Marine Pollution Bulletin 135 (2018) 83–89

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Microplastics disturb the anthozoan-algae symbiotic relationship

Nami Okubo[a,*], Shunichi Takahashi[b], Yoshikatsu Nakano[c]

[a] *Department of Economics, Tokyo Keizai University, 1-7-34 Minamimachi, Kokubunji, Tokyo 185-8502, Japan*
[b] *Division of Environmental Photobiology, National Institute for Basic Biology, Nishigonaka 38, Myodaiji, Okazaki 444-8585, Japan*
[c] *Tropical Biosphere Research Center, Sesoko Station, Ryukyu University, Motobu, Okinawa 905-0227, Japan*

A R T I C L E I N F O

Keywords:
Microplastics
Sea anemone
Coral
Symbiodinium
Symbiosis

A B S T R A C T

World production of plastic has dramatically increased from the 1950's and now it reaches approximately 311 million tons per year. The resulting accumulation of small plastic detritus less than 5 mm in size, termed "microplastics", has started threatening the life cycles of marine organisms. Here we show the first evidence that microplastics disturb the initiation of symbiotic relationships in anthozoan-algae symbiosis. We found in both the aposymbiotic sea-anemone *Aiptasia* sp. and the coral *Favites chinensis* that the infectivity of symbiotic algae into the host is severely suppressed by microspheres fed either directly or indirectly through microsphere-fed *Artemia* sp. Similar trends were seen when microplastics collected from commercial facewash were used instead of microspheres. Therefore, ongoing accumulation of microplastics in the ocean might disturb the healthy anthozoan-algae symbiotic relationships, which are cornerstones of the biologically enriched coral reef ecosystem.

1. Introduction

More than 300 million tons of plastic are produced per year (PlasticsEurope, 2016). Models estimate at least 5.25 trillion plastic particles weighing 268,940 tons are currently floating at sea (Eriksen et al., 2014). Recently, the effects of plastic detritus, especially microplastics which are smaller than 5 mm in size, have been investigated for many animals in the marine environment (Taylor et al., 2016; Wright et al., 2013). Invertebrates, fish, turtles and other larger animals ingest plastic detritus and potential risks for organisms are predicted from cellular to population level (Bergami et al., 2016; Browne et al., 2008; Cole et al., 2013; Galloway et al., 2017; Oliveira et al., 2013; Sussarellu et al., 2016; Talsness et al., 2009). For example, the alimentary canal in birds was blocked and thus the food intake was limited (Pierce et al., 2004). Similarly, adult oysters with two months exposure to polystyrene microbeads showed significant reduction in sperm motility, fecundity, and oocyte sizes (Sussarellu et al., 2016). Furthermore, toxic chemicals, particularly hydrophobic drugs, can absorb to the plastic surfaces and thus microplastics work as a vector (Mato et al., 2001).

Coral reefs are not exceptions. More than 11 billion plastic items are entangled on coral reefs across the Asia-Pacific and the likelihood of coral disease increases when corals are wrapped in plastic (Lamb et al., 2018). Past studies show that corals take microplastics into the gastric cavity (Allen et al., 2017; Hall et al., 2015). These microplastics were wrapped in mesenterial tissues (Hall et al., 2015), however, their movement within and influence on corals are still unknown.

Reef-building coral species form the endosymbiotic relationship with dinoflagellate of the genus *Symbiodinium*. Most coral species recruit them from environment in each generation; their offspring are released without symbionts and uptake free-living *Symbiodinium* during early developing stages in both larvae and juveniles (Baird et al., 2009). The recruitment of *Symbiodinium* from environment occurs also in adult polyps (Baker, 2001), e.g., after losing symbionts due to thermally induced coral bleaching. Since corals rely most of energy on their symbionts, reef-building coral species cannot survive without successful symbiont recruitment. *Symbiodinium* include genetically diverse types and they are grouped into 9 clades from A to I (Pochon and Gates, 2010). Each *Symbiodinium* types, or sometimes clades, show different physiological characteristics, such as photosynthetic activity and heat and high light stress tolerances. Therefore, it is assumed that the recruitment of new *Symbiodinium* types is crucial for adapting environmental changes.

Recently, we found that the sea anemone incorporate microspheres into their host cells (Biquand et al., 2017). This finding provides the hypothesis that microplastics might interrupt the uptake of new *Symbiodinium* from environment. However, it has not been experimentally tested. In the present study, we examined the effects of microspheres and microplastics in commercial facewash, on the initiation of the symbiotic relationship in anthozoan-algae symbiosis. Our results demonstrated that these microparticles significantly suppress it, suggesting that increase in microplastics in coral reefs threatens the cornerstone of coral reef ecosystems through disturbing the coral-

* Corresponding author.
E-mail address: nokubo@tku.ac.jp (N. Okubo).

https://doi.org/10.1016/j.marpolbul.2018.07.016
Received 12 May 2018; Received in revised form 2 July 2018; Accepted 3 July 2018
0025-326X/ © 2018 Elsevier Ltd. All rights reserved.

Symbiodinium symbiosis.

2. Material and methods

2.1. Samples

The sea anemone *Aiptasia* sp. used in this study is a monoclonal strain originating from a single polyp and has been grown in the laboratory for 15 years. This species has *Symbiodinium* in the endodermal cells. *Aiptasia* was artificially bleached (aposymbiotic *Aiptasia*) by incubating them at 33 °C in complete darkness for 3 weeks and then returning them to normal growth temperature for more than 3 weeks before the experiment. Bleaching was confirmed by the inability to detect any symbiotic algae under a fluorescence microscope. The aposymbiotic sea anemones were incubated at a continuous temperature of 25 °C in complete darkness. Freshly hatched *Artemia* nauplii were fed to *Aiptasia* once a week and the seawater was changed after feeding.

The primary coral polyps were obtained from the stony coral *Favites chinensis* in Sesoko Island, Okinawa, Japan. The three colonies were taken with the permission of Okinawa Prefecture Government and incubated in the Sesoko Marine Station at University of the Ryukyus. They spawned on 24 June 2016; egg-sperm bundles were collected and incubated for a few hours to allow fertilization to occur. Embryos then were transferred to fresh seawater that had been filtered through a 0.22 µm filter (Merck Millipore). Developed planula larvae were maintained at 25 °C and metamorphosis was naturally induced without adding any chemicals. All the primary polyps were cultured in filtered artificial seawater (sea salt; Sigma-Aldrich) until use.

Symbiodinium cells CS164 were grown in filtered (0.22 µm pore filter; Steritop-GP Filter Unit, Merck Millipore) artificial seawater (sea salt) containing Daigo's IMK medium for marine microalgae (Wako). *Symbiodinium* cells were grown at a continuous temperature of 23 °C under photosynthetically active radiation (PAR) at 80 µmol photons $m^{-2} s^{-1}$ with 12 h light (white color, fluorescence tubes) in a day. *Symbiodinium* cells that had reached the mid-logarithmic phase of growth were harvested by centrifugation at 2000 ×*g* for 3 min and suspended in fresh seawater media.

2.2. Incorporation of fluorescent microspheres into the planktons, the sea anemone Aiptasia sp. and the coral Favites chinensis

Fluoresbrite carboxylate yellow-green microspheres with diameters of 3, 6, and 11 µm (Polysciences, Inc., 2.5% solids-latex, excitation max. = 441 nm; emission max. = 486 nm) were used instead of microplastics except of the experiment using commercial microplastics.

Artemia nauplii, *Pontogeneia* sp. and *Calanus* sp. were cultured in the seawater containing 1.5×10^5 microspheres of 3.1 µm diameter per mL. The digestive tract of *Artemia* nauplii was easily seen to be full of microspheres compared to the other two species, thus *Artemia* nauplii of five individuals with microspheres were fed to *Aiptasia* in the experiment of Fig. 1e–i.

For the experiments of Figs. 1a–d, 2 and 3, about 2.2×10^4 of microspheres suspended in 4 µl of fresh water were mixed with 5 fresh *Artemia* nauplii. This paste was formed into a pellet of approximately 1 mm in diameter. A single pellet was given to tentacles of individual *Aiptasia* by using forcep (n = 3 for each treatment).

We also tested the effect of microplastics on symbiosis in corals using an aposymbiont primary polyp of *Favites chinensis* before symbiosis started after settlement (n = 21, Fig. 4). For corals, yellow-orange fluorescent microspheres (Polysciences, Inc., 2.5% solids-latex, excitation max. = 529 nm; emission max. = 546 nm) with the diameter of 3 µm were used, because *F. chinensis* has similar auto-fluorescence as yellow-green microspheres around the mouth. Individual *Artemia* nauplii (approximately 0.1 mm in diameter) were cut in half, mixed with microspheres, and fed to the primary polyps. At the same time, a shrimp pellet without microspheres was fed to the aposymbiont primary polyps as controls without microspheres (n = 22).

2.3. Infection of Symbiodinium

Thick agglomerates of *Symbiodinium* CS164 (ca. 5×10^4 cells) mixed with five freshly chopped *Artemia* nauplii were sucked up by a pipette and then gently blown on the mouth and tentacles of *Aiptasia*. Pellets made from a mixture of *Symbiodinium* and *Artemia* did not work for the experiments because *Symbiodinium* was killed while drying the pellets.

2.4. Incorporation of microplastics of commercially available facewash into Aiptasia

We also tested the effects, if any, of microplastics in a commercially available facewash on the symbiosis (Fig. 5). The paste of 1 cm in diameter and 5 cm in length of facewash was diluted with 0.5 L warm water and the supernatant was filtered through 0.22 µm Millipore filter. The particles recovered from the filter were suspended in 1 L warm water, and this procedure was repeated twice. The microplastics (ca. 3.5×10^3, the average size was 17.3 µm from 3 to 60 µm of size gating) thus obtained were mixed with five freshly chopped *Artemia* nauplii, and the mixed pellets of approximately 1 mm in diameter were fed by using forceps to aposymbiotic *Aiptasia* (n = 4) once a day for two days. The *Artemia* pellets without microplastics were fed similarly to the controls (n = 3).

2.5. Quantification of microspheres and Symbiodinium incorporated into the sea anemone and coral

The numbers of microspheres and *Symbiodinium* were counted with an Olympus cell counter model 1. Incorporation of microspheres into *Aiptasia* was confirmed by imaging yellow-green fluorescence using a fluorescence stereomicroscope (Olympus SZX16) fitted with a GFP (excitation: 460/90 nm, emission: 510 nm) and *Favites* by yellow-orange fluorescence using RFP1 filter (excitation: 530/50 nm, emission: 575 nm) because *Favites* have auto fluorescence of GFP. Incorporation was frequently monitored using a digital camera (EOS 600D, Canon). The *Aiptasia* polyp was determined to be infected when more than 10 foci of *Symbiodinium* could be seen within a tentacle.

For the measurement of Fig. 5f we followed the method by Krediet et al. (2013). An *Aiptasia* polyp was placed in 1 mL of distilled water containing 0.01% sodium dodecyl sulphate (SDS) (Sigma-Aldrich) in a 1.5 mL tube and homogenized with a VH-10 violamo homogenizer (VIOLAMO) with the S10N05G (IKA) attachment at 30,000 rpm for 10 s on ice. The homogenate was further homogenized by passage five times through a 25-gauge needle affixed to a 1 mL syringe. The number of *Symbiodinium* cells in the homogenate was then estimated by using an Attune Flow Cytometer (Life Technology). The homogenate was also used to measure the total protein content using the Pierce BCA protein assay kit (Thermo Scientific). Then, the *Symbiodinium* cell number in an *Aiptasia* polyp was normalized by protein content, because the polyp size corresponds with the total protein content.

3. Results

(a) Incorporation of microspheres

We confirmed that microspheres were incorporated into the host cells of aposymbiotic *Aiptasia*, as reported by Biquand et al. (2017) (Fig. 1a-d). Each pellet was successfully moved from the mouth into the gastric cavity (Fig. 1b) and incorporated into the mesenterial filaments (Fig. 1c). The incorporated microspheres gradually dispersed throughout the body and tentacles (Fig. 1d). At this stage, microspheres were within the host cells. Next, we examined whether microspheres

Download English Version:

https://daneshyari.com/en/article/8870618

Download Persian Version:

https://daneshyari.com/article/8870618

Daneshyari.com