Blood Properties of the Japanese Flounder (Paralichthys olivaceus) Exposed to Smectite Suspended in Seawater

Koji KAWANA1, *, Nakahiro IWATA2, Takeshi HANDA3, Yoshihiko BABA4, Kazumasa UEMATSU1 and Kenji NAMBA2

Abstract: The effects of smectite suspended in seawater on the blood properties of the Japanese flounder (Paralichthys olivaceus) were examined. The fish were exposed to smectite (0, 4.5, 10, 18 and 56 g/l) seawater solutions at 20°C. All the fish died within 4.5 h exposure to a 56 g/l smectite solution, and almost all fish within 24 h exposure to a 18 g/l smectite solution. Blood was collected from the dead fish soon after death and from the live fish after 24 h exposure, and was chemically analyzed. Hemoglobin and plasma protein concentrations did not vary significantly after exposure to the smectite solutions. Compared to the live fish and the control fish, the dead fish showed markedly high plasma lactate, blood total ammonia, and plasma electrolyte (Na+, Cl−, K+, Ca2+, Mg2+) concentrations and significantly low plasma glucose concentrations. Taken together, changes in the blood properties revealed that the lethal physiological disturbances were attributable to hypoxia caused by exposure to the high concentrations of smectite.

Key words: Japanese flounder; Paralichthys olivaceus; Suspended smectite; Blood properties

Inflow of eroded soils through rivers into estuarine environments causes elevated suspended solid concentrations in seawater on occasions such as heavy rain fall, and can have eco-physiological effects on fish. Smectite is one of the major clay mineral species of soil component and was studied as suspended matter for its effects on the Japanese flounder Paralichthys olivaceus, which is an important fish species in Japanese estuarine fisheries. Baba et al. (2006) have estimated that the 96hLC50 value for flounder exposed to smectite suspension in seawater was 37 g/l. Furthermore, Kawana et al. (2008) have reported that the percent of oxygen utilization and consumption decreased due to ventilation failure when flounder were exposed to concentrations of smectite higher than the 96hLC50 value.

Blood properties have often been studied to depict the state of fish physiology under environmental stresses, but little is known on those of fish exposed to a suspended solid. Sherk et al. (1975) have reported elevated hematocrit value, hemoglobin concentration, erythrocyte count and plasma osmolality in some estuarine fish species exposed to sublethal concentrations of suspended fuller’s earth. Changes in blood hematocrit levels with suspended sediment exposure are also reported in rainbow trout Oncorhynchus mykiss (Reid et al. 2003). McLeay et al. (1987) have studied stress response with the Arctic grayling Thymallus arcticus from the Yukon River and found effects on sugar level and leucocrit level of blood after 1-4d exposure.
to 50 mg/l organic sediment suspension. These studies were carried out under sublethal concentrations of suspended solids and did not yield enough information on blood properties under lethal conditions. This study aims to reveal the lethal physiological disturbance of fish based on the blood properties of fish exposed to smectite.

Materials and Methods

**Suspended solid**

The suspended solid used in the experiments was smectite (Kunipia-F, Kunimine Industries Co., Ltd., Tokyo, Japan). The particle-size distribution of smectite suspended in seawater was determined with a laser scattering particle-size distribution analyzer (LMS-30, Seishin Enterprise Co., Ltd., Tokyo, Japan). The median diameter of the smectite was 16.1 μm.

**Experimental fish**

Cultured Japanese flounder were purchased from a commercial fish farm (Hota Fishermen’s Cooperative, Kyonan-machi, Chiba, Japan). The fish were transported to the experimental site (Central Research Institute of Electric Power Industry, Abiko, Chiba, Japan) and held at least one week prior to the experiments in large fiberglass tanks supplied with circulating seawater at 20°C. They were fed daily with commercial Japanese flounder pellets (Higashimaru Co., Ltd., Kagoshima, Japan), but were starved one day prior to and during the experiments.

**Experimental protocol**

The smectite was added in seawater (salinity 31-32) obtained at a shore in the vicinity of Onjuku, Chiba, Japan. The fish (standard length, 398 ± 1.2 mm; body weight, 606 ± 4.5 g; n = 75) were confined individually in a net cage (18 cm × 30 cm × 35 cm) set in a 50 l experimental tank capable of maintaining smectite in suspension (Baba et al. 2006; Kawana et al. 2008) and were exposed to smectite for 24 h at 20°C. Five smectite treatments (0 [control], 4.5, 10, 18 and 56 g/l) were randomly assigned to the cumulative total 75 experimental tanks (15 replicates per treatment). The experimental tanks were partially submerged in a water bath consisting of a FRP tank (1,810 cm × 910 cm × 700 cm), a recirculation pump (RSD-40, REI-SEA Co., Ltd., Tokyo, Japan), a water cooler (LX150CX, REI-SEA Co., Ltd., Tokyo, Japan) and a thermo-controller (TC-100, REI-SEA Co., Ltd., Tokyo, Japan). A constant temperature was maintained in the experimental tanks using a water bath. The survival of the fish in the control group, 4.5 g/l group and 10 g/l group during the exposure experiment was confirmed every 2 hours, except for 9 hours during the night. The survival of the 56 g/l group was checked every one hour until 3 hours of exposure, and subsequently every 30 minutes. Fish confirmed dead were measured in body weight and standard length, and blood was drawn from them. Following 24 h of exposure, all surviving fish were anesthetized with a MS-222 (100 mg/l) seawater solution, and their body weight and standard length were measured, and blood drawn.

**Water quality**

Water temperature, dissolved oxygen (DO), pH and viscosity were checked once in each experimental tank over 24 hours of exposure. The water temperature and the dissolved oxygen were measured with a dissolved oxygen meter (DO-25A, DKK-TOA Corporation, Tokyo, Japan), pH with a pH meter (InLab410, Mettler Toledo International Inc., Tokyo, Japan), and viscosity with an analog viscosity meter (LVT, Brookfield Engineering Laboratories, Inc., MA, USA). At the end of an exposure test or at a time of mortality, total ammonia levels were determined in the experimental tanks with an ion-meter (9001K-SR, Toko Kagaku Co., Ltd., Tokyo, Japan).

**Blood analyses**

About 2 ml blood was collected from the caudal vessels in a heparinized 2.5 ml syringe. After measurements of hemoglobin and ammonia in whole blood, the remaining blood was centrifuged at 3,500 rpm for 10 min with a centrifuge (himac CF15D, Hitachi Koki Co.,
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Ltd., Tokyo, Japan) and the plasma sample was removed to microfuge tubes. The whole blood sample and some of the plasma sample were analyzed using commercial clinical investigation kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan or Kyowa Medex Co., Ltd., Tokyo, Japan) using the following methods: hemoglobin, the cyanmethemoglobin method (Hemoglobin-test Wako); blood total ammonia, the indophenol method (Ammonia-test Wako); plasma glucose, the glucose oxidase method (Glucose C II-test Wako); total plasma protein, the biuret method (A/G B-test Wako); plasma calcium (Ca2+), the MXB method (Calcium E-test Wako); plasma magnesium (Mg2+), the xylidil blue method (Magnesium B-test Wako); and plasma lactate, the lactate oxidase method (Determiner LA Kyowa). Plasma sodium (Na+), potassium (K+) and chloride (Cl−) ions were measured with a biochemical analyzer (Fuji DRI-CHEM 800, FUJIFILM Corporation, Tokyo, Japan).

Statistics

Data shown in figures and table 2 or given in the text are presented as mean ± S. E. M. (n = number of fish); values in table 1 are as means ± SD. The significance of differences at P<0.01 and P<0.05 was examined using the Kruskal-Wallis analysis of variance, followed by Steel-Dwass multiple comparisons test (KyPlot4.0, KyensLab Inc., Tokyo, Japan).

Results

The temperature, dissolved oxygen concentration, pH, viscosity, and total ammonia level of the test water in each group during smectite exposure are shown in Table 1. The water temperature was maintained in the immediate vicinity of 20°C, and DO was sustained at 90% or more. The water pH of the smectite groups was slightly higher than that (8.21 ± 0.04) of the control group, and the highest water pH of all groups was 8.88 ± 0.02 in 18 g/l group. Total ammonia levels in the water of the smectite groups were modestly lower than those of the control group (0.6 ± 0.2 mg/l, NH3-N<0.05 mg/l), and the lowest total ammonia level was 0.2 ± 0.0 mg/l in the water of the 56 g/l group. Viscosities in the test water increased with increasing smectite concentrations: 1.2 ± 0.0 mPas in the control group; 1.3 ± 0.0 mPas in the 4.5 g/l group; 1.4 ± 0.0 mPas in the 10 g/l group; 2.0 ± 0.0 mPas in the 18 g/l group; and 4.8 ± 0.3 mPas in the 56 g/l group.

No mortalities were recorded in the control, 4.5 g/l or 10 g/l groups during 24 h exposure but the initial mortalities occurred at about 6 h in the 18 g/l group and about 2 h in the 56 g/l group. All fish except one fish in the 18 g/l group and all fish in the 56 g/l group died during 24 h and 4.5 h exposures, respectively.

The blood properties of all fish treated are given in Table 2. In Figs. 1 and 2, then, those blood properties have been categorized in three groups: those of the control group; the live fish which survived 24 h exposure (i.e., the 4.5 and 10 g/l groups); and the dead fish which died during 24 h exposure (i.e., the 18 and 56 g/l groups). Hemoglobin concentration did not differ significantly among all groups (e.g., the control group, 6.5 ± 0.28 g/100 ml; the live fish group, 6.7 ± 0.26 g/100 ml; and the dead fish

<table>
<thead>
<tr>
<th>Smectite (g/l)</th>
<th>Water temperature (°C)</th>
<th>Dissolved oxygen (%)</th>
<th>pH</th>
<th>Total ammonia (mg/l)</th>
<th>Viscosity (mPas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>20.0 ± 0.4</td>
<td>95.8 ± 1.1</td>
<td>8.21 ± 0.04</td>
<td>0.6 ± 0.2</td>
<td>1.2 ± 0.0</td>
</tr>
<tr>
<td>4.5</td>
<td>19.9 ± 0.1</td>
<td>94.6 ± 0.9</td>
<td>8.61 ± 0.03</td>
<td>0.4 ± 0.1</td>
<td>1.3 ± 0.0</td>
</tr>
<tr>
<td>10</td>
<td>20.3 ± 0.2</td>
<td>96.0 ± 0.5</td>
<td>8.63 ± 0.01</td>
<td>0.2 ± 0.1</td>
<td>1.4 ± 0.0</td>
</tr>
<tr>
<td>18</td>
<td>19.9 ± 0.1</td>
<td>94.5 ± 0.8</td>
<td>8.88 ± 0.02</td>
<td>0.3 ± 0.2</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>56</td>
<td>20.5 ± 0.2</td>
<td>98.6 ± 1.6</td>
<td>8.39 ± 0.09</td>
<td>0.2 ± 0.0</td>
<td>4.8 ± 0.3</td>
</tr>
</tbody>
</table>

Water temperature, dissolved oxygen, and pH were measured at the start of the experiment and total ammonia and viscosity were measured either after 24 hours exposure or immediately after fish death.
The data are presented as means ± SD.
Plasma glucose concentrations were significantly higher in the 10 g/l group than in the control group whereas lower in the 18 g/l and 56 g/l groups. The live fish group showed significantly higher plasma glucose concentrations (18.7 ± 0.70 mg/100 ml) than the control group (14.8 ± 0.70 mg/100 ml) or the dead fish group (5.8 ± 0.92 mg/100 ml).

Additionally, the dead fish group indicated a significantly low plasma glucose concentration relative to the control group. Plasma lactate concentrations in the 4.5 g/l group and in the 10 g/l group were significantly low compared to those in the control group (2.1 ± 0.39 mg/100 ml). However, plasma lactate concentrations in the dead fish group (82.1 ± 4.97 mg/100 ml) were markedly higher than those in the control group. Furthermore, the dead fish group had significantly low plasma lactate concentration relative to the control group.

The data are presented as means ± S.E.M.; for each variable, values that do not share a common letter are statistically different from each other (P<0.01).

Hb, hemoglobin; Glu, plasma glucose; T-Pro, total plasma protein; LA, plasma lactate; NH₃, total ammonia; Na⁺, plasma sodium; Cl⁻, plasma chloride; K⁺, plasma potassium; Ca²⁺, plasma calcium; Mg²⁺, plasma magnesium.

The table below shows the blood properties of the Japanese flounder exposed to suspended smectite.

### Table 2. Blood properties of the Japanese flounder exposed to suspended smectite

<table>
<thead>
<tr>
<th>Smectite (g/l)</th>
<th>0.0 (n=15)</th>
<th>4.5 (n=14)</th>
<th>10 (n=14)</th>
<th>18 (n=13)</th>
<th>56 (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/100 ml)</td>
<td>6.5 ± 0.29a</td>
<td>6.5 ± 0.29a</td>
<td>6.9 ± 0.41a</td>
<td>6.7 ± 0.22a</td>
<td>6.7 ± 0.17a</td>
</tr>
<tr>
<td>Glu (mg/100 ml)</td>
<td>14.8 ± 0.71a</td>
<td>17.2 ± 0.76ab</td>
<td>20.1 ± 1.06b</td>
<td>3.4 ± 0.42c,1</td>
<td>8.0 ± 1.54c</td>
</tr>
<tr>
<td>T-Pro (g/100 ml)</td>
<td>4.2 ± 0.11a</td>
<td>4.3 ± 0.09a</td>
<td>5.2 ± 0.31a</td>
<td>4.4 ± 0.19a</td>
<td>4.3 ± 0.17a</td>
</tr>
<tr>
<td>LA (mg/100 ml)</td>
<td>2.1 ± 0.38a</td>
<td>0.2 ± 0.04b</td>
<td>1.5 ± 0.10a</td>
<td>87.8 ± 8.76c</td>
<td>71.1 ± 5.34c</td>
</tr>
<tr>
<td>NH₃ (µg/g/100 ml)</td>
<td>307 ± 11.8a</td>
<td>296 ± 20.0a</td>
<td>310 ± 22.2a</td>
<td>870 ± 63.4b,2</td>
<td>810 ± 69.8b</td>
</tr>
<tr>
<td>Na⁺ (mM)</td>
<td>155 ± 1.1 a</td>
<td>151 ± 1.0 a</td>
<td>156 ± 0.7 a</td>
<td>188 ± 2.6 b</td>
<td>195 ± 2.1 b</td>
</tr>
<tr>
<td>Cl⁻ (mM)</td>
<td>181 ± 3.0 a</td>
<td>176 ± 0.9 a</td>
<td>175 ± 1.8 a</td>
<td>211 ± 2.4 b</td>
<td>202 ± 2.5 b</td>
</tr>
<tr>
<td>K⁺ (mM)</td>
<td>2.8 ± 0.10a</td>
<td>2.4 ± 0.06b</td>
<td>2.7 ± 0.09ab</td>
<td>6.7 ± 0.25c</td>
<td>6.2 ± 0.31c</td>
</tr>
<tr>
<td>Ca²⁺ (mM)</td>
<td>6.4 ± 0.10a</td>
<td>6.3 ± 0.07a</td>
<td>6.5 ± 0.09a</td>
<td>6.9 ± 0.21a</td>
<td>13.1 ± 1.01b</td>
</tr>
<tr>
<td>Mg²⁺ (mM)</td>
<td>1.3 ± 0.13a</td>
<td>0.9 ± 0.11a</td>
<td>1.4 ± 0.19a</td>
<td>5.9 ± 0.36b</td>
<td>5.8 ± 0.46b</td>
</tr>
</tbody>
</table>

The values are means ± 1 S.E.M. (* P<0.01; ** P<0.001) from each other (Steel–Dwass test).

**Fig. 1.** Hemoglobin (A), plasma glucose (B), plasma lactate (C), total plasma protein (D) and blood total ammonia (E) of the Japanese flounder exposed to suspended smectite. Open bars, control fish; hatched bars, live fish; filled bars, dead fish. Values are means ± 1 S.E.M. (n=15–28). Asterisks indicate significant difference (* P<0.01; ** P<0.001) from each other (Steel–Dwass test).
remarkably higher plasma lactate concentrations than the live fish group (0.9 ± 0.13 mg/100 ml). Total plasma protein concentration showed no significant differences among all groups (e.g., the control group, 4.2 ± 0.10 g/100 ml; the live fish group, 4.7 ± 0.19 g/100 ml; and the dead fish group, 4.3 ± 0.13 g/100 ml). Blood total ammonia concentrations in the dead fish group were significantly high compared to those in the control group (303 ± 11.8 μg/100 ml). The dead fish group had markedly higher blood total ammonia concentrations (841 ± 46.4 μg/100 ml) than the live fish group (303 ± 15.0 μg/100 ml). No significant differences in the blood total ammonia concentration were observed between the live fish group and the control group.

Plasma Na⁺ concentrations significantly increased in the dead fish group (192 ± 1.8 mM) compared to those in the control group (155 ± 1.1 mM). The dead fish group showed significantly higher plasma Na⁺ concentrations than the live fish group (154 ± 0.7 mM. The concentrations of plasma Cl⁻ and plasma K⁺ in the fish exposed to smectite changed similarly to the concentrations of plasma Na⁺, and were significantly higher in the dead fish group (Cl⁻, 206 ± 1.9 mM; K⁺, 6.5 ± 0.20 mM) than in the control group (Cl⁻, 181 ± 2.9 mM; K⁺, 2.8 ± 0.10 mM). Concentrations of plasma Ca²⁺ and plasma Mg²⁺ (divalent ions) in the dead fish group were significantly higher than those in the control group. Both plasma Ca²⁺ and plasma Mg²⁺ in the dead fish group (Ca²⁺, 10.2 ± 0.81 mM; Mg²⁺, 5.8 ± 0.30 mM) showed remarkably higher concentrations than those in the live fish group (Ca²⁺, 6.4 ± 0.06 mM; Mg²⁺, 1.2 ± 0.11 mM).

Discussion

There were no adverse influences of dissolved oxygen, pH in the test water (Suzuki et al. 2000) and total ammonia (U.S. Environmental Protection Agency 1989; Ip et al. 2001) on the experimental fish during a 24 h exposure.

The viscosities of the 18 g/l and 56 g/l groups were approximately 1.7 and 4 times that of the control, respectively. The higher viscosity of the test water was associated with gill ventilation failure. The reduced gill ventilation
flow decreased the oxygen uptake from the test water through the gill surface and caused progressive hypoxia (Kawana et al. 2008). Moreover, the energy cost of gill ventilation in the high viscosity water was expected to be larger than that of routine gill ventilation, which is generally around 10% of routine oxygen consumption (Jones and Randall 1978; Milsom 1989; Perry and McDonald 1993).

Almost all the Japanese flounder (606 g mean body weight) exposed to 18 g/l smectite died within 24 h, whereas none of the flounder (4.1 g mean body weight) exposed to the same concentration of smectite by Baba et al. (2006) died during 96 h. This difference in tolerance to suspended smectite appears to be related to fish size or fish life stage (Servizi and Martens 1991).

Release of erythrocytes from their storage points, the spleen and the liver, occurred in yellowtail (Seriola quinqueradiata) (Yamamoto et al. 1983) or rainbow trout (Wells and Weber 1990) in response to acute hypoxia, and the hemoglobin concentration increased. Although the fish exposed to 18 g/l or 56 g/l of smectite probably suffocated to death due to gill ventilation failure (Kawana et al. 2008), such an increase in hemoglobin concentration was not observed. Unlike active swimmers, such as yellowtail and rainbow trout, the Japanese flounder, which is a relatively inactive and demersal fish, may not have sufficient erythrocyte-storage for urgent oxygen supply in burst swimming or hypoxia. Furthermore, severe dehydration or hemoconcentration did not occur in the fish exposed to smectite, since hemoglobin concentrations and plasma protein concentrations did not significantly change even in the dead fish group.

When compared to the concentrations of plasma glucose and plasma lactate in the control group, plasma glucose concentrations were significantly high in the live fish group and significantly low in the dead fish group, while the plasma lactate concentrations were significantly low in the live fish group, especially in 4.5 g/l group, and markedly high in the dead fish group. Plasma glucose concentrations in adult sockeye salmon reportedly increased 150 and 39% as a result of exposure to 1,500 and 500 mg/l, respectively, of fine sediment (Servizi and Martens 1987), and plasma cortisol concentrations were elevated in coho salmon and steelhead after exposure to 2–3 g/l of suspended topsoil (Redding et al. 1987). Presumably, the exposures to the suspended sediments were stressors in salmonids. Similarly, the exposure to 4.5 g/l or 10 g/l smectite behaved as a stressor and increased the plasma glucose concentration in the live fish group higher than that in the control group.

The decrease in the plasma lactate concentration in the live fish group was probably caused by the activation of gluconeogenesis due to catecholamines and/or cortisol released in response to the stress (Wright et al. 1989; Reid et al. 1998; Perry and Bernier 1999; Mommsen et al. 1999; Barton 2002). With the 18 g/l and 56 g/l smectite solutions, the high viscosities of the test waters probably demanded a large excess of energy for gill ventilation. The gill musculature of the fish consumed glucose in large quantities, and consequently, the plasma glucose concentration in the dead fish group became lower than that in the control group. Furthermore, owing to gill ventilation failure, the fish were subjected to progressive hypoxia, and a marked rise in the plasma lactate—a major end product of anaerobic metabolism—occurred (Burton and Heath 1980; Boutilier et al. 1988) and probably resulted in metabolic acidosis (Wood 1991).

The blood total ammonia concentrations in the dead fish group were about 2.8 times higher than those in the control group and the live fish group. The gill ventilation failure inhibited blood total ammonia excretion through the gill epithelium (Evans et al. 2005; Weihrauch et al. 2009), and presumably caused marked increases in blood total ammonia concentration, since above 80% of the total nitrogen, ammonia plus urea, was excreted from the gills of many fish species (Wood 1993). Elevated blood total ammonia acts on the central nervous system of fish causing coma, convulsions, and finally death (Ip et al. 2001).

Tazaki et al. (2003) have reported that K
and Ca contents were reduced in the gills of rainbow trout exposed to smectite. Conversely, with flounder, plasma electrolytes (Na⁺, Cl⁻, K⁺, Ca²⁺, Mg²⁺) concentrations were all elevated in the dead fish group; and the concentrations of K⁺, Ca²⁺ and Mg²⁺ were highly increased in other groups. Plasma Na⁺ is extruded across the leaky tight junctions between the mitochondrion-rich cell and the accessory cell of the gill epithelium, while plasma Cl⁻ is extruded across the apical membrane via a Cl⁻ channel of the mitochondrion-rich cell (Evans et al. 2005). The elevation of plasma Na⁺ and plasma Cl⁻ concentrations was probably due to the hypofunction of the mitochondrion-rich cell caused by low ATP levels resulting from anaerobic metabolism. The exact mechanism by which K⁺ in the plasma is excreted is at present not known. However, several K⁺ excretion channels are likely to exist in the mitochondrion-rich cell of the gill (Marshall and Grosell 2006). The elevation of plasma K⁺ may therefore be due to the hypofunction of the mitochondrion-rich cell, similar to as occurred in both the plasma Na⁺ and in the plasma Cl⁻ concentrations. Excessive plasma Ca²⁺ and Mg²⁺ derived from the oral ingestion of seawater must be excreted by renal and branchial mechanisms (Evans et al. 2005) so that the renal and branchial insufficiency in the dead fish group elevated the plasma Ca²⁺ and Mg²⁺ concentrations.

In conclusion, changes in the blood properties of the Japanese flounder exposed to high 18 g/l and 56 g/l concentrations of smectite revealed that the lethal physiological disturbances were attributable to hypoxia due to ventilation failure; the anaerobic metabolism yielded by hypoxia resulted in ATP deficiency from the depletion of plasma glucose, metabolic acidosis from the elevation of plasma lactate concentrations, neural pathologies from the elevation of blood total ammonia concentrations, and plasma electrolyte (i.e., Na⁺, Cl⁻, K⁺, Ca²⁺, Mg²⁺) transport disorders mainly from ATP deficiency.

References


海水に懸濁するスメクトライトに曝露されたヒラメの血液性状

川那公士・岩田伸弘・半田岳志・馬場義彦・植松一真・難波憲二

海水（20℃）に懸濁するスメクトライト（0, 4.5, 10, 18, 56 g/l）にヒラメを24時間曝露し、その血液性状の変化を調べ、スメクトライトがヒラメに及ぼす生理的障害を推定した。24時間の曝露でも、スメクトライト濃度 4.5および10 g/lでは、全ての魚が死亡した。一方、スメクトライト濃度56 g/lでは全ての魚が曝露4.5時間以内に死亡し、18 g/lでは、15尾中14尾が24時間以内に死亡、残り1尾は24時間曝露後に死亡した。ヘモグロビン濃度と血漿タンパク質濃度はコントロール、生残魚、死亡魚の間に有意な相異は認められなかった。死亡魚はコントロールおよび生残魚に比較して、血漿中乳酸量、血中全アンモニア濃度および血漿中の無機イオン（Na⁺, Cl⁻, K⁺, Ca²⁺, Mg²⁺）濃度が著しく有意に増加し、血漿中グルコース濃度は有意に減少した。血液性状の変化から、スメクトライトがヒラメに及ぼす致死的生理障害は、高濃度のスメクトライトによる窒息に起因するものと推定された。