Respiratory, Ionoregulatory and Cardiovascular Responses of the
Yellowtail *Seriola quinqueradiata* to Exposure
to the Red Tide Plankton *Chattonella* *1*

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To clarify the mechanisms by which the yellowtail *Seriola quinqueradiata* are killed by the red tide plankton *Chattonella*, an analysis was made on physiological responses of the fish exposed to the plankton. Fish were chronically cannulated in the dorsal aorta and gill cavity. Changes in respiratory, ionic and cardiovascular parameters were followed until death. Soon after exposure, a severe hypoxemia developed which was accompanied by a marked increase in ventilatory pulse pressure, though the latter subsequently subsided. No acidosis occurred until shortly before death despite the persistent hypoxemia. Plasma concentrations of Na⁺, K⁺, Cl⁻, Mg²⁺, and Ca²⁺ were all elevated to different magnitudes. Blood pressure responses varied among fish, but arrhythmia was the predominant response. Heart rate gradually declined or remained unchanged until shortly before death. Gill ventilatory frequency changed very little during most of the exposure period. These results were critically discussed in the light of data reported previously.

In the summer of 1987, another extensive bloom of *Chattonella* (Rhaphidophycea) broke out in the Seto Inland Sea, Japan. This resulted in loss of 1.7 million yellowtail *Seriola quinqueradiata*, the most intensively cultured fish species of the country. This very fact stressed that there are virtually no effective countermeasures to prevent those mass mortalities in spite of nearly two decades of severe damage to aquaculture.

Surprisingly, only a small number of studies have investigated the possible causes of fish death by exposure to *Chattonella*. Okaichi and Nishio*4* attributed the toxic action to the free fatty acid content in the cells (C. sp.). Onoue and Nozawa*3) claimed that they could separate three toxic fractions, neurotoxic, hemolytic, and hemagglutinative, from *C. marina*. Shimada et al.2) on the other hand, found that *C. antiqua* reduced cytochrome c, which they thought was in part due to superoxide produced by the plankton. A few papers have been published as to the possible mechanisms of death*3-*6) and the histology of the killed fish.7,8) Only one pioneering study by Kobayashi*9) investigated one time course of physiological changes of plankton-exposed fish. The purpose of the present study was to conduct a detailed analysis of the physiological responses of chronically catheterized yellowtail exposed to bloom concentrations of *Chattonella* under controlled conditions in the hope that such knowledge would form a scientific basis to develop countermeasures to the negative impact of plankton blooms on aquaculture in the future.

**Materials and Methods**

**Experimental Animals**

Yellowtails *Seriola quinqueradiata* weighing about 1 kg were purchased from local fishermen, and kept in netcages (3 m × 3 m × 3 m) settled in Nomo Bay for several months before use. Fish were fed on chopped sardine twice a week. Fish were transferred into indoor tanks with a flow-through seawater supply approximately 1 week before use and kept at the experimental temperature of 25°C without feeding.

**Plankton Culture**

*Chattonella marina* were generously provided by Professor K. Hirayama, Nagasaki University, and batch-cultured according to the method of Okaichi et al.1) This strain was originally

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centration and medium pH were checked every other day during the culture period. The medium pH tended to rise due to metabolism of the plankton and hence was adjusted back to the original pH of 8.2 by CO₂ bubbling. Temperature was kept at 25°C. In our hands, over 40,000 cells/ml of plankton concentration were routinely attained in the final culture bottles of 10 l capacity.

**Animal Surgery**

Fish were chronically cannulated in the dorsal aorta and branchial cavity to allow repetitive blood sampling and pressure measurement. The dorsal aorta was cannulated by the method of Soivio et al. The fish were first anesthetized by immersing them in a 0.02% sea water solution of MS 222 (ethyl m-aminobenzoate methanesulfonate, Sigma) neutralized with 0.1 N NaOH. Temperature of the solution was kept at 25°C. The solution was bubbled with pure O₂. During surgery, the gills of the fish were irrigated with an oxygenated MS 222 solution of 0.01%. Cannulas were filled with heparinized (50 i.u./ml) seawater fish saline. After the aortic cannulation, the branchial cavity of one side was cannulated with another piece of polyethylene tubing (1 m long) with one end flared by heat. Following surgery, the fish was quickly transferred into a Plexiglass box with circulating normoxic water and allowed to recover overnight. During the recovery period, fresh sea water was continuously supplied into the experimental setup with the flow rate of approximately 8 l/min. Hematocrit value was regularly monitored during this period, and only fish with stable hematocrit were subjected to experimentation.

**Experimental Setup**

In the course of preliminary studies, we have noticed that *Chattonella* cells are extremely sensitive to mechanical disturbances such that the cells were deformed and sank in a short period by the use of ordinary rotary pumps or by vigorous aeration. On the other hand, yellowtail, as an active species, need a rather high water flow rate (4-5 l/min) and sufficient oxygen to be kept in a good condition in the experimental chamber. The present setup was designed to meet these two conflicting demands as shown in Fig. 1. Firstly, we used a peristaltic pump with a large pumping head (Delasco BZ-20, Paris). Due to the large diameter of the head (ca. 140 mm) as well as the large bore of the pump tubing (I.D. 20 mm), sufficient flow could be obtained with low rotation speed (5.0 l/min at 50 rpm under zero counterpressure). Secondly, we used two aeration columns connected in series which allowed the reduction of air flow to 20 l/h to attain air saturation. Fig. 2 demonstrates that both water Po₂ and plankton

![Diagram of the experimental setup](image)
separated in Kagoshima in 1978. Plankton concentration were successfully kept constant during the experimental period of up to 7 h.

**Fig. 2.** Water Po2 (Pwo2, above) and plankton concentration (below) in eight experimental runs. Filled circles indicate runs in which fish were killed, open ones those in which fish survived. F denotes open flow-through condition. Arrowhead indicates system flushing at 3.5 h.

**Protocols**

1. Controls Considering rather small volume of the present setup relative to fish size, possible effects of the confinement of fish on their physiological state were studied using 7 fish. Following the recovery period of approximately 20 h, duplicate blood analysis and pressure measurement were made under open flow-through condition. The system was then closed and 5 l of autoclaved plankton culture medium was added to the system. Blood samples were taken at 5 min, 15 min, 30 min, and then with 30 min intervals until 5 h from the start of an experiment followed by sampling at 6 and 7 h. At each sampling time, 0.35 ml of blood was anaerobically withdrawn into a 1 ml tuberculin glass syringe with specially reduced dead space volume, and immediately analyzed for Po2 and pH. Another 0.6 ml was withdrawn into a nonheparinized plastic syringe for plasma ion analysis. Additionally, two hematocrit tubings were filled with blood to obtain hematocrit value. Water samples were also taken for Po2 measurement. Blood and ventilatory pressures were recorded continuously throughout the experiment. Water pH tended to decrease due to acid excretion of the fish and was thus kept constant by the addition of 0.1 N NaOH. In some preliminary experiments, slight disturbances in ventilatory pressure were observed 3–4 h after system closure, and hence the system was flushed at 3.5 h.

2. Plankton Exposure After the same recovery period and initial sampling and pressure measurements under the open flow-through condition, the system was closed and the plankton was added to the system to attain concentrations of around 4,000 cells/ml (usually 4.5–5.0 liters was added from 10 l bottle). Blood and water samples were taken with the same sampling schedule as in controls until the fish died. When muscular activity ceased, it was quickly removed from the chamber. The heart was exposed and the terminal blood sample was taken by cardiac puncture. Experiments were terminated at 7 h if the fish survived.

**Analytical Techniques**

Po2 and blood pH were measured with an IL-213 blood gas analyzer (Instrumentation Laboratory, Mass.) thermostatted at 25°C. Plasma was obtained by centrifugation at 11,000 rpm for 5 min. Plasma Na+ and K+ were analyzed by flame photometry (Corning 480, Essex). Plasma Ca2+ and Mg2+ concentrations were measured on diluted samples by atomic absorption spectrophotometry (Hitachi polarized Zeeman Z-8000, Tokyo). Plasma Cl− concentration was determined coulometrically (Joukou 200-A, Tokyo). Hematocrit was determined using a microcentrifuge. For pressure recording, the cannulas were connected to Gould P 23XL transducers (Gould Statham Instruments Inc., Calif.). The output was amplified with a polygraph (Nihondenki-Sanei Model 366, Tokyo) and recorded using a four-channel Recti-Horiz 8K20 series recorder (Nihondenki-Sanei). Mean blood pressure was calculated as diastolic pressure plus 1/3 pulse pressure.13) Instantaneous heart rate was obtained by feeding the pressure signal into a tachometer (Nihondenki-Sanei Model 1332). Plankton concentrations were determined by triplicate counting of the plankton number in 20 μl aliquots under a dissecting microscope. Sea water salinity was estimated by determinations of specific gravity.

**Statistical Analysis**

Data are expressed in means ± S.D. wherever possible. Statistical comparisons were made using paired or unpaired Student’s t-test where appropriate.
Results

Controls

Figs. 3–6 show changes in 13 variables measured here. All but hematocrit value (Hct) were kept nearly constant throughout the 7 h period. Statistically significant difference from mean values under the open flow-through condition could be detected for most of the experimental period in two variables (plasma Ca\(^{2+}\) (Fig. 4) and mean dorsal aortic pressure (mean \(P_{\text{DA}}\), Fig. 5)). Absolute differences in mean values were, however, rather small (<0.3 mM for Ca\(^{2+}\) and <8 cmH\(_2\)O for mean \(P_{\text{DA}}\)) so that we considered these physiologically insignificant. Hct showed a slight, gradual decrease due to repetitive blood sampling (Fig. 3). Based on these observations, we concluded that both our blood sampling schedule and confinement in our experimental setup exerted little, if any, influences on physiological status of the fish. Table 1 gives the summary of blood analysis and pressure data determined under the open flow-through conditions (denoted F in the Figs. 2–15).

Plankton Exposure

In 12 trials of plankton exposure, fish were killed in 8 runs and survived in the other 4. The responses of these fish will be separately described below.

1. Responses of killed fish Figs. 7–10 show the physiological responses of the fish killed by Chattenella exposure. We presented individual plots, rather than means and S.D.s, in these Figures because the time course to eventual death was quite variable among fish. In spite of the strictly controlled experimental conditions employed in this study, time to death largely varied ranging from nearly 30 min to slightly less than 3.5 h. Devastating effects of plankton exposure were obvious for most of the variables when comparison was made between the resting values and the
terminal ones. Thus, blood Po₂ dropped nearly down to zero (Fig. 7), pH by almost 1 pH unit (Fig. 7), and all the plasma ion levels increased considerably (Fig. 8). In all cases, blood Po₂ started to decline immediately after the addition of plankton. Some fish withstood hypoxemia for some period, but others did not. Cardiovascular responses varied among individuals. Mean PDA elevated or dropped individually while the pulse pressure (Pulse PDA) increased in most of the fish (Fig. 9). Heart rate (HR) remained unchanged or progressively declined before eventual stop (Fig. 9). Ventilatory pulse pressure (Pulse P) responded quickly to the plankton exposure invariably showing large but transient increase (Fig. 10). Ventilatory frequency (f₀) revealed little change except sharp decline except toward the death (Fig. 10). Similar but less pronounced increase in Pulse P was observed in a preliminary study.*

Fig. 5. Mean (above) and pulse (middle) blood pressure in the dorsal aorta (PDA) and heart rate (HR, below) in control experiments. N is 7 except three points in HR. Mean±S.D.

Fig. 6. Ventilatory pulse pressure (Pulse P₀, above) and ventilatory frequency (f₀, below) in control experiments. N is 7 except the last point in Pulse P₀ and two points in f₀. Mean±S.D.

Table 1. Mean±S.D. of the measured variables in recovered, chronically cannulated yellowtail in normoxic water at 25°C (N=7)

<table>
<thead>
<tr>
<th>Blood gas and electrolytes</th>
<th>Cardiovascular and ventilatory variables</th>
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<tbody>
<tr>
<td>Pao₂ (mmHg)</td>
<td>Mean PDA (cmH₂O)</td>
</tr>
<tr>
<td>pH</td>
<td>Pulse PDA (cmH₂O)</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>Plasma [Na⁺] (mm)</td>
<td>Pulse P₀ (cmH₂O)</td>
</tr>
<tr>
<td>[K⁺] (mm)</td>
<td>f₀ (breaths/min)</td>
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<tr>
<td>[Cl⁻] (mm)</td>
<td></td>
</tr>
<tr>
<td>[Mg²⁺] (mm)</td>
<td></td>
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<tr>
<td>[Ca²⁺] (mm)</td>
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Mean PDA: mean dorsal aortic pressure.  
Pulse PDA: dorsal aortic pulse pressure.  
Pulse P₀: ventilatory pulse pressure.  
f₀: ventilatory frequency.

Fig. 7. Water and arterial Po2 (above), arterial pH (pHa, middle) and hematocrit (Hct, below) in individual fish that died from plankton exposure. Triangles represent values of the terminal samples taken by cardiac puncture immediately following fish death. F denotes open flow-through condition.

Fig. 8. Plasma ion levels in individual fish that died from plankton exposure. Symbols are the same as in Fig. 7.

Fig. 11 illustrates one example of direct pressure tracings of a fish killed at about 200 min after the onset of exposure. Substantial changes in profiles of both blood and ventilatory pressure wave forms occurred in response to exposure. Rhythmic cardiac beating and regular contour of the blood pressure wave forms changed into arrhythmic and irregular patterns. Ventilatory wave forms showed even more marked responses. Ventilatory pressure fluctuation of resting fish was composed of a major downward excursion caused by opercular suctioning and a subsequent small positive rebound attributable to opercular adduction. After plankton exposure, pressure depression became much deeper accompanied by a more distinct positive peak. In many cases enhancement of Pulse Po2 occurred within short time period while its timing after the onset of exposure varied from experiment to experiment. Characteristically, ventilatory pressure, after the stimulation of ventilatory activity, often became superimposed on blood pressure tracings as distinct spikes. In the last stages of exposure, heart beat became slower and even more arrhythmic. Finally rhythmic ventilatory activity ceased followed by sporadic convulsion prior to total cessation of respiratory muscle activity. All the other fish died by exposure showed similar responses.

2. Responses of survived fish Figs. 12-15 summarize the responses of the survived 4 fish. In these experiments, the experimental system was totally flushed at 3.5 h as in control experiments while plankton concentration was kept constant throughout. Blood Po2 declined to below 30 mmHg in these fish: two fish showed rapid decrease while in the other two it declined only after 2-2.5 h of exposure. After flushing the system, however, Po2 of two fish recovered (Fig. 12). No change at all could be seen for pHa and plasma electrolyte levels (Figs. 12 and 13). Cardiovascular variables showed some disturbances but these too
became more or less stabilized after system flushing with the exception of one fish which showed large increase in Pulse $P_{DA}$ at 7 h (Fig. 14). $f_0$ was quite stable throughout the experiments, but Pulse $P_o$ increased in response to exposure (Fig. 15). The two fish showing rise in Pulse $P_o$ at 2 h correspond to those with lowering $P_o$ at that time shown in Fig. 12. After system flushing, Pulse $P_o$ declined and stabilized in two fish, but kept elevated in the other two. The former correspond to those with recovered $P_o$ after flush (Fig. 12).

**Discussion**

The present study demonstrated that exposure to the red tide plankton *Chattonella* provoked severe perturbations in respiratory, ionoregulatory and cardiovascular functions of fish in cases where mortality occurred. A question remains why some fish survived under the same conditions. We have treated the fish and cultured the plankton in a consistent way throughout this study. But there must have been some uncontrolled factors which resulted in unknown physiological or biochemical variabilities either in fish or plankton, or in both. Seeking possible correlation between such variabilities in fish and/or plankton and fatality should give an important clue to understand the causes of fish death by *Chattonella*.

It may be considered that the observed physiological disturbances occurred more or less simultaneously, but close inspection of the data clarifies that they occurred with different time courses. Thus, the development of hypoxemia (Fig. 7) and rise in Pulse $P_o$ (Fig. 10) are the earliest responses occurred whereas the lowering of blood pH (Fig. 7) and decrease in $f_0$ (Fig. 10) are the last among the observed. It is conceivable that the latter perturbations are likely to be secondary responses to preceding physiological disturbances. Direct, immediate effects of *Chattonella* exposure should be identified in order to understand the causes of death in fish and thereby seek an effective countermeasure against the mass mortalities. For this purpose, time course study using chronic preparations as reported here is essential as it enables us to follow in vivo perturbations in desired variables after the onset of plankton exposure with predetermined sampling schedule.

The precipitous decline in arterial $P_o$ (Fig. 7) is the most consistent and quickly developed response observed in all of the killed fish as pre-
previously reported. As water Po2 was always kept above 130 mmHg in these as well as in the other experiments, it is evident that oxygen diffusion was impaired by plankton exposure. Published histological studies as well as our own microscopic observations showed that this was not caused by clogging of gas exchange surface of the gills with plankton cells. Nor did we observe excessive mucus secretion as has been frequently reported.

Fig. 11. Direct recording of the dorsal aortic pressure (PDA), ventilatory pressure (Pv) and instantaneous heart rate (HR) of a fish that died some 200 min after the start of exposure. Sequence of events is from top left triad to top right, bottom left and bottom right. Chart running speed is indicated at the top with brackets. Elapsed time after the onset of plankton exposure is also shown above the dorsal aortic pressure trace with arrows. Blood pressure and heart rate measurements were interrupted by blood sampling. Soon after the start of exposure, no apparent change was seen both in PDA and Pv (top left triad). In this fish, sudden increase in ventilatory activity occurred at 60 min (top right).

It is noteworthy in this context that edema in the gill lamellae and filaments is one of the most pronounced histological changes induced by Chattonella exposure. This must interfere with O2 transfer across the gill by increasing the blood-water diffusion distance. In fish gill, the situation may well be even more complicated by the presence of the osmotic and ionic gradients across the epithelium and by active transepithelial transport. Tsyoshima et al. observed disintegration of chloride...
cell in fish exposed to *Chattonella* and suggested this to be the possible cause of edema. Chloride cell is the definite site of Cl⁻ extrusion in seawater fish gill.¹⁷) The observed rises of plasma ion levels which invariably occurred in our fish that were killed (Fig. 8) are in apparent agreement with histological deduction by Toyoshima et al.⁸) Granted that chloride cell disintegration would result in edema which in turn impairs oxygen diffusion, it may be considered that the rises in plasma ion levels are too slow to account for the more quickly occurring Po₂ decline. There should be, however, a time lag between development of local ionic imbalance and rise in plasma ion levels considering large body pool of electrolytes. Physiological significance of the inhibition of branchial carbonic anhydrase activity by *Chattonella* exposure⁴,⁵ should probably be considered in this connection. Branchial edema formation is not a unique histological response to exposure to *Chattonella*, but it can be induced also by

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**Fig. 13.** Plasma ion concentrations of fish surviving plankton exposure. Symbols are the same as in Fig. 12.

**Fig. 14.** Mean (above) and pulse (middle) pressure of the dorsal aorta (PdA), and heart rate (HR, below) in individual fish surviving plankton exposure. Symbols are the same as in Fig. 12.

**Fig. 15.** Ventilatory pulse pressure (Pulse Pd, above) and ventilatory frequency (fₐ, below) in individual fish surviving plankton exposure. Symbols are the same as in Fig. 12.
other plankton species like *Gyrodinium* and *Gymnodinium*. Fig. 16 shows the relative increases in plasma ion concentrations against preexisting concentration gradients between plasma and surrounding seawater for the fish that were killed. Fig. 16 shows the relative increases in plasma ion concentrations against preexisting concentration gradients between plasma and surrounding seawater for the fish that were killed. It is of interest to note that K⁺ showed by far the highest value of 0.76 compared with those of the other ions (0.09–0.27). Plasma K⁺ levels increased very close to the seawater concentration of 10 mm in spite of the persistent large concentration gradients for the other monovalent ions (Fig. 8). Ionic diffusion from sea water has undoubtedly contributed to the observed rise in plasma ions in the face of probable malfunctioning of chloride cell, but the distinct behavior of K⁺ might be brought about by additional mechanisms. As K⁺ is the major ionic constituent of intracellular fluid in fish as in other animals, it seems possible that transmembrane electrochemical gradient was disturbed by *Chattonella* exposure. Mg²⁺ showed much higher increase (×6.0 from control) than Ca²⁺ (×1.7), but this is likely due to larger preexisting concentration gradient for the former ion, which resulted in the lower R value for Mg²⁺.

Yamaguchi *et al.* compared O₂ transport properties of yellowtail hemoglobin with those of two other fish species and found that O₂ affinity of the yellowtail hemoglobin characteristically became insensitive to pH changes at pH ranges of either below 6.7 or above 7.4. They also reported considerable tissue and blood acidification in yellowtail that were struggling (blood pH 6.72) or just killed (4.50) in a red tide bloom. They speculated, as to the mechanism of fish kill, that struggling caused by red tide plankton would induce acidosis and this would in turn suppress oxygen transport by hemoglobin due to the lack of the Bohr effect. Our present data demonstrated that acidosis occurred only in the last stages of exposure (Fig. 7) and hence is probably of minor importance to elucidate causes of fish kill by *Chattonella*. Arterial pH was kept fairly constant for the fish that survived 7 h of plankton exposure despite the arterial Po₂ of below 30 mmHg (Fig. 12). This suggests that hypoxemia alone cannot account for the marked acidosis observed in the last stages of exposure.

The increase in Pulse Po in exposed fish (Figs. 10 and 15) is apparently a reflex response to the lowered arterial Po₂. Subsequent decreases probably reflect a general failure of the respiratory center. The control of ventilation in fish has been recently reviewed.

Recently, Endo *et al.* reported an electrocardiogram of the red sea bream Pagrus major exposed to *Chattonella*. The observed development of bradycardia, particularly in fish exposed to the concentration of 8,000 cells/ml, could be due to the effect of *Chattonella* exposure, though the small number of fish used in their study makes those results inconclusive. An analysis of the relationship between heart rate and arterial Po₂.

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*R* was calculated as:

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R = \frac{C_{\text{killed}} - C_{\text{cont}}}{C_{\text{sw}} - C_{\text{cont}}}
\]

where \(C_{\text{killed}}\) denotes plasma ion concentration at death, \(C_{\text{cont}}\) is the control plasma ion concentration, and \(C_{\text{sw}}\) is the ion concentration in seawater. Data shown in Fig. 8 were used to construct this Fig. Measured salinity of the seawater in the present study was 35.2±0.93% (N=18). Sea water ion concentrations for standard ocean water were therefore used here.

Vertical lines above bars show S.D.

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of our fish (both killed and survived) revealed that heart rate was independent of the arterial Po2 over wide range of the latter (30 to >100 mmHg). Since the cardiovascular responses of yellowtail to hypoxemia is not known, it remains uncertain if this apparent absence of hypoxic bradycardia is normal for the species or indicative of the occurrence of other physiological disorders which may have affected the regulation of the cardiovascular system.

Finally, the Po2 recovery in two of the survived fish after system flushing (Fig. 12) deserves consideration. Since the plankton concentration was kept unchanged by flushing, and 3.5 h recirculation exerted no apparently adverse effects on plankton activity, the only renewed factor must have been chemical quality of the sea water. This may indicate that some toxic substance(s) had been accumulated by interaction of the plankton and fish, and was removed by flushing to result in the observed recovery. Obviously further research is needed in this respect.

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