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Comparative toxicity of *Alexandrium affine* to finfish *Thamnaconus modestus* and shellfish *Haliotis discus hannai*

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ABSTRACT

A Korean strain of *Alexandrium affine* (TY-1509-02) was exposed to adult *Thamnaconus modestus* and *Haliotis discus hannai* for a comparative toxicity evaluation. Tests were done in two subsequent ways in the chamber (70 L carrying 30 L alga) for 24 hours. The first test was to find the first damage concentration (FDC) at 4 allocations (0, 1.25, 2.5, or 5.0 × 10^3 cells mL^{-1}) at 20℃. The second was to detail at a median concentration (MC) between FDC and its previous concentration at 20 and 25℃. In the first test FDC was 5.0 × 10^3 cells mL^{-1} for both species, calculating 3.75 × 10^3 cells mL^{-1} as an MC in common. The algal toxicities at FDC were species-specific with bigger toxicity to the finfish. Toxicities at MC were also species-specific: harmless to the shellfish and temperature-dependently toxic to the finfish with mortalities of 20% at 20℃ and 0% at 25℃. These results elucidated that *A. affine* is no longer harmless with an estimated potent concentrations near MC for the finfish and close to FDC for the shellfish. In the toxicity evaluation, particularly to *T. modestus*, temperature should be taken into account as it might be a parameter influencing the algal damage potential.

Key words: *Alexandrium affine*, toxicity evaluation, algal damage potential

Introduction

Many species of *Alexandrium* can produce a number of toxins and/or toxic candidates (Shang *et al*., 2021). In exposure or feeding tests, however, results are disparate; some species are toxic (Castrec *et al*., 2020,2021; Haque *et al*., 2021), but not to all the marine life (Kamiyama *et al*., 2005). Toxicity of *Alexandrium affine* is more controversial. Unlike other *Alexandrium* species, *A. affine* contains no or negligible levels of PSTs (Band-Schmidt *et al*., 2003; Anderson *et al*., 2012).

The controversy with *A. affine* toxicity exist in the Korean waters. *A. affine* found in the Korean waters also synthesizes PSTs, but the levels are negligible (Kim *et al*., 2023). Without apparent identification of causative agents, the species has been blamed for causing aquaculture species mortality among fish farmers.

An early study advanced an estimation in which *A. affine* toxicity might have an allelopathic pathway as in the other *Alexandrium* species (Tillmann and John, 2002). However, studies supporting the estimation are lacking, roughly grading it as a potentially harmful alga (Nguyen-Ngoc, 2004). To put it forward a more persuasive argument, laboratory exposure to adult animals might be necessary. Laboratory exposure using animals in early life stages are easier to put it in practice than those in adult stages. But the data from earlier stages might not represent the real physiology of the test species because toxic physiology

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might be different stage by stage, mostly with earlier stages more susceptible (Rolton *et al*., 2015; Castrec *et al*., 2020; Pease *et al*., 2022).

Susceptibilities to harmful algae are manifestly different between finfish and shellfish (Rolton *et al*., 2022). Finfish *Thamnaconus modestus* and shellfish *Haliotis discus hannai* are farmed in pen or land-based system on the Korean coasts where *A. affine* blooms annually occur. In our previous studies *A. affine* at a realistic concentration in Korean waters appeared to be harmful to a certain extent to juvenile abalones (Lee *et al*., 2018). Comparative exposures of the alga to marine animals, particularly to adult finfish and shellfish, are still lacking.

A. affine is difficult to culture in mass in laboratory. The algal exposure to adult animals, however, need a large quantity of the species at a time, which imposes a common constraint on the test. A stepwise test design might be a good approach to compromise the culture difficulty and the mass necessity. In our tests, the first step is to find first damage concentration (FDC) in the roughly allocated test concentrations in a temperature, followed by detailed test at a median concentration (MC) between FDC and its previous concentration. For further evaluation of the algal damage potential in the second test, we employed a reference exposure of *Karenia mikimotoi* known to be particularly toxic to species of abalones (Satake *et al*., 2002; Botes *et al*., 2003; Kim *et al*., 2020).

Materials and Methods

1. Test algae and the culture

Harmful algae, *Alexandrium affine* (TY-1509-02) and *Karenia mikimotoi* (KM02KSS) were cultured in mass in the laboratory as test and reference algae, respectively. They were cultured in specially designed culture chambers (carrying capacity, 500 L) using f/2 medium under conditions of illumination 50 μE $m^{-2}s^{-1}$, LD cycle 24:0, and temperature 23℃. All the culture details otherwise mentioned followed NIFS Harmful Algae Culture Protocol (NIFS, 2018).

2. Test animals and the captive acclimation

Two-year old black scrapers *Thamnaconus modestus* (132.1 ± 26.9 g) as a test finfish were delivered into the laboratory from a commercial fish pen. Two-year old abalones *Haliotis discus hannai* (4.9 ± 0.6 g) as a test shellfish were from a commercial raceway. After a captive acclimation in the laboratory, the animals were maintained at two temperatures 20 or 25℃, feeding on formulated diets or salted seaweeds in the running-through tanks (500 L). Two days earlier the test, healthy animals were selected and kept in new tanks (500 L) without feeding.

3. First test

The first test was to find the first damaging concentration (FDC) in roughly allocated test algae at 20℃ for 24 hours. In brief, *A. affine* at about 6,000 cells mL^{-1} grown at 23℃ were placed in the test room at 20℃ for about 12 hours prior to use. The cultures were regularly agitated to keep the algae in suspension during the whole temperature adjustment. As soon as the cultures reached 20℃, they were diluted into 4 test concentrations (0, 1.25, 2.5, and 5.0 × 10^3 cells mL^{-1}) in test chamber (30 L in 70 L capacity) which was specially designed for keeping the algae in suspension. The test animals starved for 2 days were then exposed to each of the 4 dilutions. Animal numbers per chamber were 5 for the finfish and 10 for the shellfish. Animal mortality was monitored according to the following timeline: 0, 1, 2, 3, 6, 12, 18, and 24 hours after exposure. The abalones detached from substrates without showing muscle contraction for minutes in the air (Lee *et al*., 2018) and the black scrapers without respiration behavior were counted as dead ones. The tests were replicated 3 times.

4. Second test

The second tests were conducted at a median concentration (MC) between FDC and its previous non-toxic concentration which were from the first test. The second test at MC was done at two temperatures 20 and 25℃ together with *K. mikimotoi* as a reference. Briefly, *A. affine* at 6,000 cells mL^{-1} and *K. mikimotoi* at 20 × 10^3 cells mL^{-1} grown at 23℃ were placed in

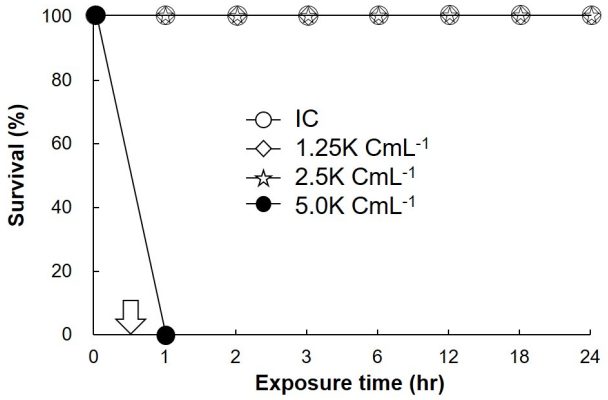

Fig. 1. Effects of *Alexandrium affine* on the survival of finfish black scraper *Thamnaconus modestus* at 20℃. Abbreviations for symbol: IC, for intact control; KCmL^{-1} for 10^3 cells/mL. Arrow symbol indicates the actual time the finfish first reached 0% survival at algal concentration 5.0 x 10^3 cells mL^{-1}.

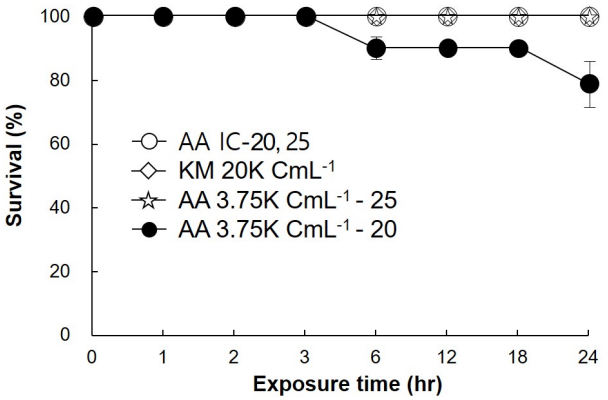

Fig. 2. Temperature-dependent *Thamnaconus modestus* survival at MC of *Alexandrium affine*. Symbol: vacant circle for IC at 20 and 25℃; vacant diamond for reference *Karenia mikimotoi* 20K CmL^{-1} at 20 and 25℃; vacant star for solid circle for 20℃; vacant circle for 25℃; vacant star for *A. affine* 3.75K CmL^{-1} at 25℃; solid circle for *A. affine* 3.75K CmL^{-1} at 20℃. Abbreviations for symbol are the same as in Fig. 1.

two test rooms each with fixed temperature 20℃ or 25℃ for about 12 hours. Test animals acclimated to each temperature and starved for 2 days were, then, exposed to each algal concentration and monitored in survival as in the first test. All the exposures were replicated 3 times.

5. Statistics

Data were statistically analyzed in two-way ANOVA (SPSS ver. 18).

Results and Discussion

Fig. 1 shows survival of *Thamnaconus modestus* exposed to 4 concentrations of *Alexandrium affine* (0, 1.25, 2.5, or 5.0 × 10^3 cells mL^{-1}) at 20℃ for 24 hours. The profile was simple: a total mortality at 5.0 × 10^3 cells mL^{-1} in an hour and no mortalities below the concentration. Speaking of the time the finfish first reached total mortality, it happened earlier than expected. In our measurement time line, the first measurement was scheduled by hour 1 post the algal exposure. However, actual mortality occurred in 30 minutes post exposure (see the arrow symbol in Fig. 1). This simple profile revealed 5.0 × 10^3 cells mL^{-1} as an FDC and calculated 3.75 × 10^3 cells mL^{-1} as an MC.

The observed acute toxicity at 5.0 × 10^3 cells mL^{-1} insinuated that the concentration might be far beyond actual FDC. Followings support our insinuation. The first one relies on the big difference in the animal response at 2.5 × 10^3 and 5.0 × 10^3 cells mL^{-1}. The toxicity at 5.0 × 10^3 cells mL^{-1} was so acute that none of the finfish could survive over an hour, or more precisely speaking, over half an hour. The big difference, therefore, suggested that the real FDC might be to some extent away from 5.0 × 10^3 cells mL^{-1} toward 2.5 × 10^3 cells mL^{-1}. The second one is regarding the animal susceptibility to the algal toxicity. Haque *et al.* (2021) evaluated *A. affine* damage potential to sea breams *Pagrus major* using the same algal strain as used in the present study, concluding that approximately 4.0 × 10^3 cells mL^{-1} of *A. affine* might be a threshold for significant mortality and biochemical responses for the finfish. The black scrapers employed in the present study are highly susceptible to changes of environmental variables. According to unreported observations, the species is more susceptible at least than sea bream to a variety of environmental variables including harmful algal blooms. Our findings together with work of Hague (2021) and unreported observations, thus, reminded us of real FDC being a concentration in between 4.0 × 10^3 cells mL^{-1} and 2.5 × 10^3 cells mL^{-1}.

To further approximate the real FDC, a subsequent

second test was conducted at MC, a median concentration between 2.5 × 10^3 cells mL^{-1} and 5.0 × 10^3 cells mL^{-1} or 3.75 × 10^3 cells mL^{-1}. The test was done at two temperatures (20 and 25℃) for 24 hours. Fig. 2 shows the results in which the tested MC appeared to be threshold or around the concentration. At the concentration, the animal susceptibility was solely dependent on temperature. It was 20℃ at which the animals showed mortality. The damage at MC was significant ($P < 0.05$) but not acute: the first mortality of 10% by hour 6 with final mortality of 20%.

An interesting finding was that the reference alga *Karenia mikimotoi* at 20 × 10^3 cells mL^{-1} was harmless to the finfish. It is surprising that *A. affine* at 3.75 × 10^3 cells mL^{-1} was more toxic than *K. mikimotoi* at 20 × 10^3 cells mL^{-1}, considering the algal capacity to to synthesize the notorious gymnocins (Satake *et al*., 2002, 2005), This harmlessness of the reference *K. mikimotoi* at the concentration needs further verification.

Results of Fig. 2 suggest that real FDC of the alga is 3.75 × 10^3 cells mL^{-1} or around the concentration. However, there is a question that should be answered to make our suggestion more reasonable. It is about the culprit responsible for the temperature-dependent mortality at MC. Is it the test animal more susceptible at 20℃ or the test alga more influential at the temperature, or both? It appears that the alga is responsible for the mortality. Harmful algal toxicities vary with variation of environmental parameters, including temperature (Jeff and Michalak, 2019; Vidyarath *et al*., 2020). This is particularly true for *A. affine* as the algal toxicity becomes more toxic at 20℃ than at 25℃ in the Korean waters (Kim *et al*., 2019; Lim *et al*., 2019). On the other hand, *T. modestus* is a temperate species with optimum temperatures of 15-28℃ for normal growth in the Korean waters (Lee *et al*., 2014). The two test temperatures 20 and 25℃, thus, appear to be correspondent to the very favorable range within the window of tolerable temperature, suggesting that the mortality at 20℃ might be solely due to enhanced algal toxicity at lower temperature.

Now that *A. affine* is no longer harmless to *T. modestus* and its FDC to the species is around 3.75 × 10^3 cells mL^{-1}, another question is matters responsible for the toxicity even though it is beyond our experiment subject. A possible explanation is that *A. affine* can also synthesize highly toxic compounds collectively called allelochemicals (Tillmann and John, 2002) which are even more toxic than other types of toxins produced by *Alexandrium* species (Tillmann and John, 2002; Shang *et al*., 2021). Therefore, *A. affine* toxicity in our study might be attributed to the presence of these compounds rather than PSTs and/or other toxins. The estimated toxicants and their toxic mechanisms remain as a future study.

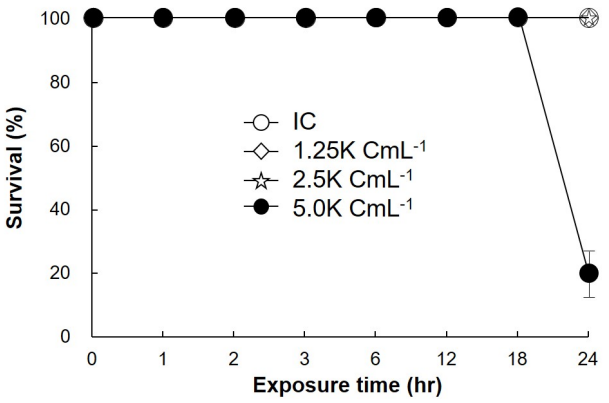

Fig. 3. Effects of *Alexandrium affine* on the survival of shellfish abalone *Haliotis discus hannai* at 20℃. Abbreviations for symbols are the same as in Fig. 1.

Fig. 3 shows survival of *Haliotis discus hannai* exposed to 4 concentrations of *A. affine* (0, 1.25, 2.5, or 5.0 × 10^3 cells mL^{-1}) at 20℃ for 24 hours. *A. affine* was less toxic in terms of the shellfish mortality (compare with Fig, 1). Only did the algal concentration at 5.0 × 10^3 cells mL^{-1} induce 87% mortality, but it happened suddenly at the end of the test. Even though not acute, the alga at the concentration calculated 3.75 × 10^3 cells mL^{-1} as an MC for the shellfish.

As in *T. modestus*, a subsequent second test was conducted at MC. The test was also done at two temperatures (20℃ and 25℃) for 24 hours. However, none of the shellfish were susceptible to all the test algal concentrations and a reference *K. mikimotoi* at 20 × 10^3 cells mL^{-1} (figure, not shown). These results imply that the estimated actual FDC for *H. discus hannai* might be far beyond 3.75 × 10^3 cells mL^{-1} or

slightly lower or around 5.0×10^3 cells mL^{-1}.

In conclusion, the Korean strain of *A. affine* (TY-1509-02) was toxic both to *T. modestus* and *H. discus hannai* only at 5.0×10^3 cells mL^{-1} in the 4 test concentrations (0, 1,25, 2.5, and 5.0×10^3 cells mL^{-1}). The alga was no longer harmless at the highest concentration to both of the species. The damage potentials, however, were species-specific with an estimated actual FDC at or a little bit lower than 3.75×10^3 cells mL^{-1} for *T. modestus* and at or slightly lower than 5.0×10^3 cells mL^{-1} for *H. discus hannai*. In the algal toxicity evaluation, particularly to *T. modestus*, temperature should be taken into account as it might be a parameter influencing the algal toxicity potential.

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