



NOTE

Inhibitory effects of four typical bloom-forming algae species on metamorphosis of the abalone *Haliotis discus hannai*

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ABSTRACT: In recent years, harmful algal blooms (HABs) have occurred frequently along the coast of China and have caused massive shellfish mortality. However, little is known about the impacts of HABs on the metamorphosis of shellfish. In this study, the effects of 4 typical harmful algae species present in Chinese waters (*Karenia mikimotoi*, *Alexandrium catenella*, *Aureococcus anophagefferens*, and *Prorocentrum donghaiense*) on the survival and metamorphosis of abalone *Haliotis discus hannai* larvae were investigated. After 96 h of treatment, un-metamorphosed larvae (veligers) were significantly affected by *K. mikimotoi*, *A. catenella*, and *P. donghaiense*, as mean \pm SD survival rates were 5.2 ± 2.1 , 27.7 ± 1.4 , and $63.8 \pm 6.9\%$, respectively, compared with $86.0 \pm 4.1\%$ for the control treatment (the non-toxic alga *Skeletonema costatum*). Survival rates of metamorphosed larvae were significantly affected by *K. mikimotoi*, *A. catenella*, and *A. anophagefferens* (4.5 ± 2.8 , 22.3 ± 5.8 , and $26.5 \pm 3.6\%$, respectively, compared with $46.2 \pm 1.3\%$ for the control), and metamorphosis was significantly inhibited by *K. mikimotoi* and *A. catenella* (5.0 ± 2.2 and $24.5 \pm 3.1\%$, respectively, compared with $49.3 \pm 1.5\%$ for the control). *A. anophagefferens* was more toxic to metamorphosed larvae than to un-metamorphosed larvae. These results suggest that all 4 species of harmful algae have detrimental effects on the metamorphosis of *H. discus hannai* and that *K. mikimotoi* and *A. catenella* are more toxic to abalone larvae than *A. anophagefferens* and *P. donghaiense*.

KEY WORDS: Harmful algae · *Haliotis discus hannai* · Metamorphosis · Toxic impact

1. INTRODUCTION

In recent decades, harmful algal blooms (HABs) have occurred continuously in coastal waters of China, and are characterized by their high frequency

of occurrence, large scale, and diverse species (Yu et al. 2018). HABs have caused substantial financial losses to the marine aquaculture industry due to frequent mortalities of valuable fish and shellfish (Yu et al. 2018). For example, *Karenia mikimotoi* blooms

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caused losses of more than 2 billion yuan (US \$290 million) to the abalone culture industry along the coast of Fujian Province in 2012 (Li et al. 2017). *Aureococcus anophagefferens* blooms were responsible for large-scale diapauses and mortalities of the bay scallop *Argopecten irradians*, which resulted in 200 million yuan (US \$29 million) in losses along the coast of Qinhuangdao in the Bohai Sea (Zhang et al. 2012). Annual *Alexandrium* spp. and *Prorocentrum donghaiense* blooms also threaten the local marine aquaculture industry in the Changjiang River estuary (Gu et al. 2013, Lin et al. 2014). Many harmful algae species are toxic to abalone, and they can damage tissues, affect immune activities, and impact survival rates (Botes et al. 2003, Shi et al. 2012, Lin et al. 2016a). However, the effects of harmful algae on metamorphosis, a critical process for larval abalone, have not been described.

Metamorphosis is a critical process in the life cycle of shellfish; it plays an important role in shellfish population dynamics and determines the success of their breeding in culture and reproduction in the wild (Burke 1983, Hadfield & Paul 2001). Metamorphosis of both cultured and wild abalone in China occurs from April to July, which coincides with the period of high incidence of HABs. Moreover, the culture area for abalone in China is located mainly along the coast of the Bohai Sea and the East China Sea, where HABs occur frequently every year (Yu et al. 2018). HABs may inhibit activity of shellfish larvae or cause direct shellfish larval mortality (Tang & Gobler 2012, Basti et al. 2015a,b). Therefore, HABs can be a great threat to metamorphosis of both cultured and wild abalone.

To explore whether and how harmful algae species affect metamorphosis of abalone *Haliotis discus hannai*, 4 typical species present in Chinese waters (*K. mikimotoi*, *Alexandrium catenella*, *Aureococcus anophagefferens*, and *P. donghaiense*) were selected and their effects on survival and metamorphosis of abalone at the late veliger to peristome larval stages examined. Non-toxic *Skeletonema costatum* served as the control algal species.

2. MATERIALS AND METHODS

Abalone larvae were maintained in corrugated plastic plates, with diatoms as the food source. All test algae were isolated from coastal waters of China and cultured in IOCAS with sterilized f/2 seawater medium (Guillard & Ryther 1962) at a temperature of $20 \pm 1^\circ\text{C}$, pH of 8.09 ± 0.05 , and salinity of

30 ± 1 ppt. Cultures were kept under a 12:12 h photoperiod using cool-white fluorescent lamps (2650 ± 100 Lux).

The corrugated plates were cut and transferred into plastic petri dishes (9 cm diameter). Late veliger stage abalone larvae were counted under a dissecting scope, and 200 individuals were added to each dish. Algae were counted using a hemocytometer under a microscope and diluted to 60 ml at different densities (*Karenia mikimotoi*, *Alexandrium catenella*, *Aureococcus anophagefferens*, *Prorocentrum donghaiense*, and *Skeletonema costatum* at 5×10^3 , 4×10^3 , 1×10^7 , 4.4×10^4 , and 2.3×10^5 cells ml^{-1} , respectively). The density of *K. mikimotoi* was chosen as the common bloom density along the coast of China, and the densities of the other algae were set based on their cell size to create a biomass equal to that of *K. mikimotoi*; these densities were also roughly similar to their common bloom densities. The non-toxic alga *S. costatum* served as the control, as this species is not directly toxic, especially at a low density (Lin et al. 2016b), and seawater alone was used as the blank. Each treatment was replicated 3 times.

All larvae were observed under a microscope after 96 h. Those individuals showing no stress reaction (i.e. no movement in response to stimulation with a needle) were considered to be dead, and those with a shell were considered to have metamorphosed. Mortality was calculated as the total percentage of individuals that died, and total metamorphosis was calculated as the percentage of all larvae that had metamorphosed. Final metamorphosis totals were calculated as the percentage of metamorphosed individuals that survived. Experimental data were analyzed using 1-way ANOVA followed by a Dunnett's test, and tests were conducted using SPSS 19.0. Sample differences were considered to be statistically significant at $p < 0.05$ and highly significant at $p < 0.01$. Values are presented as means \pm SD.

3. RESULTS

After 96 h of incubation, both survival and metamorphosis of *Haliotis discus hannai* were influenced by all 4 algae tested. Final metamorphosis was significantly inhibited (ANOVA, $F_{5,12} = 111.27$, $p < 0.01$) by *Karenia mikimotoi*, *Alexandrium catenella*, and *Aureococcus anophagefferens* (Fig. 1). Percentage of surviving metamorphosed individuals in the *K. mikimotoi* treatment was the lowest ($4.5 \pm 2.8\%$), followed by the *A. catenella*

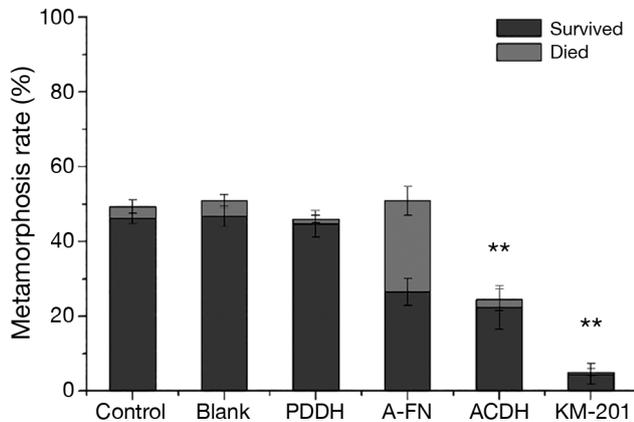


Fig. 1. Mean percentage of live and dead abalone *Haliotis discus hannai* larvae which had metamorphosed at 96 h when treated with *Karenia mikimotoi* (IOCAS code: KM-201), *Alexandrium catenella* (ACDH), *Prorocentrum donghaiense* (PDDH), and *Aureococcus anophagefferens* (A-FN). Vertical bars represent SD and asterisks denote significant differences in total metamorphosis from controls (* $p < 0.05$, ** $p < 0.01$). Control treatments included the non-toxic alga *Skeletonema costatum*; blanks consisted of seawater alone

(22.3 ± 5.8%) and *A. anophagefferens* (26.5 ± 3.6%) treatments.

Both *K. mikimotoi* and *A. catenella* significantly inhibited total metamorphosis after 96 h (5.0 ± 2.2 and 24.5 ± 3.1%, respectively; $p < 0.01$; Fig. 1). In contrast, total metamorphosis in the *A. anophagefferens* and *Prorocentrum donghaiense* treatments (50.8 ± 4.9 and 46 ± 4.0%, respectively) did not differ significantly from that observed for the control treatment (49.3 ± 1.5%).

All test algae species were significantly lethal to abalone larvae (ANOVA, $F_{5,12} = 89.69$, $p < 0.01$; Fig. 2). Total mortalities in the *K. mikimotoi*, *A. catenella*, *A. anophagefferens*, and *P. donghaiense* treatments were 95.3 ± 2.8, 74.5 ± 2.8, 48.5 ± 12.1, and 37.5 ± 6.4%, respectively, compared with 17.2 ± 3.1% for the control. However, different algae species had different effects on larvae at different stages. *K. mikimotoi*, *A. catenella*, and *P. donghaiense* were toxic to abalone larvae at the late veliger stage. Compared with the control (14.0 ± 4.1%), veliger mortality rates in these 3 treatments were significantly higher (ANOVA, $F_{5,12} = 143.83$, $p < 0.01$), at 94.8 ± 2.1, 72.3 ± 1.4, and 36.2 ± 6.9%, respectively. However, *A. anophagefferens* was more toxic to metamorphosed larvae (larvae with a peristome) than to late veliger stage larvae, as 24.3 ± 3.9% of metamorphosed larvae died after 96 h of treatment with *A. anophagefferens* (which was nearly half of total metamorphosed individuals).

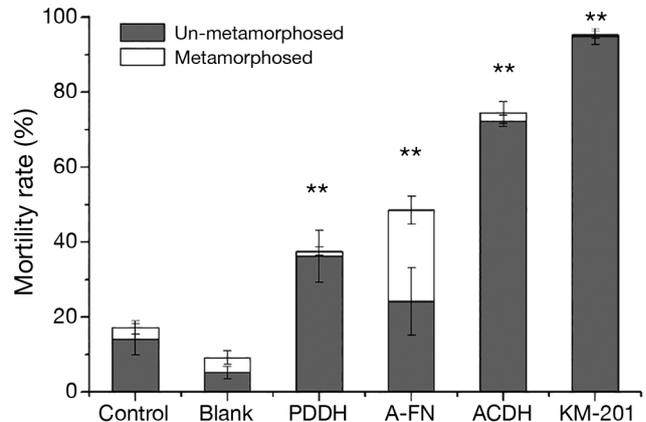


Fig. 2. Mean percentage of metamorphosed and un-metamorphosed abalone *Haliotis discus hannai* larvae which had been killed at 96 h when treated with toxic algae. Vertical bars represent SD and asterisks denote significant differences in mortality from controls. See Fig. 1 for further details

4. DISCUSSION

Although our results suggested that all 4 selected algae have negative impacts on abalone, they also clearly demonstrated that responses varied among algal species. The effects of *Karenia mikimotoi* and *Alexandrium catenella* on abalone larvae were similar; both were extremely lethal to un-metamorphosed larvae, and they were the only 2 species that significantly inhibited metamorphosis. *K. mikimotoi* is cytotoxic, hemolytic, and ichthyotoxic, and it can cause mortality to many fish, shellfish, and other invertebrate species (Cross & Southgate 1980, Botes et al. 2003, Silke et al. 2005, Mitchell & Rodger 2007, Li et al. 2017). *A. catenella* can produce paralytic shellfish poisoning toxin, and is also lethal to fish and invertebrates (Chen et al. 2007a,b). Both species can cause veliger larvae to die before metamorphosis begins (Shumway & Cucci 1987, Mu & Li 2013). Basti et al. (2015a) also found that they significantly inhibited activity of the Japanese pearl oyster *Pinctada fucata martensii* larvae at low densities (500 cells ml⁻¹); however, no lethal effect was observed, which indicates that algal toxicity may be species specific.

Aureococcus anophagefferens did not affect metamorphosis, but was toxic to metamorphosed larvae, suggesting that algal toxicity changes with abalone ingestion method pre and post metamorphosis. *A. anophagefferens* inhibits ingestion rates in many copepods and shellfish, and causes mortality (Greenfield & Lonsdale 2002, Caron et al. 2004, Padilla et al. 2006, Bricelj & MacQuarrie 2007). During metamorphosis, feeding processes change from lecithotrophic

at the veliger stage to heterotrophic after metamorphosis when they ingest primarily diatoms (Ino 1952). *A. anophagefferens* may affect mortality of metamorphosed larvae by inhibiting ingestion, which explains why the percentage of dead metamorphosed individuals was higher in this treatment than in the others.

Prorocentrum donghaiense had no significant effects on total metamorphosis or final survival, but it was toxic to veliger larvae. *P. donghaiense* is generally considered to be a nontoxic species because no known toxin has been detected. However, it does have impacts on survival of some zooplankton species, such as brine shrimp *Artemia salina* and copepods (*Moina mongolica*; Chen et al. 2007a), and on the life cycle of rotifers (*Brachionus plicatilis*; Lin et al. 2016b). In the present study, *P. donghaiense* was toxic to abalone larvae, as it reduced the number of veligers, which may have effects on the population.

Metamorphosis is a crucial period in the life cycle of abalone. During this process, the ingestion method, body structures, physiological functions, and behavior change drastically within a short period of time, and this process can be affected by changing environmental parameters such as temperature, salinity, and substrate (Wang et al. 2006). During the veliger stage, abalone larvae do not have a shell, which means that they are fragile and susceptible to toxic algae by direct contact (Botes et al. 2003, Basti et al. 2015a). Basti et al. (2015a) suggested that pre-settling larvae of *Pinctada fucata martensii* were more sensitive to these same 4 harmful algae than were D-stage larvae. In our study, we observed mortality of abalone veliger larvae exposed to *K. mikimotoi*, *A. catenella*, and *P. donghaiense*. In contrast, metamorphosed individuals at the peristome stage, which are protected by a shell, are much more tolerant to toxic algae. However, the change in nutritional mode from autotrophic to heterotrophic in metamorphosed larvae made them more susceptible to *A. anophagefferens*, suggesting that it might inhibit ingestion after metamorphosis.

All 4 selected algae are typical and common bloom-forming species in coastal waters of China, including the Bohai Sea, northern Yellow Sea, and East China Sea, and have caused direct shellfish mortality in most abalone, but also in clam and scallop farms, in this region (Yu et al. 2018). Our results suggest that these algae may also further harm abalone by inhibiting metamorphosis. Compared with mature individuals, abalone larvae were more sensitive to harmful algae, especially to toxic species like *K. mikimotoi* and *A. catenella*, because signifi-

cant mortality and inhibition were observed at a lower density than that observed in HABs (Botes et al. 2003, Lin et al. 2016a). Inhibition of ingestion by *A. anophagefferens* also caused heavy post-metamorphic damage to abalone larvae. Culture of shellfish larvae including abalone could thus collapse when toxic algae bloom nearby or when the algae are pumped with water into indoor breeding pools. Even algae like *P. donghaiense*, which was previously reported to be non-toxic, may have long-term effects on wild abalone populations. Thus, these HAB-forming species should be considered harmful to shellfish farms, including abalone and shellfish breeding ponds, as well as fish farms.

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