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研究課題名（英文）Impacts of multi-specific blooms of harmful algae (HAB) on reproduction of bivalves

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研究成果の概要（和文）：数種類の赤潮原因植物プランクトンは日本産真珠貝の初期発生の段階に影響を与えることがしられている。養殖場でしばしば数種のプランクトンが同時にブルーミングを形成することがある。そこで、本研究では孵化後1日、6日、16日目の幼生に対して、複数の赤潮原因プランンクトンと共存することでどのような影響を受けるかについて調べた。具体的には、幼生を次の４種のプランクトンのうちの２種類と共存させ、成長に与える影響を調べた。その結果、次の４種の組み合わせが成長に悪影響を与え、高齢になるほど影響を受けやすかった。このような複数の赤潮を共存することが、二枚貝の発生に影響を与えることを示した世界初の研究結果である。

研究成果の概要（英文）：In previous studies, we have shown that several species of harmful algae affect the early-life development of Japanese pearl oyster, Pinctada fucata martensii. In the present study, the effects of HAB species forming multi-specific blooms in the culture area of Japanese pearl oyster on 1 day-old, 6 day-old, and 16 day-old larvae were tested. Larvae were affected by exposure to H. circularisquama, C. marina or K. mikimotoi. Mixed blooms of K. mikimotoi and K. papilionacea, K. mikimotoi and C. marina, H. circularisquama and C. marina and, H. circularisquama, C. marina and K. mikimotoi differentially affected the three stages of larvae with increased sensitivity with elder larvae. This is the first study to examine effects of multispecific bloom-forming species of harmful algae on reproduction of a representative bivalve mollusc. The effect reported warrant further investigation.

研究分野：Harmful Algal Blooms

キーワード：Harmful Algal Blooms　Japanese pearl oyster　Reproduction　Karenia spp.　Chattonella marina　H. circularisquama　Alexandrium spp.

Impacts of multi-specific blooms of harmful algae (HAB) on reproduction of bivalves

1. 研究当初の背景

Several species of microalgae forming harmful algal bloom in Japan have been shown to affect the reproduction of bivalve molluscs (Landsberg, 2002). Recent research has reported that the early-life stages of Japanese pearl oyster, *Pinctada fucata martensii*, are differentially affected by species of noxious and toxic dinoflagellates and raphidophytes forming blooms in the major cultivation areas of pearl oysters in Japan (Basti et al., 2015a). The shellfish-killer, *Heterocapsa circularisquama* has been shown to severely affect gaemetes and all early development stages of the oysters (Basti et al., 2011; 2013). The cosmopolitan species of dinoflagellates *Alexandrium affine, A. catenella, Karenia mikimotoi* and *K. papilionacea* affect both the fertilization and embryo development of the oysters, and the early- and late-stage larvae (Basti et al. 2015b). In addition, the raphidophytes *Heterosigma akashiwo*, *Chattonella marina* and *Chattonella antiqua* have recently been found to affect larvae of the oysters (Basti et al., 2016). Most of these harmful species form recurrent mono-specific blooms in Japan; however, records of blooms where two to three species of the harmful algae co-occur have been reported in the culture areas of the oysters during their reproductive season. The aim of the present project was to assess the effects of muti-specific blooms on the early-life development of bivalve molluscs, using the Japanese pearl oyster as a bivalve model.

2. 研究の目的

The objectives of the study were to assess the responses of early-developmental stage of bivalves, using Japanese pearl oysters as model organism, to exposures to multi-specific blooms of harmful algae that has been recently increasing in frequency of occurrence in areas of bivalve culture. The species of harmful algae that were considered are *Heterocapsa circularisquama*, *Chattonella marina*, *Karenia brevis*, and *Karenia papilionacea*.

3. 研究の方法

3.1. Rearing of the oysters

Sexually mature male and female pearl oysters, *Pinctada fucata martensii*, were reared at the K. Mikimoto & Co, Ltd. farm located in Ago Bay, Mie Prefecture, Japan. Gametes were stripped from the gonads, activated with ammonia seawater, and fertilization was carried out as described in previous publications (Basti et al., 2011; 2013).

3.2. Microalgae culture

The activities of 6-day old larvae exposed to *H. circularisquama* and *C. marina* were also affected, following 24, 48 and 72 h of exposure (Fig. 5). Similar to the results with 1 day-old larvae, exposures to all three mixtures of *K. mikimotoi* and *K. papilionacea* and to mixtures of *K. mikimtoi* and *C. marina*, where *K. mikimotoi* was dominant (75% of the total cell density), resulted in a further decrease in the activities of the larvae.

The dinoflagellates *Heterocapsa circularisquama*, *Karenia papilionacea*, and *Karenia mikimotoi* and the raphidophyte *Chattonella marina* were grown in autoclaved F/2 medium. All cultures were maintained under a 12 h light: 12 h dark photoperiod, at 23 °C.

3.3. Mixed-bloom experiments

Gametes, fertilized eggs, and three larval stages (1 day-old larvae, 6 day-old larvae, and 16 day-old larvae) were used for the experiments. Gametes, fertilized eggs and the three larval stages were exposed to either one species or to two to three species of harmful algae, at realistic cell densities, as shown in Table 1. In all experiments, the maximum cell density of the harmful algae was set at 10^4 cells ml^{-1}. Except for the exposures to the mixture of *H. circularisquama*, *K. mikimotoi*, and *C. marina* which reflect the dominance of *H. circularisquama* in such a mixture in nature, the other experiments of exposure to mixtures of *K. mikimotoi* and *K. papilionacea*, *K. mikimotoi* and *C. marina*, and *H. circularisquama* and *C. marina* were conducted considering three scenarios of cell densities. All experiments were conducted in triplicates, in 12-, 24- or 48- well plates, for 1 h to 72 h. In addition to direct observation of the effects, samples were regularly taken and preserved immediately either at -80 °C or in 4% formalin solution for later analyses.

4. 研究成果

The effects of the exposures to the multi-specific blooms were variable, depending on the species and the densities of each species of the harmful alga. For larvae, the exposure experiments to both the mono-specific and multi-specific harmful algae did not lead to mortalities and the survival rates for all three

Table 1. Details of the different exposure conditions of harmful algae adopted in this study

Treatments	Abbreviation	Cell densities (cells ml^{-1})
Seawater	*SW*	0
Culture medium	*F/2*	0
Heterocapsa circularisquama	*H*	1×10^4
Chattonella marina	*Cm*	1×10^4
Karenia mikimotoi	*Km*	1×10^4
Karenia papilionacea	*Kp*	1×10^4
Heterocapsa circularisquama and *Chattonella marina*	*HCm1* *HCm2* *HCm3*	7.5×10^3 *H. circularisquama* + 2.5×10^3 *C. marina* 5×10^3 *H. circularisquama* + 5×10^3 *C. marina* 2.5×10^3 *H. circularisquama* + 7.5×10^3 *C. marina*
Karenia mikimotoi and *Karenia papilionacea*	*KmKp1* *KmKp2* *KmKp3*	7.5×10^3 *K. papilionacea* + 2.5×10^3 *K. mikimotoi* 5×10^3 *K. papilionacea* + 5×10^3 *K. mikimotoi* 2.5×10^3 *K. papilionacea* + 7.5×10^3 *K. mikimotoi*
Karenia mikimotoi and *Chattonella marina*	*KCm1* *KCm2* *KCm3*	2.5×10^3 *C. marina* + 7.5×10^3 *K. mikimotoi* 5×10^3 *C. marina*

		+ 5×10^3 *K. mikimotoi* 7.5×10^3 *C. marina* + 2.5×10^3 *K. mikimotoi*
Heterocapsa circularisquama and *Karenia mikimotoi* and *Chattonella marina*	*HKCm*	8.5×10^3 *H. circularisquama* + 1×10^3 *K. mikimotoi* + 5×10^2 *C. marina*

larval stages were well above 90%, within the exposure time.

The activity rates of the larvae, however, were affected by both mono-specific and multi-specific exposures . The activity rates of 1 day-old larvae exposed to *Heterocapsa circularisquama* were particularly decreased (Fig. 4). The activities of larvae exposed to *Chattonella marina*, and to a lesser extent those of larvae exposed to *Karenia mikimotoi*, were also affected. Exposures to all three mixtures of *K. mikimotoi* and *K. papilionacea* and to mixtures of *K. mikimtoi* and *C. marina*, where *K. mikimotoi* was dominant (75%) of the total cell density, resulted in a further decrease in the activities of the larvae.

The activity of 16 day-old larvae were the most affected by exposures to the harmful algae. A 24 h-exposure to *H. ciruclarisquama*, *C. marina*, and *K. mikimotoi* resulted in decreased activity rates. Additionally, exposure to mixtures of *H. circularisquama* and *C. marina*, *K. mikimotoi* and *K. papilionacea*, and *K. mikimotoi* and *C. marina* dominated by *K. mikimotoi*, resulted in decreased activities. The 16 day-old larvae that were exposed for 48 h to 72 h were additionally more sensitive in terms of activity to mixtures of *K. mikimotoi* and *C. marina*, and to the mixed blooms of *H. circularisquama*, *C. marina* and *K. mikimotoi*. The sensitivity of larvae of pearl oysters to *H. circularisquama*, *C. marina* and *K. mikimotoi* reported in previous studies was confirmed in the present study. Additionally, negative effects on larvae exposed to mixed blooms were shown. In particular, elder larvae that could have fed on the harmful algae were more sensitive to more mixed blooms than younger larvae.

The present study is the first attempt to understand the responses of early-life stages of a bivalve mollusc to mixed blooms of harmful algae that occur in the natural environment during the reproductive season of the bivalve. Pearl oysters larvae showed differential sensitivity to the exposure to all mixed blooms depending on their age and therefore to their physiological state. Further results are being confirmed with other developmental stages, and research looking into the impacts of multispecific blooms of harmful algae on aquatic organism should be considered.

5. 主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

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6. 研究組織

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