

Note

Dietary effects of the red-tide raphidophyte *Heterosigma akashiwo* on growth of juvenile Manila clams, *Ruditapes philippinarum*SHIGERU TAGA¹, YASUHIRO YAMASAKI^{2,*} & MASANOBU KISHIOKA¹¹Yamaguchi Prefectural Fisheries Research Center, 437–77, Aiofutajima, Yamaguchi, Yamaguchi 754–0893, Japan²Laboratory of Environmental Biology, Department of Applied Aquabiology, National Fisheries University, 2–7–1 Nagata-Honmachi, Shimonoseki, Yamaguchi 759–6595, Japan

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Abstract: We studied the effects of *Heterosigma akashiwo*, a harmful algal species, in the diet of juvenile Manila clams (*Ruditapes philippinarum*), because it often blooms in coastal waters where aquaculture of the clams is carried out. Weight of soft tissue of clams increased when they were fed *H. akashiwo* added at 8,000 cells mL⁻¹, with weight growth about 8% higher than that of clams fed only *Chaetoceros neogracile*. In addition, a significant increase in the body-mass index and glycogen content was observed when they were fed *H. akashiwo* compared with *C. neogracile*. Analysis of the chemical composition of *H. akashiwo*, *C. neogracile*, *Pavlova lutheri* and *Nannochloropsis* sp. showed a higher total sugar and acidic sugar content in *H. akashiwo* than in the other species, whereas the protein content was almost the same for all species except *Nannochloropsis* sp. These observations suggest the possibility that the sugar content of phytoplankton is an important factor affecting the growth of juvenile clams.

Key words: growth promotion, harmful algal bloom, *Heterosigma akashiwo*, *Ruditapes philippinarum*, sugar content

In Japan, the annual catch of the Manila clam (*Ruditapes philippinarum* Adams & Reeve, 1850) in coastal areas continues to decrease drastically as it has since the 1980s (Matsukawa et al. 2008). Several factors have been identified as causes of the dramatic decrease, such as the pejouration of bottom sediment (Tsutsumi 2006), mass depletion of larvae (Sekiguchi & Ishii 2003), the effects of predators (Segawa 1997, Yamaguchi et al. 2005), and possible reduced egg production due to infection by the protozoa *Perkinsus* (Park et al. 2006) in addition to overfishing. Thus, the development of a mass culture method and a new algal diet for the clam is extremely important for the conservation of the clam resource and to enable a stable market supply. On the other hand, in coastal areas of Yamaguchi Prefecture, aquaculture grounds for the clams, *Heterosigma akashiwo* (Hada) Hada ex Hara & Chihara, 1987 often forms red tides during June. This species is a harmful algal bloom (HAB) species that has caused serious damage to cultured fish in many coastal areas throughout the world (Honjo 1993, Smayda 1998, Imai & Itakura 1999), but there are no reports that this species affects bivalves, including *R. philippinarum*. Recently, Nagasoe et al. (2011) examined the effects of the harmful raphidophytes *Chattonella*

marina (Subrahmanyam) Hara & Chihara, 1982 and *C. anti-qua* (Hada) Ono, 1980 on the Pacific oyster, *Crassostrea gigas* Thunberg, 1793, and found that no oysters died, even after exposure to high cell densities of both species. In addition, they reported that both species of *Chattonella* are good oyster diets, and note that the oyster is useful because it has the potential to clear the fish-killing flagellates from the environment (Nagasoe et al. 2011). Thus, as well as the genus *Chattonella*, it is possible that *H. akashiwo* could be a good diet for *R. philippinarum*. In this study, we examined the effects of *H. akashiwo* when supplied as a diet in juvenile Manila clams.

For rearing tests with juvenile clams, *H. akashiwo* (cell length: 8–25 µm, cell width: 6–15 µm) and the bacillariophyte *Chaetoceros neogracile* VanLandingham, 1968 (cell length: 4–8 µm) were maintained in 30-L tanks containing 20 L filtered seawater enriched with KW21 marine alga growth medium (Daiichi Seimo Co., Ltd., Kumamoto, Japan). Cultures were grown at 20°C under continuous light. Juvenile *R. philippinarum* that were raised on a diet of *C. neogracile* and the eustigmatophyte *Nannochloropsis* sp. were also used in the rearing tests. For growth test, 30 clams (initial average shell length: 13.26 ± 0.73 mm) were placed into each of three 5-L suspensions of *H. akashiwo* (8,000 cells mL⁻¹) and as fed controls three of *C. neogracile* (80,000 cells mL⁻¹), and

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reared for 30 d; the suspensions were replaced every day. The test was conducted under ambient conditions with a mean water temperature of 22–25°C. Aerated, filtered natural seawater (pore size: 1 μm) with a mean salinity of 30–31 was used for these tests. The pH of the water was checked every day when the water was changed, and no significant pH changes were observed in any of the rearing tests. The glycogen analysis of each test group was conducted by the anthrone-sulfuric acid method, as described previously (Horikoshi 1958), and about 0.5 g of the whole tissue was used. This quantitation assay was performed in triplicate.

Strains of *H. akashiwo*, *C. neogracile*, *Nannochloropsis* sp., and the haptophyte *Pavlova lutheri* (Droop) Green, 1975 were inoculated at a density of 5,000 cells mL^{-1} into 200-mL glass flasks ($n=3$) containing 150 mL modified SWM-3 medium (Yamasaki et al. 2007). After incubation for 12 d at 25°C under 150 (± 10) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of cool-white fluorescent illumination on a 12 : 12 h light : dark cycle, 100 mL from each of the three replicate flasks was centrifuged at 700 $\times g$ for 15 min, and the cell pellet from each sample was frozen at -30°C until used for total sugar and acidic sugar determination. The total sugar content was determined by the modified phenol-sulphuric acid method (Mckelvy & Lee 1969), and the acidic sugar content was determined by the carbazol-sulphuric acid method (Bitter & Muir 1962). The remaining 50-mL sample from the three replicate flasks was centrifuged at 700 $\times g$ for 15 min, and the cell pellets were frozen at -30°C until used for protein determination. The concentration of protein in each wet sample was determined using a Pierce BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA). All quantitation assays were performed in triplicate.

For the results of the rearing tests, statistical analyses was performed using the Mann-Whitney *U* test to assess differences between groups because there was no proof of data homoscedasticity. For the results of the analyses of the chemical composition, the data were analyzed with a one-way analysis of variance (ANOVA) and then subjected to Tukey's post hoc tests.

Our field observations (unpubl.) and previous studies (Yokote & Honjo 1985, Honjo 1993, Nagasoe et al. 2011) suggested the potential of *Heterosigma akashiwo* as an algal diet

for *Ruditapes philippinarum*. The body-mass index and glycogen content of the group fed *H. akashiwo* were significantly higher than the control group fed *Chaetoceros neogracile* (Table 1, Fig. 1), but the shell length, height, width, and weight of the soft tissues of the group fed *H. akashiwo* were not significantly different (Table 1). The survival of three groups of *R. philippinarum* under different diet conditions (no diet, *C. neogracile* and *H. akashiwo*) ranged from 93% to 98%. The results from a preliminary experiment indicated the growth of clams fed only *H. akashiwo* tended to be promoted in a concentration-dependent manner (data not shown). Thus, *H. akashiwo* possesses a growth promoting effect on *R. philippinarum*.

Grazing of animals is known as a major factor decreasing phytoplankton populations in the field (Johansson & Coats 2002, Smayda 2008). Based on our results, we suggest, as did Nagasoe et al. (2011), that the Manila clam can potentially prevent red-tide flagellates such as *H. akashiwo* from bloom-

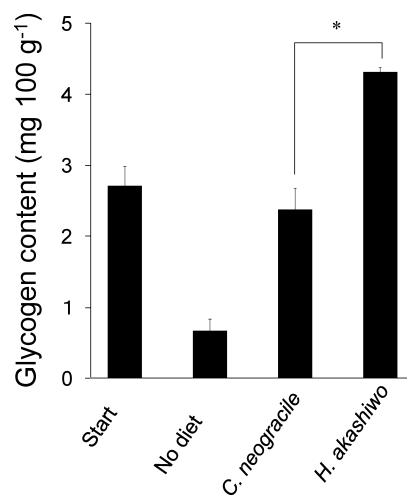


Fig. 1. Glycogen contents of the juvenile Manila clam *Ruditapes philippinarum* at the start of culture and after the 30-day cultures without diet, with *Chaetoceros neogracile* (80,000 cells mL^{-1}) and with *Heterosigma akashiwo* (8,000 cells mL^{-1}). The data are mean \pm SD of triplicate measurements. *The difference between *C. neogracile* and *H. akashiwo* was significant ($p < 0.05$).

Table 1. Shell size (length, height, and width), soft tissue weight and body-mass index of the juvenile Manila clam *Ruditapes philippinarum* after the 30-day cultures without diet, with *Chaetoceros neogracile* (80,000 cells mL^{-1}), and with *Heterosigma akashiwo* (8,000 cells mL^{-1}). Data are means \pm SD of triplicate measurements.

	No diet	<i>C. neogracile</i>	<i>H. akashiwo</i>	Difference between <i>H. a.</i> and <i>C. n.</i>
Shell length (mm)	13.27 \pm 0.05	13.39 \pm 0.18	13.27 \pm 0.12	N.S.
Shell height (mm)	9.39 \pm 0.08	9.52 \pm 0.14	9.45 \pm 0.09	N.S.
Shell width (mm)	5.28 \pm 0.14	5.31 \pm 0.09	5.36 \pm 0.12	N.S.
Soft tissue weight (mg wet weight)	55.37 \pm 3.53	85.57 \pm 6.13	92.24 \pm 0.99	N.S.
Body-mass index	8.37 \pm 0.62	12.66 \pm 0.36	13.72 \pm 0.16	Significant ($p < 0.05$)

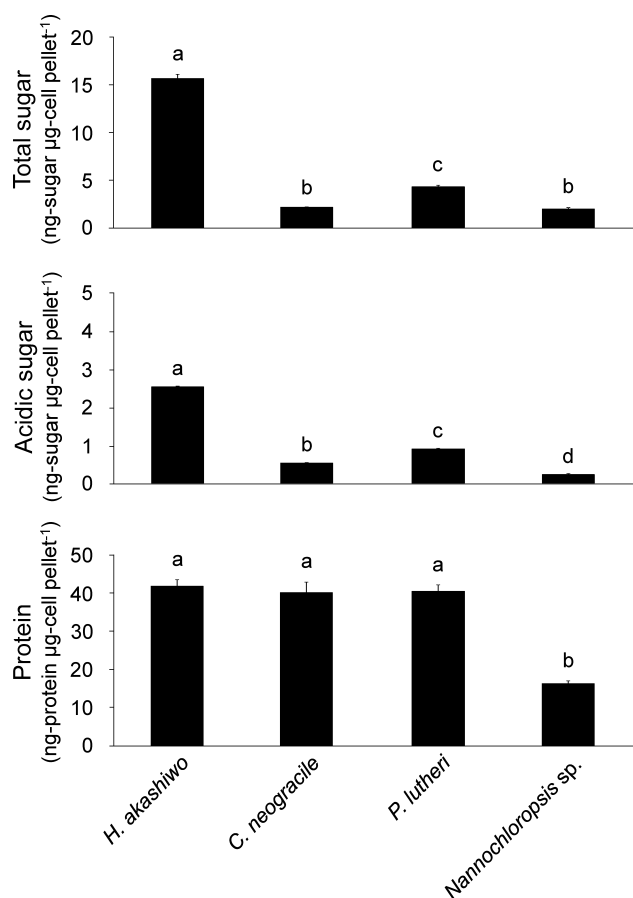


Fig. 2. Total sugar, acidic sugar, and protein contents of *Heterosigma akashiwo*, *Chaetoceros neogracile*, *Pavlova lutheri*, and *Nannochloropsis* sp. The data are mean \pm SD of triplicate measurements. The bars with the same letter are not significantly different.

ing in the field. Therefore, the recovery of the stock of *R. philippinarum* is an important issue, not only because of declining catches but also because of environmental problems related to red tides and eutrophication.

To clarify which microalgal components might have a beneficial effect on *R. philippinarum* growth, we determined the concentrations of protein, total sugar, and acidic sugar in *H. akashiwo*, *C. neogracile*, *Nannochloropsis* sp., and *P. lutheri*. The total sugar and acidic sugar content of each species was dramatically different, whereas the protein content was almost the same, although the protein content of *Nannochloropsis* sp. was lower than those of the other three species (Fig. 2). The surface of *H. akashiwo* cells is draped with glycocalyx (extracellular polymeric material) that contains at least two types of acidic complex-carbohydrates (sulfated and non-sulfated), together with a neutral carbohydrate-protein complex (Yokote & Honjo 1985, Honjo 1993). One moiety of the acidic complex-carbohydrates in the *H. akashiwo* glycocalyx is believed to be hyaluronic acid or a closely related substance (Honjo 1993). In the present study, the total sugar and acidic sugar contents of *H. akashiwo* were the highest of the four

species, and these results are in good agreement with previous studies (Honjo & Tabata 1985, Honjo 1993). Jørgensen (1983) reported that uptake of DOM in seawater by clams occurs through epidermal tissue located in the mantle and gills. Welborn & Manahan (1990) showed that the larvae of the bivalve *Crassostrea gigas* can take up glucose, maltose, cellobiose, and cellotriose, but not rhamnose or maltotriose. Recently, Uchida et al. (2010) demonstrated that the growth rate of soft tissue in *R. philippinarum* was significantly promoted by supplementing a diet of *Chaetoceros calcitrans* with glucose. Thus, sugar content of phytoplankton may be an important indicator to determine the growth of clams, and to evaluate the nutritional environment for the clams in the field.

On the other hand, Kawamura et al. (1995) examined the dietary effects of several benthic diatoms on the growth of post-larval abalone *Haliotis discus hannai*, and demonstrated that fragility of the silicified cell walls could allow ready absorption of the diatom cell contents. Thus, the observed positive high dietary effect of *H. akashiwo* may, in part, result from differences in the strength of the extracellular matrix, though we did not examine the digestion efficiencies of clams on *H. akashiwo* and *C. neogracile*. Future studies are required to investigate the effects of cell size, extracellular matrix, and absorption efficiency of microalgae on the growth of the clams.

Our results indicate that the red tide flagellate *H. akashiwo* can promote the growth of the Manila clam *R. philippinarum* and suggest the possibility that the sugar content of phytoplankton is an important factor affecting the growth of juvenile *R. philippinarum*. Future studies are required to demonstrate the strength of a correlation between *R. philippinarum* growth and sugar content of the natural phytoplankton population. Furthermore, it is also necessary that studies aimed at developing new and improved methods for rearing bivalves such as *R. philippinarum* should include a focus on the sugar content of diet algae, and the mode of action of sugars on the growth of bivalves.

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