Comparison of Physiological Responses to Exposure to *Chattonella marina* in Yellowtail, Red Sea Bream and Japanese Flounder†

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We compared tolerance of three aquaculture fishes (yellowtail, red sea bream and Japanese flounder) to *Chattonella marina*, and studied physiological responses to exposure to the alga. Resting oxygen uptake (Mo2) was also determined for the three species. All yellowtails were killed by exposure to *C. marina*, whereas mortality was 33% and 0% for bream and flounder, respectively. Arterial oxygen pressure (Pao2) declined rapidly in yellowtail and bream soon after the onset of the exposure. The Pao2 gradually fell and remained lower than the control values in two flounders, whereas it was close to the pre-exposure levels in the other four flounders at the end of the exposure. Ventilatory response during *C. marina* exposure was consistently intense in yellowtail, variable among individuals in bream while nearly absent in flounder. Resting Mo2 was highest in yellowtail, and lowest in flounder. We consider that the observed difference in tolerance to *C. marina* resulted from the different oxygen requirements of the species and their physiological responses in hypoxia (oxygen regulator/conformer). It appears that venous oxygen extraction as a means to maintain oxygen uptake during hypoxia does not function efficiently in fish with a higher oxygen requirement like yellowtail.

Key words: *Chattonella*, Japanese flounder, red sea bream, yellowtail, fish kill mechanism, oxygen uptake

Blooming of harmful algal species *Chattonella* spp. (Rhaphidophyceae) has occasionally resulted in mass mortality of yellowtail in the Seto Inland Sea or along the coasts of Kyushu, Japan. In contrast to the high susceptibility of yellowtail to *Chattonella*, some other aquaculture species, such as red sea bream and Japanese flounder, are thought to be highly tolerant to the blooming, and this received knowledge is supported by two reports on aquaculture damage by *Chattonella* blooming.*1,2 However, the species difference in tolerance to *Chattonella* has not been verified by laboratory experiments and no comparable data are available on physiological responses of different fish species to exposure to *Chattonella*.

The mechanism of fish kill by the alga has not been fully understood in spite of a number of previous investigations.1-7) Matsusato and Kobayashi10 reported a rapid decline of blood oxygen levels in yellowtail exposed to *Chattonella* spp., and ascribed it to interception of gill ventilatory flow by mucus secreted from the algae. These authors concluded that the asphyxiation would lead to the death of fish. On the other hand, Endo et al.7) argued that bradycardia observed in bream exposed to *C. marina* was the major cause of fish death. In contrast, bradycardia did not develop in yellowtail exposed to *C. marina* until shortly before death while blood oxygen levels declined soon after the onset of exposure to *C. marina*.6,8) The difference in cardiac response could be explained by the use of the two different fish species.

We believe that comparison of physiological responses to *Chattonella* in fishes with different susceptibility provides useful knowledge on the mechanism of fish kill by this alga. In the present study, we exposed three fish species (yellowtail, bream and flounder) to *C. marina* to confirm difference in their tolerance to the alga and studied physiological responses under controlled laboratory conditions.

Materials and Methods

*Chattonella* Exposure

1. Experimental Fish *Seriola quinqueradiata* (982 ± 237(SD) g, N=5) was purchased from a local fisherman or offered by Aquaculture Research Laboratory, Nagasaki Prefectural Institute of Fisheries, and kept in netcages or in indoor or outdoor concrete pools with feeding as described previously.6) Red sea bream *Pagrus major* (1091 ± 259 g, N=6) was purchased and kept in a similar way as used for yellowtail. Yellowtail and bream were not fed for 3 days before use. Japanese flounder *Paralichthys olivaceus* (494 ± 29 g, N=6) was purchased 3 to 5 days before use and kept in an indoor tank with a flow-through sea water supply without feeding. Fishes were acclimated.

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to the experimental temperature of 25 ± 1°C.

2. Preparation of Fish and Alga Yellowtail and bream were chronically cannulated in the dorsal aorta under anesthesia for blood sampling and pressure recording.4) Flounder was cannulated in the caudal artery through a lateral incision in the peduncle.5) Fishes were also cannulated in the branchial cavity to sample expired water. Ventilatory pressure (VP) was recorded only for flounder and bream because the data were already available for yellowtail.6) Fishes were then allowed to recover for about 24 h in a fish chamber supplied by continuous flow of well aerated sea water.

*Chatastonella marina* isolated in Kagoshima in 1985 was batch-cultured using ESM medium (Erd-Schreiber modified) as previously described.6)

3. Protocol The details of the setup and analytical techniques were described in Ishimatsu et al.6) Following duplicate blood and water sampling under resting conditions, the fish was exposed to *C. marina* (plankton concentration; 4000 cells/ml, temperature; 25 ± 1°C, water P02 = 155 mmHg). In yellowtail experiments, blood and water samples were taken at 5 min, 30 min, and then with 30 min intervals until fish death. We sampled about 0.8 ml of blood at one sampling time. A half volume of the blood was used for blood gas measurements and rein fused to the fish at the next sampling time. The rest of the blood was centrifuged to obtain plasma for later determinations of Na+, K+ and Cl− concentrations.6) It was reported that blood sampling of this magnitude little influenced the physiological status of the fish.6) At each sampling time, arterial Po2 (Pao2), arterial pH (pHa), hematocrit (Hct), and the P02 of expired water (Peo2) were measured. In bream and flounder experiments, sampling was done at 5 min, 30 min, and then with 60 min intervals until 210 min. Then the experimental setup was flushed with fresh sea water containing the same concentration of *C. marina* cells.6) Further samples were taken at 215 min, 240 min, and then with 60 min intervals until 420 min. In addition to the above measurements, VP, ventilatory frequency (VF), mean blood pressure (BP), and heart rate (HR) were determined for these two species. Data for VP, VF, BP and HR in yellowtail were taken from Ishimatsu et al.6)

**Oxygen Uptake**

Yellowtail (857 ± 183 g, N=6), bream (1247 ± 180 g, N=6) and flounder (554 ± 50 g, N=6) were acclimated as above. An intact fish was transferred into a tank (water volume; 150 l in yellowtail and bream, 60 l in flounder) with a flow-through supply of well aerated sea water about 24 h before measurement. A plastic sheet was placed below water surface. At the start of measurement, water supply was stopped and water samples were siphoned into 100 ml DO bottles with 20 min intervals until 180 min. Water P02 was measured with an ULTRA-DO METER (CENTRAL KAGAKU Inc.). Oxygen uptake (Mo2) was calculated according to Itazawa.9)

**Results**

Data on respiratory and circulatory variables under control conditions for yellowtail, red sea bream and Japanese flounder are summarized in Table 1. Percent oxygen utilization at gills (Ug), ventilation volume (Vg) and ventilatory stroke volume (VsV) were calculated according to Itazawa.9) Yellowtail showed the highest pHa, Hct, BP, VP, VF and Mo2 but the lowest Pao2 among the three species (Bonferroni test). Most variables were not significantly different between bream and flounder, except VF and Mo2. Pao2 and Ug were not significantly different among the three species. Only a single estimate of Vg is given for each species because an average Mo2 value for the species determined under somewhat different conditions was used for calculation. Vg of yellowtail was more than twice that of bream and flounder. Vg/Mo2 was nearly identical in yellowtail and bream, which was about 63% of the value for flounder.

All individuals of yellowtail were killed with a mean survival time of 83 ± 22 min of *C. marina* exposure. Bream showed a mortality of 33%; two fish died at 165 and 253 min while four fish survived till the end of the exposure (420 min). No mortality was observed for flounder at all. Yellowtail showed a rapid decline of Pao2 after the start of *C. marina* exposure. The Pao2 was nearly zero when fish were killed (Fig. 1A). Bream also showed a similar fall of Pao2 at the early stage of the exposure (Fig. 1B). Then, the Pao2 of four surviving fish remained at the low level until the end of experiments, although some fluctuations were obvious. In the killed fish, the Pao2 fell close to zero at death, although we failed to take the terminal blood sample in one of them. Most flounder showed slight increases in Pao2 immediately after the onset of the exposure (Fig. 1C). Then, the Pao2 of three fish progressively decreased to 45–65 mmHg until the flush at 210 min, after which the Pao2 of one fish was elevated to above 80 mmHg. The Pao2 of the other flounder remained at or above the control levels. The Pao2 difference between control and 30 min values was similar in yellowtail (59 ± 8 mmHg) and bream (55 ± 20). The pHa sharply dropped in yellowtail and bream at death (Fig. 2A, B), whereas it remained stable throughout the exposure in surviving bream and flounder (Fig. 2B, C). Hct increased considerably only in yellowtail at death (51.0 ± 6.2%), but did not change in bream (neither in those which died nor in those which survived)

| Table 1. Ventilatory, respiratory and cardiovascular parameters of yellowtail, red sea bream and Japanese flounder in the control conditions and O2 consumption (mean±SD) |
|-----------------|-----------------|-----------------|
|                 | Yellowtail      | Red sea bream   | Japanese flounder |
| Pao2 (mmHg)     | 80.5 ± 9.5      | 102.8 ± 15.2    | 116.0 ± 11.9      |
| pHa             | 7.927 ± 0.039   | 7.747 ± 0.021   | 7.803 ± 0.054     |
| Hct (%)         | 31.2 ± 5.2      | 17.8 ± 3.0      | 15.7 ± 2.1        |
| Pao2 (mmHg)     | 56.7 ± 9.0      | 66.6 ± 13.9     | 82.5 ± 24.6       |
| HR (beats/min)  | 96 ± 12.5       | 77.7 ± 17.4     | 96.1 ± 23.0       |
| BP (cmH2O)      | 59 ± 5.1        | 33.4 ± 4.3      | 32.3 ± 2.7        |
| VP (cmH2O)      | 3.2 ± 1.0       | 1.0 ± 0.4       | 1.2 ± 0.4         |
| VF (breaths/min)| 92 ± 8.6        | 60.3 ± 8.0      | 43.5 ± 5.9        |
| Ug (%)          | 63.4 ± 5.8      | 57.0 ± 8.9      | 46.8 ± 15.8       |
| Vg (ml/kg/min)  | 1099.6          | 436.5           | 384.0             |
| VsV (ml/kg)     | 12.0            | 8.1             | 8.8               |
| Mo2 (mmol/kg/h) | 8.8 ± 2.4       | 3.8 ± 0.4       | 1.9 ± 0.3         |
| Ug/Mo2 (ml/μmol)| 7.5             | 7.7             | 12.1              |

\* a: data from Ishimatsu et al.,6) #: significantly different from the corresponding values for yellowtail (P<0.05), ¶: significantly different from red sea bream (P<0.05).
Fig. 1 Arterial P\textsubscript{o\textsubscript{2}} (Pao\textsubscript{2}) of yellowtail (A), red sea bream (B) and Japanese flounder (C).

Open and filled symbols represent surviving and killed fish, respectively. C represents pre-exposure environment.

or flounder (data not shown). In yellowtail, the Peo\textsubscript{2} increased (Fig. 3A) when Pao\textsubscript{2} fell (Fig. 1A). The Peo\textsubscript{2} of three surviving bream remained at the pre-exposure level before the flush (Fig. 3B). Then, it elevated to and remained at a higher level in one fish, while it increased only transiently in the other two. In one killed bream the Peo\textsubscript{2} increased as in yellowtail. In the remaining bream (one which died and one which survived), the Peo\textsubscript{2} could not be measured due to cannula blockade. In flounder, Peo\textsubscript{2} after C. marina exposure became higher than the pre-exposure values, except in two fish (Fig. 3C).

The VP as well as VF increased markedly in only three bream (two which killed and one which survived, Fig. 4A, B). The VP of flounder hardly changed, while the VF gradually, but only moderately, increased in three fish (Fig. 4C, D). The HR in control conditions widely varied among individuals in both bream and flounder (Fig. 5A, C). In bream, changes in HR during the exposure were variable among fish except for sharp declines before death. Four flounder showed bradycardia soon after the onset of the exposure. The BP gradually decreased in both surviving bream and flounder (Fig. 5B, D). The BP of the two killed bream was at or above the control levels during the exposure and precipitously declined towards the death of fish (Fig. 5B).

Control plasma Na\textsuperscript{+}, K\textsuperscript{+} and Cl\textsuperscript{-} concentrations were 183.4±4.3 mmol/l, 3.4±0.1 and 172.2±4.5, respectively, in bream and 163.3±2.7, 3.1±0.1 and 143.7±2.0, respectively, in flounder. C. marina exposure did not affect plasma levels of the three ions in surviving bream or flounder. In one of the two killed bream, all plasma ion levels increased considerably shortly before death, whereas only plasma K\textsuperscript{+} increased in the other fish (data not shown).

**Discussion**

Our results demonstrated that the tolerance to C. marina differs among the three fish species used in this study. All individuals of yellowtail died when exposed to C. marina, while only one third of bream and none of flounder were killed under the same experimental conditions. The low tolerance of yellowtail to Chattonella has been repeatedly demonstrated.\textsuperscript{1,6} Matsusato and Kobayashi\textsuperscript{10} previously reported mortality of bream exposed to Chattonella sp. (species not identified) under closely similar experimental conditions as used in our study, although their fish weighed only about 30 g. Endo et al. also reported death of bream exposed to C. marina, but only at much higher plankton concentrations (8,000\textsuperscript{3} or 9,700 cells/ml\textsuperscript{7}). Un-
fortunately, the number of fish used in their study is too
too low to draw a convincing conclusion about tolerance of
bream to *C. marina*. To our knowledge, no previous data are available on survival/death of flounder exposed to

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**Chaptonella.**

The present *Pao* under control conditions for yellowtail is similar to the previous value reported by Yamamoto, whereas the *Pao* for bream is higher than the previous data by Azuma and Itazawa (both studies conducted at 20°C). To our knowledge, the *Pao* has not been reported for the flounder. Wood et al. reported a considerably lower *Pao* of 34.9±3.0 mmHg in starry flounder *Platichthys stellatus* at a lower temperature of 7.5–10.5°C. The difference in species and experimental temperature must be important contributing factors for the *Pao* difference. Additionally, Wood et al. reported for starry flounder that the *Pao*, as well as *Pao* and *Vg*, continued to fall for at least 48 h after surgery, and consistently stable levels could only be recorded after three days. Usually, 24 h is sufficient for recovery after surgical implantation of cannulae, and therefore we allowed our fish, including the flounder, to recover for 24 h. Sluggish species like flounders may need a longer recovery period.

The present *Peo* values of yellowtail and bream under control conditions were higher than the previously reported values (ca. 30 mmHg in yellowtail, 37.3±11.3 mmHg in bream), although the measurements were made at slightly lower temperature in those studies (20°C). The *Peo* for starry flounder was reported to be 34.9±3.0 mmHg. These differences in *Peo* may be due to the sampling technique of expired water with a branchial cannula. It is known that *Peo* can vary depending on sampling sites due to incomplete mixing of expired water at the opercular cavity, although Dejours pointed out that this technique can give a reasonably accurate estimate of *Peo* at least for smaller (100–300 g) carp. But this technique allows the animal to move freely and the surgical procedure is minimal. Particularly, for active pelagic fish like yellowtail, the use of other techniques for a more accurate measurement of *Peo* is impractical. For the purpose of this study, we considered this advantage outweighs the inherent inaccuracy of the technique. The *Ug* was calculated to be lower, whereas *Vg* and *Vg/MO* was estimated to be higher, in this study than in the previous studies (yellowtail: *Ug* 77.2±5.3%, *Vg* 619±59 ml/kg/min, *Vg/MO*...
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Fig. 6 Relative VP (control = 100) and VF as a function of Pao2. (A, B) yellowtail, (C, D) bream, (E, F) flounder.

Open and filled symbols represent surviving and killed fish, respectively. Data for yellowtail were taken from Ishimatsu et al.6)

Fig. 7 Relative HR (control = 100) and BP as a function of Pao2. (A, B) yellowtail, (C, D) bream, (E, F) flounder.

Open and filled symbols represent surviving and killed fish, respectively. Data for yellowtail were taken from Ishimatsu et al.6)

Relationships between Pao2 and relative values (control = 100) of VP, VF, HR and BP are diagrammed in Fig. 6 (VP and VF) and Fig. 7 (HR and BP). In general, fish respond to hypoxemia by increasing ventilatory water flow. The ventilatory flow is determined by the frequency (VF) and stroke volume.9,14-16) In the present study, we did not directly measure ventilatory flow as stated above, but used VP as an index for changes in stroke volume. VP of yellowtail sharply increased in response to a fall of Pao2. Relative VP was significantly higher for the surviving fish than for the killed ones in the Pao2 range of 20-40 mmHg, while no such difference was detected at higher Pao2. VF ranged from slightly less than 100% to 150% of the control values when Pao2 was approximately 20-70 mmHg, while the slope for the relationship appears to be close to zero. It appears that modulation of VP is important for the regulation of ventilatory flow in yellowtail. In bream, VP of the killed bream increased with a similar magnitude as found for yellowtail, while the response in the surviving bream was much weaker. Relationship of VF to Pao2 is not discernible between the killed and surviving individuals, VF ranging from 150% to 200% at the lowest Pao2. Ventilatory responses in flounder are clearly different from those observed in the two other species. Neither VP nor VF showed increases in response to lowering Pao2 (Fig. 6). Although there are some points which are as high as above 200% (VP) or 150% (VF) at Pao2 of about 40-80 mmHg, same magnitude of variations occurred at the highest range of Pao2. We therefore consider that ventilation is relatively insensitive to Pao2 in this species, at least for the Pao2 range observed in this study.

Figure 7 demonstrates occurrence of hypoxic bradycardia in killed yellowtail, while HR in surviving yellowtail appears to increase in response to moderate fall of Pao2. HR in bream failed to show a consistent response to *C. marina* exposure. HR clearly declined at an early stage of the exposure in four flounder, while no such bradycardia developed in bream (Fig. 5). When plotted against Pao2, HR of both bream and flounder declined to a similar extent.

There are two types of responses in oxygen uptake to hypoxia in fish.16) Yellowtail (data not shown) and bream17) can maintain oxygen uptake (oxygen regulator) down to water Po2 of approximately 40 mmHg. Oxygen uptake during environmental hypoxia has not been determined for
flounder, but, judging from available data on other flatfishes, we suspect that oxygen uptake in flounder would lower in response to hypoxia (oxygen conformer). This contention is supported by the absence of hyperventilation during C. marina exposure observed in this study. Kobayashi demonstrated that oxygen uptake increased when yellowtail was exposed to Chattonella sp. Similarly, oxygen uptake in killed bream is thought to have increased, deduced from a more than three-fold increase in VP and maintained Ug during an early phase of C. marina exposure (Fig. 4, 8). However, oxygen uptake in surviving bream is unlikely to have increased because of insignificant increase in ventilation (VP or VF little increased) and unchanged Ug until Pao2 was lowered down to 40 mmHg. In contrast to the response in oxygen uptake in the other two species, flounder appears to have reduced its oxygen uptake during C. marina exposure because of unchanged ventilation and a reduction in Ug. As stated above, flounder is probably an oxygen conformer, and this ability to reduce metabolism under C. marina-exposed conditions is probably responsible for the highest tolerance of flounder to C. marina.

Fish can maintain its O2 uptake by reducing venous P02 (Pvo2) under hypoxia. Thomas et al. demonstrated that Pvo2 was reduced when arterial O2 saturation fell down to 40%. This mechanism of adaptation to hypoxia may be less efficient for fish with a higher Mo2 because venous oxygen level can only be lowered to a limited extent. O2 uptake can be met by increasing cardiac output (Vb) when difference in O2 saturation between arterial and venous blood is reduced. However, Vb usually does not increase or even decreases under hypoxia. We speculate that the surviving bream, in which Mo2 did not supposedly increase after the start of exposure, maintained its O2 uptake by this mechanism. If so, this mechanism is expected to function more efficiently for bream than yellowtail when the Pao2 quickly decreased in response to C. marina exposure. Furthermore, when O2 uptake cannot satisfy its requirement as during severe hypoxia, an animal needs to consume body oxygen store, for which blood constitutes by far the largest portion. Based on the blood volume and hemoglobin concentration of yellowtail and calculated to be 411 mg/100 g for yellowtail and 317 for bream, the amounts of hemoglobin per body weight are calculated to be 411 mg/100 g for yellowtail and 317 for bream, the ratio (1:0.77) being different from that for oxygen uptake (1:0.43). Thus, the differences in oxygen requirement and the size of oxygen store between the two species may well explain their significant difference in tolerance to C. marina.

The difference in Vg (Table 1) implies that the number of C. marina cells which contact gills is different among three fishes, being the highest in yellowtail and the lowest in flounder even though fishes are exposed to the same concentration of C. marina. This fact may also account for the species difference in the tolerance to C. marina.

Further research is needed to elucidate mechanisms underlying the difference in VP of killed and surviving yellowtails. It is also unclear why two bream were killed despite their higher response in VP like the surviving yellowtail. To fully understand mechanisms of fish kill by Chattonella, it seems important to obtain data on oxygen uptake during the exposure, more detailed blood gas levels including venous blood, and cardiac output.

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