Morphometric Changes in Fish Gill upon Chronic Exposure to *Chattonella marina*

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Introduction

Fish gill is the multifunctional organ responsible for respiration, osmoregulation and nitrogenous waste excretion. Existing information on harmful algal bloom and fish gill pathology are exclusively based on acute exposure to high bloom concentration. Moreover, only qualitative descriptions were provided for gill alterations in these studies. Results of morphometry provide more useful data on pathological changes of gill in response to toxic algae. The raphidophyte *Chattonella marina* (Hada) has caused mass kills of fish worldwide. Hyperplasia and hypertrophy of gill lamellae, branchial edema, excessive mucus discharge were reported on the gill surface of fish upon acute exposure to *C. marina*. However, it is not known whether similar pathological symptoms may develop when fish are exposed to a low concentration of *C. marina*. The objectives of this study are:

1. to study mortality of fish upon chronic exposure to *C. marina*, and to measure blood osmolality and oxygen levels of fish at regular time intervals;
2. to investigate quantitative pathological changes in fish gill, with an attempt to elucidate the functional impairment from structural modifications.

Information obtained from this study will allow us to ascertain whether chronic exposure to *C. marina* will cause significant pathological damage to gill, which may lead to poor fish health and mortality.

Materials & Methods

Goldlined seabream, a commonly cultured species in Southern China and South East Asia, were exposed to two concentrations of *Chattonella marina* at 2000 cells/ml (Low dose) and 8000 cells/ml (High dose). Two groups of control fish were also set up. One group was exposed to the non-toxic strain of *Dunaliella* sp. and the other control group was exposed to seawater only. Exposure experiments were terminated when 50% of mortality observed. Ten survival fish were sacrificed from each treatment group. For each fish, blood sample was collected for osmolality (Wescor 5500) and oxygen measurements (Balter blood gas analyser), gill tissues were fixed (in 2% paraformaldehyde and 2.5 % glutaraldehyde in 0.1M cacodylate buffer, then post-fixed in 1% OsO_4) for morphological study. For scanning electron microscopy, fixed tissues were dehydrated in ethanol and acetone, then dried with HMDS, sputter coated with gold and examined with a Philips XL-30 ESEM-FEG scanning electron microscope at 20kV. For transmission electron microscopic study, dehydrated tissues were infiltrated and embedded in Spurr's resin, sectioned and stained before examined with a Philips Tecnai 12 transmission electron microscope at 80kV. For morphometric analysis at the SEM level, 10 image at the interlamellar region from the middle portion of each gill filament were captured at 2000x magnification. Three filaments were examined for each fish. Quantitative measurements on the number and size of chloride cell were conducted with the aid of the analySIS 3.1 image analysis software.

Results

A. Survival-Blood parameters

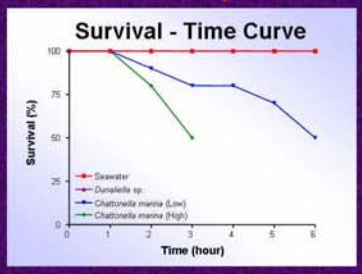

Fig 1. Mortality study of seabream

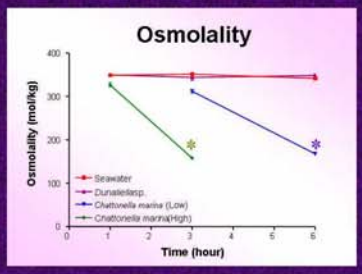

Fig 2. Osmolality of fish blood

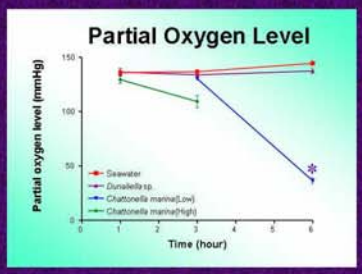

Fig 3. Oxygen level in fish blood

B. SEM observations

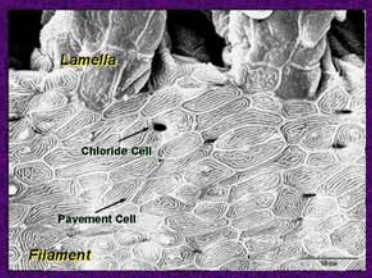

Fig 4a. Control fish gill after exposure to *Dunaliella* sp. for 6 hours.

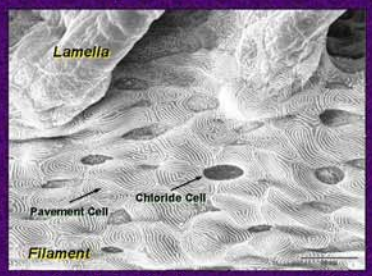

Fig 4b. Fish gill after exposure to 2000 cell/ml of *C. marina* for 6 hours.

C. Morphometric measurements

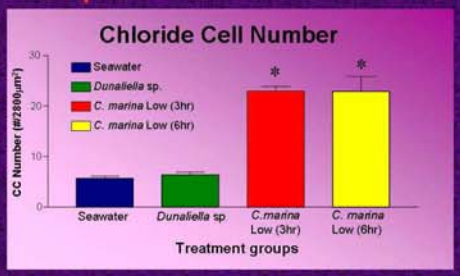

Fig 5a. Changes in number of chloride cell on the gill of fish exposed to *C. marina* at 2000 cell/ml

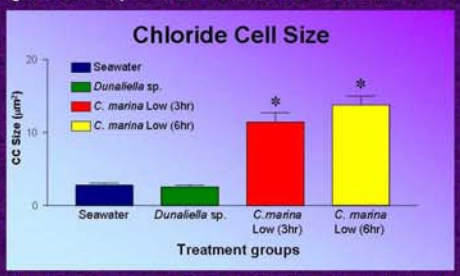

Fig 5b. Changes in size of chloride cell on fish gill of fish exposed to *C. marina at* 2000 cell/ml density

D. TEM observations

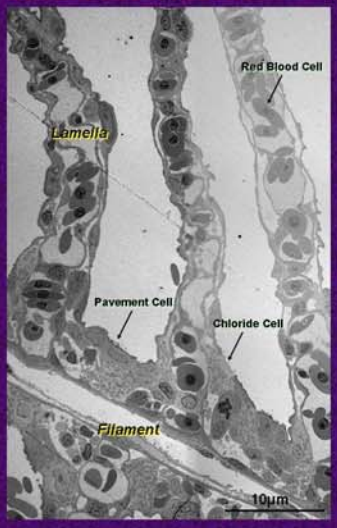

Fig 6. Fish gill after exposure to 2000 cell/ml of *C. marina* for 6 hours. Epithelia of gill filaments and lamellae remain intact.

Discussion

- 50% mortality of seabream was resulted after exposure of fish to low concentration (2000 cell/ml density) of *C. marina* for 6 hr; while it occurred at 3hr for high concentration (8000 cell/ml) of *C. marina* (Fig 1). A good dose response relationship between fish mortality and algal concentration is exhibited.

- After exposure to 2000 cell/ml density of *C. marina* for 3 hr, fish mortality remained low (Fig 1), blood osmolality and O_2 levels of fish were similar to that of the controls (Figs 2-3). However, significant increases in number and size of chloride cells were observed on the gill as compared to the controls (Figs 4a-b; Figs 5a-b). This may indicate an onset of adaptive response in fish to osmotic stress induced by exposure to sublethal level of *C. marina*.

- After exposure to 2000 cell/ml density *C. marina* for 6 h, blood osmolality and O_2 levels dropped significantly in moribund fish exposed to *C. marina* (Figs 2-3), and the number and size of chloride cells on gill remained high (Figs 5a-b). Preliminary observation of gill internal structure at the TEM level showed the epithelia of gill filaments and lamellae remained intact (Fig 6), which did not indicate significant respiratory impairments in the gill of moribund fish.

- Our pathological findings suggest that osmoregulatory failure may be the main reason causing death of seabream when fish were exposed to low concentration of *C. marina*.

Acknowledgement

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