J. Fish. Sci. Tech., 7(2), 96-98, 2004

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY
© The Korean Fisheries Society

Notes

Direct Evidence for the Reduction of Water Flow Across the Gills of Jack Mackerel (*Trachurus japonicus*) by a Harmful Alga *Chattonella marina*

Kyoung Seon Lee[1]*, Atsushi Ishimatsu[1] and Tatsuya Oda[2]

[1]*Marine Research Institute, Nagasaki University, Taira, Nagasaki 851-2213, Japan*
[2]*Division of Biochemistry, Faculty of Fisheries, Nagasaki University, Bunkyo, Nagasaki 852-8521, Japan*

Key words: Water flow, Gills, *Chattonella*, Arterial PO_2, Mucus

Blooming of harmful algal bloom (HAB) species belonging to the genus *Chattonella* (Raphidophyceae) has extensively caused mass mortalities of marine organisms (Okaichi, 1989). Yellowtail (*Seriola quinqueradiata*) one of the most intensively cultured fish species in Japan, was particularly susceptible to these plankton species, and therefore *Chattonella* blooms have caused the immense economic loss to the aquaculture industry in Japan (Okaichi, 1989; Ishimatsu et al., 1990; Hishida et al., 1997). Although blooms of raphidophyceae such as *Chattonella* spp., *Heterosigma akashiwo*, and *Fibrocapsa japonica* have also been recorded in the coastal waters of Korea, no mass mortality of fish has been so far reported. Hence there is a need to consider their potential harmful effects on coastal marine organisms.

A number of studies have proposed possible fish kill mechanisms by *Chattonella*, but the precise mechanism is not yet understood. There is a general agreement that lowering of arterial oxygen pressure (PO_2) in fish is the crucial mechanism (Ishimatsu et al., 1990; Lee et al., 2003). Several studies focussed on the decrease in arterial PO_2 to the mucus blockade in the interfilamental space, and it is presumed that such blockade inhibits water flow through the gills and enhances diffusion distance between blood and water (Ishimatsu et al., 1996a; Hishida et al., 1997; Kim et al., 2001), although this hypothesis has not been directly tested. In the present study, we measured water flow across the excised gills that had been exposed to *Chattonella marina* to answer this question.

*Corresponding author: sotome2002@hotmail.com

Jack mackerel, *Trachurus japonicus*, was purchased from a fish market and acclimated to 25℃. Fish were fed on shrimps and commercial pellet feeds (Higashimaru Foods, P-2, Kagoshima, Japan), but food was withheld for at least 24 hr prior to use. Before starting an experiment, fish was killed by decapitation, and then the first gill arch on either side was immediately cut off and rinsed in seawater. The first gill arch on one side was placed in ESM medium (Erd-Schreiber modified culture medium; see Okaichi et al., 1982) in a 100 mL beaker for 30 min as a control. The gill arch on the other side was placed in ESM medium containing *C. marina* cells (ca. 4,000 cells/mL; the cells in the exponential growing phase after 10 days from inoculation were used) for 30 min. The treated gill arch was placed on an acrylic plate (6×4 cm, 5 mm thick) with two holes (inner diameter, 4 mm). A Y-shaped connector was glued to the lower side of the plate so that outflow from the holes was directed to the connector. To prevent leakage from around the gill, a thin plastic film (ca. 0.5 mm thick) with a square window (2×1 cm) was put on the gill, and then the edge of the plastic film and the uncovered portion of the gill were sealed with silicone grease. The acrylic plate with the gill was placed in a 10 L water bath with a hydrostatic head of 2 cm. The outflow from the plate was collected in a 50 mL beaker for 1 min and then weighed. Duplicate measurements were done for each gill preparation. The water weight was converted to water volume using a specific gravity of 35 psu seawater at 25℃ (1.023 g/mL; Fofonoff and Millard, 1983).

Water flow decreased by 26-83% in the gills

Table 1. Effect of mucus on the water flow across the gills exposed to *Chattonella marina*. ESM medium was used as a control medium

Trial	Water flow (mL/min)		Ratio of II to I (%)	Cell density (cells/mL)
	(I) ESM	(II) *Chattonella*		
1	22.5	14.4	64.0	3,825
2	36.2	30.0	82.9	4,100
3	31.0	8.1	26.3	4,350
4	18.6	11.1	60.0	5,050
5	27.2	7.2	26.5	4,500
6	35.5	27.5	77.5	4,050
7	39.8	19.7	49.4	4,000
8	32.3	12.5	38.7	4,250
Mean±SE	30.4±2.6	16.3±3.0	53.2±7.7	4,266±135

exposed to *C. marina* as compared with those exposed to ESM alone (Table 1). Fish gills exposed to *C. marina* were covered with mucus containing plankton cells (Fig. 1).

Ultsch and Gros (1979) estimated the effect of mucus coating on water flow across fish gills. According to their calculation, a 5 μm layer of mucus halved the water flow through the gills, and oxygen diffusion resistance was increased by about 81%.

Cryofixation of gill tissue demonstrated that the secondary lamellae were blocked by mucus to 98.6± 0.014% for the yellowtail exposed to *Chattonella*, as compared to 1.0±0.003% for the control (Hishida et al., 1997). Ishimatsu et al. (1996a) reported that interfilamental spaces of yellowtail were already blocked by mucus when gill tissues were sampled as soon as the arterial PO_2 fell to 30 mmHg. In the immunohistochemical study of fish gill using an anti-glycocalyx antiserum, Kim et al. (2001) demonstrated that glycocalyx from *Chattonella* cell surface was easily discharged when *Chattonella* cells passed across the gills, and then aggregated on the gill surface with *Chattonella* cells.

Chattonella cells generate reactive oxygen species (ROS) such as O_2^-, H_2O_2 and $\cdot OH^-$ from glycocalyx structure on the cell surface (Shimada et al., 1993; Oda et al., 1997). ROS caused pathological changes in the fish gills and mucus secretion from gills (Oda et al., 1998; Kim et al., 2001). On the other hand, fish mucus enhanced ROS production by *C. marina* (Nakamura et al., 1998). It is noted that the itchyotoxicity of *Chattonella* was observed only for the intact-motile cells with high O_2^- production (Ishimatsu et al., 1996b). Tanaka et al. (1994) demonstrated that the motility of *Chattonella* was lost under dark conditions. Moreover, fish mortality by *Chattonella* was remarkably influenced by light conditions

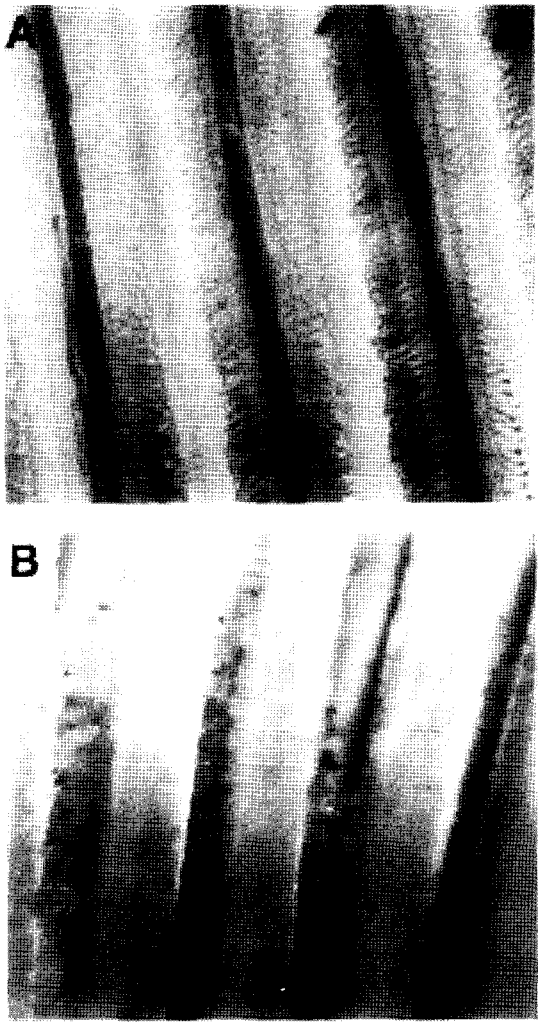

Fig. 1. Photographs of the gill exposed to ESM (A) and *Chattonella marina* (B) for 30 min at 25℃. *Chattonella marina* concentration was maintained at about 4,000 cells/mL. Mucus with plankton cells was found in the interfilamental space of the gill exposed to *Chattonella marina*.

(Ishimatsu et al., 1996b). Nevertheless, it is not clear in this study that light will have any effect on the fish kill mechanism by *Chattonella*.

Gas exchange in fish gills is achieved through ventilation, diffusion between water and blood, and perfusion (Piiper, 1998). Low arterial PO_2 in the fish exposed to *Chattonella* is most likely attributable to blockade of respiratory water flow through the gills and increased diffusion distance, both of which were caused by the accumulation of the mixture of mucus and *Chattonella* cells on the gills. Ventilatory effort was greatly enhanced during *Chattonella* exposure, as judged from the marked increase in ventilatory pressures (Ishimatsu et al., 1990), in response to the decreased arterial PO_2. But such effort is probably less effective when the respiratory surface of the gills is covered by thick layers of mucus-*Chattonella* mixtures. Cardiac output was maintained at a higher level during *Chattonella* exposure than during arterial PO_2 was lowered by environmental hypoxia to the comparable levels (Lee et al., 2003). Lee et al. (2003) suggested that O_2 transport ability of hemoglobin might be impaired during *Chattonella* exposure, as hemoglobin contained less oxygen in the *Chattonella*-exposed fish than in the hypoxia-exposed fish when compared at the same PO_2 levels.

In conclusion, the present study showed that respiratory water flow through the fish gills was severely reduced by the exposure to *Chattonella*. This should result in the reductions of gas exchange capacity of the gills followed by the lowered arterial PO_2. Further studies on the effects of *Chattonella* on O_2 transport ability of hemoglobin should be helpful to understand the ichthyotoxic mechanism of HAB species.

References

Fofonoff, P. and R.C. Millard, Jr. 1983. Algorithms for computation of fundamental properties of seawater. UNESCO Technical Papers in Marine Science, 44, 53.

Hishida, Y., A. Ishimatsu and T. Oda. 1997. Mucus blockade of lamellar water channels in yellowtail exposed to *Chattonella marina*. Fish. Sci., 63, 315-316.

Ishimatsu, A., H. Maruta, T. Tsuchiyama and M. Ozaki. 1990. Respiratory, ionoregulatory and cardiovascular responses of the yellowtail *Seriola quinqueradiata* to exposure to the red tide plankton *Chattonella*. Nippon Suisan Gakkaishi, 56, 189-199.

Ishimatsu, A., M. Sameshima, A. Tamura and T. Oda. 1996a. Histological analysis of the mechanisms of *Chattonella*-induced hypoxemia in yellowtail. Fish. Sci., 62, 50-58.

Ishimatsu, A., T. Oda., M. Yoshida and M. Ozaki. 1996b. Oxygen radicals are probably involved in the mortality of yellowtail by *Chattonella* marina. Fish. Sci., 62, 836-837.

Kim, D.K., T. Okamoto, T. Oda, K. Tachibana, K.S. Lee, A. Ishimatsu, Y. Matsuyama, T. Honjo and T. Muramatsu. 2001. Possible involvement of the glycocalyx in the ichthyotoxicity of *Chattonella marina* (Raphidophyceae): immunological approach using antiserum against cell surface structures of the flagellate. Mar. Biol., 139, 625-632.

Lee, K.S., A. Ishimatsu, H. Sakaguchi and T. Oda. 2003. Cardiac output during exposure to *Chattonella marina* and environmental hypoxia in yellowtail (*Seriola quinqueradiata*). Mar. Biol., 142, 391-397.

Nakamura A., T. Okamoto, N. Komatsu, S. Ooka, T. Oda, A. Ishimatsu and T. Muramatsu. 1998. Fish mucus stimulates the generation of superoxide anion by *Chattonella marina* and Heterosigma akashiwo. Fish. Sci., 64, 866-869.

Oda, T., A. Nakamura, M. Shikayama, I. Kawano,A. Ishimatsu and T. Muramatsu. 1997. Generation of reactive oxygen species by raphidophycean phytoplankton. Biosci. Biotechnol. Biochem., 61, 1658-1662.

Oda, T., A. Nakamura, T. Okamoto, A. Ishimatsu and T. Muramatsu. 1998. Lectin-induced enhancement of superoxide anion production by red tide phytoplankton. Mar. Biol., 131, 383-390.

Okaichi, T., S. Nishio and Y. Imatomi. 1982. In: Yuudoku Purankuton (Toxic Planktons), Japan. Soc. Sci. Fish., ed. Suisangaku series, 42, pp. 22-34.

Okaichi, T. 1989. Red tide problems in the Seto Inland Sea, Japan. In: Red tide: Biology, Environmental Science and Toxicology, Okaichi T., D.M. Anderson and T. Nemoto, eds. Elsevier, New York, pp. 137-142.

Piiper, J. 1998. Branchial gas transfer models. Comp. Biochem. Physiol., 119A, 125-130.

Shimada, M., S. Kawamoto, Y. Nakatsuka and M. Watanabe. 1993. Localization of superoxide anion in the red tide alga *Chattonella antiqua*. J. Histochem. Cytochem., 41, 507-511.

Tanaka, K., Y. Muto and M. Shimada. 1994. Generation of superoxide anion radicals by the marine phytoplankton organism, *Chattonella antiqua*. J. Plank. Res., 16, 161-169.

Ultsch, G.R. and G. Gross. 1979. Mucus as a diffusion barrier to oxygen: possible role in O_2 uptake at low pH in carp (*Cyprinus carpio*) gills. Comp. Biochem. Physiol., 62, 685-689.

(Received Jenuary 2004, Accepted June 2004)