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Harmful algal blooms affect early-life stages of Japanese pearl oyster, *Pinctada fucata martensii*

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Abstract

Pearl oysters, *Pinctada fucata martensii*, are commercially important bivalve molluscs in Japan. Their spawning and larval development seasons are coincident with harmful algal blooms (HAB), both toxic and noxious. The effects of experimental exposures to *Heterocapsa circularisquama*, *Alexandrium affine*, *Alexandrium catenella*, *Karenia mikimotoi*, *Karenia papilionacea*, *Heterosigma akashiwo*, *Chattonella marina* and *Chattonella antiqua*, on early embryo development and larvae of pearl oysters were studied. The dinoflagellates *Alexandrium affine*, *A. catenella*, and *H. circularisquama* affected embryo development. Complete inhibition of early embryo division was induced by non-PST producer *A. affine* and PST-producer *A. catenella* (saxitoxin and gonyautoxin-3). D-larvae were affected only by exposure to *H. circularisquama*. The results of the present study show that *H. circularisquama* is not the only species of HAB that affect pearl oyster early life history. Further studies are required to assess the effects of *A. affine*, *A. catenella* and other recurrent HAB in the cultivation areas of *P. fucata martensii*.

Keywords: embryo, larvae, pearl oysters, HAB, dinoflagellates, raphidophytes

Introduction

Harmful algal blooms (HAB) affect aquatic organisms via physical contact, production of toxins, and/or increased biomass (Landsberg 2002). Mass mortalities to sub-lethal effects have been well-documented in several aquatic organisms, including mammals, seabirds, fish, and shellfish, during HAB events. The impacts of HAB on early-life stages of commercially and ecologically important bivalve molluscs are largely unknown. Most of the studies considered the effects of harmful dinoflagellates on Trochophores and D-larvae of commercially important species of Pacific and eastern oysters, *Crassostrea gigas* and *Crassostrea virginica*, hard clams, *Mercenaria mercenaria*, and king and bay scallops, *Pecten maximus* and *Argopecten irradians* (Fig. 1).

In Japan, farms of the pearl oyster, *Pinctada fucata martensii*, are located in areas prone to recurrent HAB development and associated fisheries damage induced by several species of dinoflagellates and raphidophytes (Fig. 2). The toxic dinoflagellate *Heterocapsa circularisquama* is considered the highest threat to pearl oyster industry due to its potent toxicity to juvenile and adult pearl oysters (Matsuyama 2012), and its extensive cytotoxicity at low cell density to the early-life development of Japanese pearl oysters have already been shown (Basti et al. 2011, 2013). However, the effects of other HAB species that form blooms during the reproductive season of pearl oyster were not studied.

In the present paper, the effects of *Alexandrium* spp. *Karenia* spp., *Heterosigma akashiwo*, and *Chattonella* spp. on early-embryos and larvae of Japanese pearl oyster, *Pinctada fucata martensii*, were investigated. Additionally, *H. circularisquama* was also tested and used as a comparative reference.

Material and Methods

The exposure experiments were conducted at the facilities of K. Mikimoto and Co. LtD., Mie Prefecture, Japan. Eggs and spermatozoa were stripped from the gonads of sexually mature male and female pearl oysters, and fertilization was artificially induced (Basti et al. 2011, Basti et al. 2013). Freshly fertilized eggs were exposed to *Alexandrium affine* and *A. catenella* (5×10^2 cells/mL), *Heterocapsa circularisquama* (10^3 cells/mL), *Karenia mikimotoi* and *K. papilionacea*

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(10^4 cells/mL), *Heterosigma akashiwo* (5×10^4 cells/mL), and *Chattonella marina* and *C. antiqua* (10^3 cells/mL), in 6-well plates (50 eggs/mL, 5 mL/well), in duplicate.

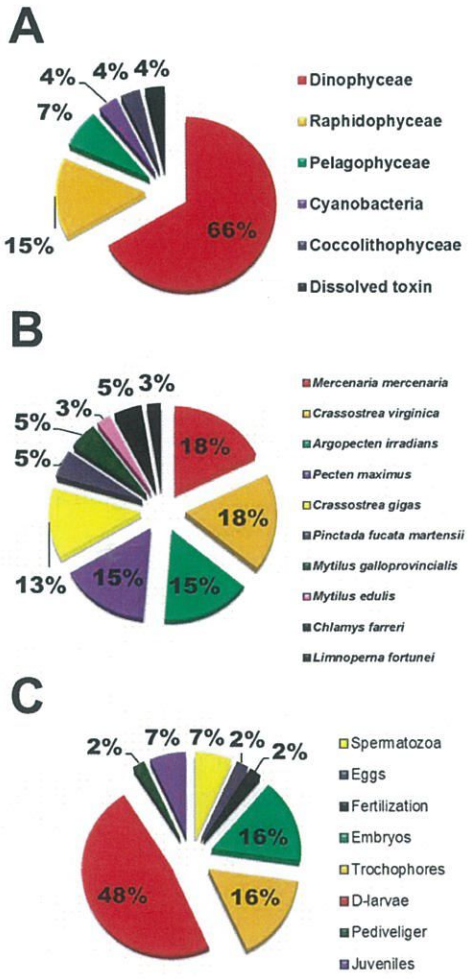

Fig. 1. Literature review of the effects of harmful algae on the early-life development of bivalves (1974–2013). (A) species of harmful algae, (B) species of bivalves, (C) developmental stages.

The harmful algae were cultured in F/2 or modified SW3 media, at 23°C, under a 12hL:12hD photocycle, and then their cells were harvested at the early to mid-stationary phases and added to the experimental seawater, at the desired cell densities.

Molecular confirmation of the strains of *A. affine*, *A. catenella*, *K. mikimotoi*, and *K. papilionacea* were conducted according to Nagai (2011) and Nagai et al. (2008, 2012). The profiles of paralytic shellfish toxins of *A. affine* and *A. catenella* were analyzed according to Ravn et al. (1995) and Oshima (1995).

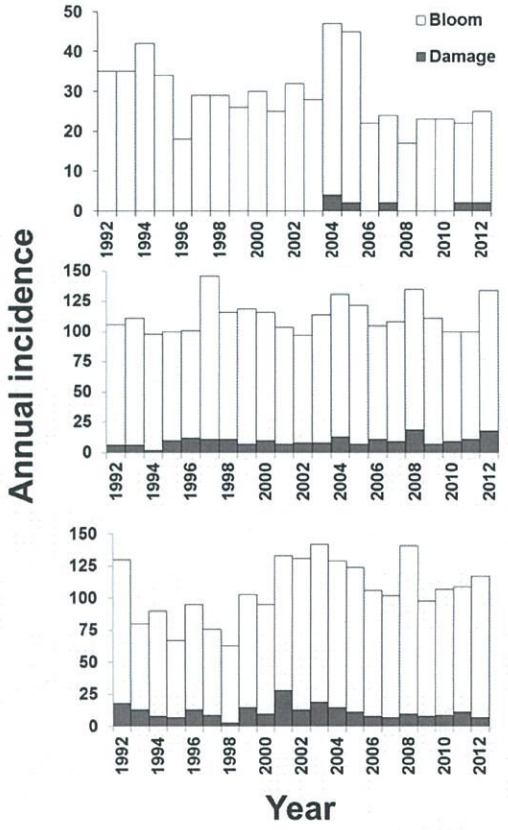

Fig.2. History of harmful algal blooms and associated fisheries damage in the cultivation areas of Japanese pearl oysters, *Pinctada fucata martensii*. (A) Mie Prefecture, (B) Seto inland Sea, (C) Kyushu Island.

The experiments were conducted at 23°C, in filtered and UV-treated seawater. The development of 4-cell embryos from fertilized eggs exposed to each harmful alga was assessed following 60 min of exposure.

In another set of experiments, D-larvae (24 h old) were exposed to the same harmful algae, at the same densities and under the same experimental conditions, to assess the effects on their swimming activity and mortality, within 24 h. In addition, 16 days-old eye-spot larvae were exposed to *H. circularisquama* at several densities to assess the effects on the activity and mortality following 24, 48, and 72 h of exposure to 10, 10^2, 5×10^2, and 10^3 cells/mL (Fig. 3).

The activity of both D-larvae and eye-spot larvae was assessed as the percentage of swimming larvae 5 min following gentle agitation of the well plates and resuspension of the larvae. Larvae were considered dead when there was complete absence of velar activity for 20s. All results were statistically compared (ANOVA and Student's *T*-test).

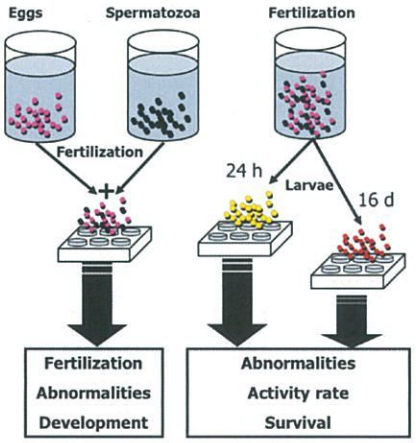

Fig.3. Schematic representation of the experimental protocol.

Results and Discussion

Exposure of freshly fertilized eggs to *Heterocapsa circularisquama*, *Alexandrium affine*, and *A. catenella* resulted in significant decrease in the percentage of 4-cell embryos following 60 min. In particular, exposures to *A. affine* and *A. catenella* resulted in a complete inhibition of embryo division and all fertilized eggs failed to reach the 4-cell embryo stage. Other harmful dinoflagellates and raphidophytes had no significant effects on early cleavage of Japanese pearl oysters (Table 1).

The activity of D-larvae was significantly decreased, and the mortality was significantly increased, only following 24 h of exposure to *H. circularisquama* (Table 2). Other harmful dinoflagellates and raphidophytes did not affect the activity and survival of D-larvae.The activity of eye-spot larvae (16 days-old) was significantly decreased following 24–72 h of exposure to *H. circularisquama* at 10–10^3 cells/mL (Fig. 4).

Table 1. Percentage of 4-cell embryos developed from fertilized eggs of *Pinctada fucata martensii* exposed to harmful algae.

HAB species	Density (cells/mL)	4-cell embryos
Heterocapsa circularisquama	10^3	74.5±1.9 *
Alexandrium affine	5×10^2	0.0±0.0 *
Alexandrium catenella	5×10^2	0.0±0.0 *
Karenia mikimotoi	10^4	39.3±7.5
Karenia papilionacea	10^4	43.5±6.5
Heterosigma akashiwo	5×10^4	69.7±7.2
Chattonella marina	10^3	55.0±7.1
Chattonella antiqua	10^3	90.0±10.0

(*) marks significance difference from respective control (P <0.05).

Table 2. Effects of harmful algae on activity (%) and survival (%) of D-larvae of *Pinctada fucata martenssi.*

HAB species	Density (cells/mL)	Activity	Mortality
Heterocapsa circularisquama	10^3	38.2±2.1*	15.2±1.5*
Alexandrium affine	5×10^2	97.5±1.0	0.1±0.0
Alexandrium catenella	5×10^2	94.9±0.2	0.0±0.0
Karenia mikimotoi	10^4	88.1±3.8	0.9±0.4
Karenia papilionacea	10^4	95.9±0.3	0.6±0.1
Heterosigma akashiwo	5×10^4	87.0±1.2	1.6±0.7
Chattonella marina	10^3	78.8±3.8	6.1±1.6
Chattonella antiqua	10^3	98.6±0.2	0.3±0.0

(*) marks significance difference from respective control (P <0.05).

Although mortalities were not observed, 45% of the eye-spot larvae exposed to 10^3 cells/mL of *H. circularisquama* for 72 h showed extensively lysed velum and hypersecretion of mucus when in contact with *H. circularisquama* cells (Fig. 5), as observed in a previous study with D-larvae of Japanese pearl oysters (Basti et al. 2011).

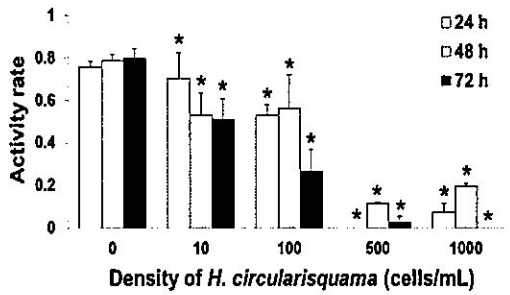

Fig. 4. Activity rate of eye-spot larvae exposed to toxic dinoflagellate *Heterocapsa circularisquama*. For (*) marks significance difference from respective control (P <0.05).

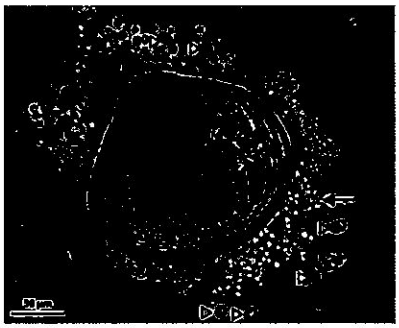

Fig. 5. Lysed velum (black arrow) of eye-spot larvae of Japanese pearl oyster, *Pinctada fucata martensii*, exposed to *Heterocapsa circularisquama* (72 h, 10^3 cells/mL). V: velum, arrow heads: vegetative cells of *H. circularisquama*.

The cosmopolitan harmful raphidophytes *H. akashiwo*, *C. marina*, and *C. antiqua*, and dinoflagellate *K. mikimotoi* are known cosmopolitan HAB associated with recurrent fish kills. Their exact mechanisms of toxicity are still not well understood. Although several toxins and metabolites were shown to affect fish, and in the case of *K. mikimotoi* shellfish, these had no effects on early embryos and larvae of Japanese pearl oysters, at the densities and exposure durations considered.

The most severe effects were observed for exposures to *Alexandrium* spp. and *H. circularisquama*. The strain of *A. affine* used in the present study was negative for PST, and *A. catenella* produced both saxitoxin (STX) and gonyautoxin-3 (GTX3); results showing the negative effects on embryo is not related to the production of paralytic shellfish toxins. In previous studies, detrimental effects on early-life stages of bivalve molluscs were found for a few species of *Alexandrium* that were also not necessarily associated with known toxins and other compounds (Matsuyama et al. 2001; Yan et al. 2001, 2003). In conclusion, the results of this study showed that, although *H. circularisquama* is the most potent HAB species tested to date for the early-life development of Japanese pearl oysters, *A. affine* and *A. catenella* severely affect embryos. Further detailed studies are necessary to address the effects of HAB on the reproduction and early-life development of Japanese pearl oyster.

References

Basti, L., Go., J., Higuchi, K. et al. (2011). J. Shellfish R. 30: 177–186.
Basti, L., Nagai, K., Tanaka, Y. & Segawa, S. (2013). Mar. Biol. 160: 211–219.
Landsburg, J.H. (2002). Rev. Fish. Sci. 10: 113–390.
Matsuyama, Y., Usuki, H., Uchida, T. & Kotani, Y. (2001). In: Harmful Algae Proceeding of the 9th ICHA, Reguera, B., Blanco, J, Fernandez, M.L. & Wyatt, T., UNESCO, Paris, pp. 411–414.
Matsuyama, Y. (2012). Harmful Algae 14:144–155.
Nagai, S. (2011). J. Phycol. 47: 703–708.
Nagai, S., Nishitani, G., Tomaru, Y. et al. (2008). J. Phycol. 44: 909–922.
Nagai, S., Yamamoto, K., Hata, N. & Itakura, S. (2012). Mar. Genomics 7: 51–56.
Oshima, Y. (1995).J. OACA Int. 78:528–532.
Ravn, H., Anthoni, U., Christophersen et al. (1995). J. Appl. Phycol. 7: 589–594.
Yan, T., Zhou, M., Fu, M. et al. (2001). Toxicon 39: 1239–1244.
Yan, T., Zhou, M., Fu, Yu, R., M. et al. (2003). Aquaculture 217: 167–178.