Anthozoans take up *Symbiodiniaceae* from the "Zone of Phagocytosis"- pathway to symbiosis revealed during microplastic experiment

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Symbiodiniaceae are symbiotic phytoplankton that live in symbiosis with various hosts. For example, *Symbiodiniaceae* live within the endodermal cells of corals and sea anemones, and they increase in number inside the cells. Nutrients obtained from coral heterotrophy and *Symbiodiniaceae* autotrophy are then exchanged.

Anthozoans acquire symbiotic algae in two ways: vertical and horizontal transmission (Muller-Parker et al., 2015; Padilla-Gamiño et al., 2012). In corals that produce aposymbiotic larvae, *Symbiodiniaceae* are vertically transmitted from parental colonies; thus, larvae receive the parental zooxanthellae before they are released into the surrounding seawater. In corals that produce aposymbiotic larvae, the mechanism through which adult hosts take up *Symbiodiniaceae* from the environment (horizontal transmission) is unknown. According to a recent review by Mullar-Parker et al. (2015), when symbiotic algae are passed to eggs via vertical transmission, free zooxanthellae in the gastrovascular cavity may be ingested by gastrodermal follicle cells (Hirose et al., 2001). However, the tissues from which *Symbiodiniaceae* is taken up after they enter the gastrovascular cavity through the mouth are unknown. In this study, I found that *Symbiodiniaceae* are phagocytosed from the "Zone of Phagocytosis" in the mesenteries of corals and sea anemones during the process of experimenting with the effect of microplastic.

The sea anemone *Exiptasia* and the coral *Seriatopora caliendrum* (Fig. 1) were used as hosts of symbionts, and *Symbiodiniaceae* (CS-164) was used as the symbiotic algae. Both were incubated to bleach at 32 ℃ for two days. In addition, fluorescent polystyrene latex microspheres (Flu-

Figure 1. *Seriatopora caliendrum*, the coral used as a symbiont host in this study.

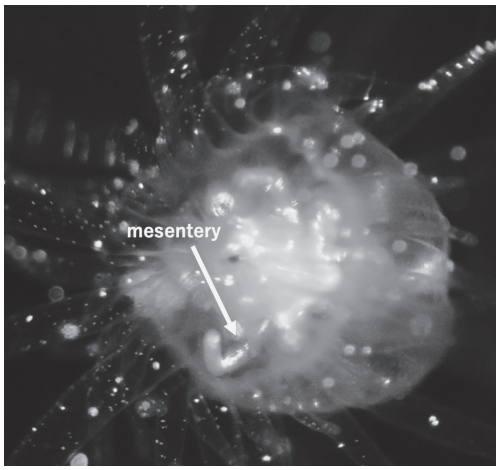

Figure 2. *Seriatopora*. Fluorescent microspheres and *Symbiodiniaceae* are found in the mesenterial filaments. Red spots: *Symbiodiniaceae*; Green-yellow spots: fluorescent microspheres.

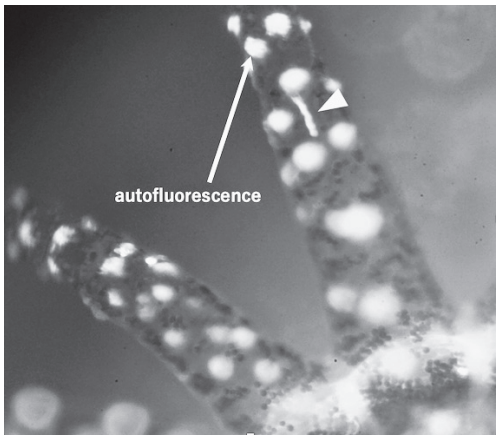

Figure 3. Fluorescent microspheres (arrow head) swept away in the lumen of tentacles of *Seriatopora caliendrum*. Red spots: *Symbiodiniaceae*. Tissue of tentacles showing the spots of autofluorescence.

oresbrite yellow-green carboxylate, Polysciences Inc.; diameter = 3 μm; 2.5% solids-latex and plastic, excitation maximum = 441 nm, emission maximum = 486 nm) were fed the nauplius of *Artemia*, which is a food source of *Exaiptasia* and *Seriatopora*. *Artemia* was placed in seawater containing microspheres (1×10^5 mL/1) and cultured for two days. Once the digestive tract of *Artemia* was filled with microspheres (Fig. 1 of Okubo et al., 2018), an *Artemia* mixed with *Symbiodiniaceae* (CS-164) was fed to each of the healthy and bleached primary polyps of *Seriatopora*. Both *Artemia* and *Symbiodiniaceae* were confirmed to be successfully localised in the gastric cavities and mesenteries of *Exaiptasia* and *Seriatopora* (Fig. 2).

After a few hours, the microspheres in the body flowed back and forth from the gastric cavity to the tentacles (Fig. 3). This is due to the water flow system of the anthozoans. During flowing, microspheres and *Symbiodiniaceae* were endocytosed from endodermal cells in the tentacle (Fig. 4a,b), whereas in the gastric cavity both were observed at the "Zone of Phagocytosis" in the mesenteries (Fig. 5). This indicates that symbiotic algae were infected from the same locations where the food was taken up.

To date, much attention has been paid to how zooxanthellae are endocytosed/exocytosed and how bleaching occurs; however, it is not known which parts of the tissue the symbionts enter. The original purpose of the Tokyo Keizai University Research Fellow Program (2020) was to determine why small microplastics are less likely to be ejected

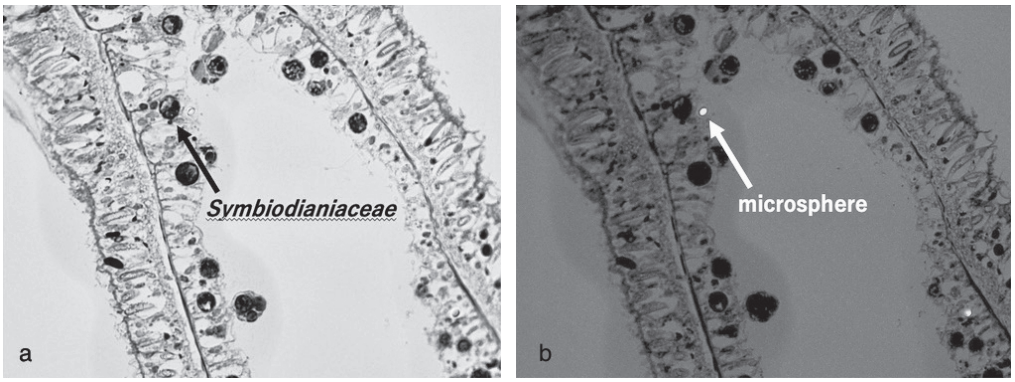

Figure 4. The tentacle of *Exaiptasia*. *Symbiodiniaceae* and fluorescent microspheres situated in the lumen and endocytosed endodermal cells. Image by a compound microscope (a) and by a fluorescence microscope (b).

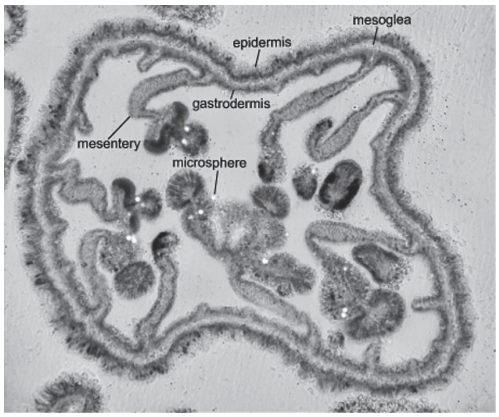

Figure 5. Fluorescent microspheres are taken up from the "Zone of Phagocytosis" of the mesenteries in the bleached *Exaiptasia*.

from cells. However, I occasionally found symbiont pathways in the anthozoan body during microplastic experiments (see also Okubo 2021). Symbiotic relationships may begin with occasional uptake of algae as food. This is a small discovery; however, the findings will be useful for studying the initiation of symbiosis in evolutionary history.

Acknowledgement

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