The toxic comparative effect of *Heterosigma akashiwo* on two kinds of zooplankton

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The raphidophyte *Heterosigma akashiwo* Hada is one of several flagellate species causing prodigious red tides. It is distributed widely in coastal seas of temperate and semitropical embayments. It is also one of the dominant red tide species along the coasts of China. It has been reported that *H. akashiwo* has lethal effects on cultured fish such as salmon, yellowtail, sea bass, black sea bream and right-eyed flounder and has caused heavy economic losses to the aquaculture industry (Kempton *et al.* 2008, Liu *et al.* 2008). Zooplankton is a key group responsible for the exchange of materials and energy in marine ecosystems and may play an important role in controlling the occurrence, propagation and abatement of harmful algal blooms. In the study reported here, we examined the effect of *H. akashiwo* on two kinds of zooplankton: *Moina mongolica* and *Neomysis awatschensis*, including their survival, fecundity and population growth under laboratory conditions. Our goal was to learn the potentially detrimental effects of large-scale harmful algal bloom events on zooplankton and the marine ecosystem.

**Study Methods**

**Organisms**

*M. mongolica* was cultured using *Chlorella* sp. *N. japonica* was provided by the laboratory of Fish Nutrition, Shanghai Ocean University. *H. akashiwo* and *Chlorella* sp. were cultured in f/2 medium flasks at a temperature of 20±1°C.

**Experimental design**

To examine the effect of different densities of *H. akashiwo* on survival of *M. mongolica*, algal cultures of *H. akashiwo* were diluted with fresh seawater to respective densities of 1×10⁴, 5×10⁴, 10×10⁴ and 20×10⁴ cells/mL. The experiment was carried out for 96h. Fifty-mL breakers were utilized and the experimental volume was 30 mL. Twenty individuals of two-day-old *M. mongolica* were placed randomly into each beaker. There was a control group that consisted of *M. mongolica* fed with *Chlorella* sp. (3×10⁶ cells/mL). All numbers of *M. mongolica* were counted every 24h, then put back into the beaker with fresh medium. The experiment was carried out in triplicate.

Similarly, algal cultures of *H. akashiwo* were diluted with fresh seawater to respective densities of 1×10⁴, 5×10⁴ and 10×10⁴ cells/mL. The experiment was performed at a temperature of 25±1°C. One thousand-mL breakers were utilized and the experimental volume was 1000 mL. Ten *N. japonica* were placed into each beaker randomly. *N. japonica* were cultured in fresh seawater as a control group. All numbers of *N. japonica* were counted every 24h, then put back into the beaker with fresh medium. The experiment was carried out in triplicate.

An *H. akashiwo* culture was diluted with *Chlorella* sp. (2×10⁶ cells/mL) to 2×10⁴ cells/mL and 4×10⁴ cells/mL. One-day old *M. mongolica* were individually placed in a 15 mL tube with 10 mL of culture medium. Every other day, the body lengths of the *M. mongolica* were measured under the microscope, after which they were placed back into the tubes with fresh medium. Throughout the experiment the numbers of juveniles and survival times of adult *M. mongolica* were also noted. The experiment continued until all of the *M. mongolica* had died.

An experiment was also conducted to determine the Effects of *H. akashiwo* on population numbers of *M. mongolica*. Once again the temperature selected was 25±1°C. Two hundred-mL breakers were utilized and the experimental volume was 200 mL. One hundred *M. mongolica* were placed into each beaker randomly. A control group of *M. mongolica* was fed with 1×10⁴ cells/mL of *Chlorella* spp. The *M. mongolica* were counted every 48h, and then put back into the beaker with fresh medium.

**Results**

Figure 1 shows that *H. akashiwo* had adverse effects on the survival of *M. mongolica*. As seen in the figure, the toxic effects of *H. akashiwo* increased when algal density increased.
When exposed to *H. akashiwo* for 96h, survival rates of *M. mongolica* were 10, 23.3, 21.7 and 0 percent at *H. akashiwo* densities of 1×10^4, 5×10^4, 10×10^4 and 20×10^4 cells/mL, respectively. This was a significant decrease compared to the survival rate, 98.4 percent, in the *Chlorella* sp. control group.

The results of the effects of *H. akashiwo* on survival of *N. japonica* are presented in Figure 2. When exposed to 1×10^4, 5×10^4 and 10×10^4 cells/mL of *H. akashiwo* for 96h, survival rates of *N. japonica* were 33.3, 27.8 and 33.7 percent, respectively, each of which was lower than the 66.7 percent survival in the control. Thus, *H. akashiwo* also had adverse effects on *N. japonica* survival.

Figure 3 shows the effects of *H. akashiwo* on body length of *M. mongolica*. *H. akashiwo* led to reduced growth of *M. mongolica* compared with the control. After 13 days, body lengths of *M. mongolica* in 2×10^4 and 4×10^4 cells/mL of *H. akashiwo* averaged 1.44 mm and 1.37 mm, while body lengths in control averaged 1.45 mm.

Figure 4 shows the effects of *H. akashiwo* on brood number and larvae number per brood of *M. mongolica*. The results showed that when exposed to 2×10^4 and 4×10^4 cells/mL of *H. akashiwo*, brood numbers of *M. mongolica* averaged 5, less than 7 averaged in the control. When exposed to 4×10^4 cells·mL⁻¹ of *H. akashiwo*, larvae numbers per brood of *M. mongolica* were also less than in the control.

The effects of 2×10^4 and 4×10^4 cells/mL of *H. akashiwo* on the fecundity of *M. mongolica* as shown in Table 1 were as follows:

1. Delayed development. The times for the first brood of *M. mongolica* were 4.0 and 4.1 d, while that in the control was 3.7d.

2. Decreased gross fecundity. Gross larva numbers were 30.2 and 26.7 in the experimental groups and 35.5 in the control.

3. Reduced average life-time: Survival times in *H. akashiwo* were 11.4 and 12.5 d in the experimental groups 14.5 d in the control.

Figure 5 shows that *H. akashiwo*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>The time for the first brood (Day)</th>
<th>Gross fecundity (Individual)</th>
<th>Lifetime (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.7±0.7</td>
<td>35.5±11.6</td>
<td>14.5±4.8</td>
</tr>
<tr>
<td>2×10^4 cells·mL⁻¹</td>
<td>4.0±0.8</td>
<td>30.2±8.4</td>
<td>11.4±3.0</td>
</tr>
<tr>
<td>4×10^4 cells·mL⁻¹</td>
<td>4.1±0.8</td>
<td>26.7±5.6</td>
<td>12.5±2.4</td>
</tr>
</tbody>
</table>

*Table 1. The effect of *H. akashiwo* on the time for the first brood, gross fecundity and lifetime of *M. mongolica*.*
had an inhibiting effect on population numbers of *M. mongolica* after 17 days. The population numbers of *M. mongolica* exposed to 2×10^4 and 5×10^4 cells/mL of *H. akashiwo* were 36.5 in the experimental groups and 0 in the control. Thus, *H. akashiwo* blooms could have strong adverse effects on the population growth of *M. mongolica*.

Through the microscopic observation, it was determined that *M. mongolica* could feed on *H. akashiwo*, which filled its intestinal tract, however, it seemed that *M. mongolica* could not digest the raphidophyte effectively. It appears that *H. akashiwo* may secrete some material that causes it to stick to the intestinal tract terminal of *M. mongolica* (Figure 6A). Moreover, *H. akashiwo* also stuck to the setae of *N. japonica* (Figure 6B). Thus, *H. akashiwo* might disturb normal physiological activities of *M. mongolica* and *N. japonica* and cause their death.

**Discussion**

In the present experiment, *H. akashiwo* had a toxic effect on *M. mongolica* and *N. japonica*. Even after *Chlorella* sp. was added, *H. akashiwo* still showed an impact on the body length and fecundity of *M. mongolica*, indicating the *H. akashiwo* species is responsible for the adverse effects on the zooplankton.

However, the toxicological mechanism of *H. akashiwo* remains controversial and unresolved. Some investigators have suggested that mucus or other lectin-like polysaccharides might cause the adverse impact on some marine organisms (Keppler et al. 2005, Twiner et al. 2004, Twiner and Trick 2000, Yan et al. 2003). Yan et al. (2003) reported that the glyco-calyx structures on the surface of the algal cells might be responsible for the inhibiting effects on the swimming activity of *Artimia salina*. Wang et al. (2006) found that long-term adhesion of the algal cells to the membranes of larval *Argopecten irradians* caused hatching failure. This implies that algal cells have a specific ability to attach to the larvae. Some studies indicate that *H. akashiwo* has glycocalyx structure on the cell surface and it is likely that those organelles have a high affinity for the surfaces of the larvae.

In this paper, we found that *H. akashiwo* may secrete some materials that cause them to stick closely to the intestinal tract terminal of *M. mongolica*. *H. akashiwo* could stick to the setae of *N. japonica*. Thus, *H. akashiwo* might disturb the normal physiological activities of marine zooplankton, and cause their death.

When *H. akashiwo* blooms occur, the density can reach 9.5×10^4 cells/mL and even 7.2×10^4cells/mL (Guo 1994, Liu et al. 2008), which is more than the algal cell densities used in our experiment. So, a *H. akashiwo* bloom could severely affect the zooplankton community, which may further impact marine ecosystem structure and function, potentially influencing populations and communities at higher-trophic levels.

**References**


