species/individuals lacking a UV-absorbing mucus layer. Marine metazoans, including sea slugs, often utilize mycosporine-like amino acids (MAAs) for UV-protection and antioxidation (*e.g.*, Karentz 2001; Shick and Dunlap 2002), whereas we did not qualify the UV-absorbing molecules in the present study. As most metazoans lack the essential pathway for MAA synthesis, they acquire MAAs from their diet or algal symbionts (Sinha et al. 2007), except for some cnidarians that possess the gene set for the shikimate pathway (Starcevic et al. 2008; Shinzato et al. 2011). However, diets are poorly known in many sea slugs. In the sea hare *Aplysia dactylomela*, the parapodial skin contains a considerable amount of MAAs that probably originated from food algae (Carefoot et al. 2000).

Seasonal fluctuation in *Plakobranchus ocellatus* and functional implications for kleptoplasty

In the Okinawan coral reefs, many of the heterobranch sea slug species occur only in the spring season (Tanamura and Hirose 2016a), but *P. ocellatus* occurs all year round while the population density fluctuates (Tanamura and Hirose 2016b). Due to the subtropical climate in Okinawa, water temperature and solar radiation annually fluctuate at the sampling site of this species (Tanamura and Hirose 2016b; Chihara et al. 2020), and the mucus layer on the body surface may be involved in tolerance to environmental changes as well as functional retention of photosynthetically active chloroplasts from food algae in the digestive gland, *i.e.*, kleptoplasty. In sacoglossans harboring kleptoplasts, photosynthesis of the kleptoplasts is involved in mucus production (Trench et al. 1970 1972; Lopes et al. 2022). Excessive radiation on the animals causes photoinhibition and/or damage to their kleptoplasts (Cartaxana et al. 2018 2019; Christa et al. 2018; Chihara et al. 2020; Donohoo et al. 2020), and the mucus layer protects kleptoplasts from photoinhibition and improves their longevity (Havurinne et al. 2022). The body mucus of these sacoglossans contains some polyketides that provide sunscreen and toxicity for chemical defense, and the animals utilize methylmalonyl-CoA, a photosynthetic metabolite from kleptoplasts, to biosynthesize these polyketides (Ireland and Scheuer 1979; Gavagain et al. 1994; Torres et al. 2020).

In 52 samples from *P. ocellatus* collected from January to November, clear seasonal trends were not found in the refractive indices of mucus varying from 1.3331 to 1.3573 (Fig. 3). On average, the refractive indices of the body mucus were slightly greater than that of sea water, probably indicating low reflectance as discussed above. Accordingly, the mucus layer may reduce the loss of solar radiation for photosynthesis of the kleptoplasts by reducing light reflection. No prominent absorptions were found in the range of visible light in the mucus from any individuals examined, indicating that the mucus layer does not absorb photosynthetically active radiation (PAR). Furthermore, in 52 absorption spectra, an absorption shoulder was found at around 330 nm in six spectra and at around 400 nm in 13 spectra. The UV absorption at 330 or 400 nm is not a species-specific feature of the *P. ocellatus* mucus, and clear seasonal trends were not found for the occurrence of these shoulders (Fig. 4, Table S1). The absorption shoulders in the mucus may be involved in the protection of the kleptoplasts in *P. ocellatus*, whereas the mucus in some individuals does not have prominent absorption shoulders. The present results showed UV-absorption of the body mucus varied considerably among individuals in *P. ocellatus*, but we could not determine what causes the differences. Since *P. ocellatus* feeds on the cytoplasm of various species of siphonous green algae (Maeda et al. 2012), the presence or absence of UV absorption in the mucus possibly depends on what the individual has fed on. If the optical properties of the mucus are variable depending on diet or other environmental factors, this variability may partly support the environmental tolerance that allows this species to occur year-round on subtropical reefs.

CONCLUSIONS

Mucus is known to have a variety of functions in gastropods (*e.g.*, Voltzow 1994; Davies and Hawkins 1998; Avila and Angulo-Preckler 2020). Because heterobranch sea slugs are shell-less or have only tiny shells, functional importance of the mucus layer as the outermost barrier would be greater than in shelled gastropod species. The present study showed that the layer of body mucus is nearly transparent in the range of visible light and the refractive index is similar or slightly greater than that of seawater. These optical properties are suitable to exactly exhibit the body colors and patterns that are often aposematic and mimic signals. Moreover, dissolution of mucus into seawater also enhances reflection reduction, forming a gradient of refractive indices. In 21 of 32 species so far examined, the mucus layer has absorption peaks/shoulders in the range of UV and potentially serves as a UV-screen. In kleptoplasty sacoglossans, the mucus layer is also expected to reduce loss of PAR for kleptoplasts and may also protect kleptoplasts from harmful radiation if it contains UV-absorbing compounds. Thus, the mucus would be important to serve as a functional optical layer for the shell-less life of these colorful animals. The optical properties of body mucus described above may also be desirable or necessary for other marine invertebrates that have a mucus layer as the outermost covering of the body, and more comprehensive surveys across many animal taxa would be needed for further discussion.

List of abbreviations

MMAs, mycosporine-like amino acids.
PAR, photosynthetically active radiation.
UV, ultraviolet.

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Consent for publication: Not applicable.

Ethics approval consent to participate: This work complies with the ethical guidelines of the University of the Ryukyus Animal Experimentation Committee.

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Supplementary materials

Fig. S1. *In situ* photographs of the three *Chromodoris* sp. collected on November 13, 2021 (A), September 10, 2022 (B), November 3, 2022 (C). (download)

Fig. S2. Absorption spectra of the body mucus listed in table 1. (download)

Fig. S3. *Chelidonura hirundinina* releasing yellowish secretions during mucus collection. (download)

Fig. S4. Absorption spectra of the body mucus from *Plakobranchus ocellatus* (black type, solid line; white type, broken line). The animals were collected from January to November 2022. White type specimens in March were collected at Odo coast and the other specimens were collected at Toguchi beach. See Table S1 for details. (download)

Table S1. List of specimens, dates, locations, refractive indices and light absorptions of body mucus. (download)