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Citation	Fisheries Science, 76(3), 529-536 https://doi.org/10.1007/s12562-010-0233-7
Issue Date	2010-05
Doc URL	https://hdl.handle.net/2115/43069
Rights	The original publication is available at www.springerlink.com
Type	journal article
File Information	FS76-3_529-536.pdf



Photosensitivity in mice caused by pyropheophorbide in the midgut gland of the scallop *Patinopecten yessoensis* observed in diarrhetic shellfish poisoning mouse bioassays

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Abstract Photosensitivity was observed in the mice used in bioassays of diarrhetic shellfish poisoning. The mice were intraperitoneally injected with the extracts from the midgut glands of cultured scallops *Patinopecten yessoensis* fished in early spring in Funka Bay, Hokkaido, Japan. The injections induced untypical symptoms in the mice within 24 h. The symptoms included piloerection, substantial eye discharge, swelling of the ears and head, and ear scratching. They were similar to those reported in study of photosensitivity caused by pyropheophorbide in midgut glands of abalones, which are herbivorous gastropods. The problematic scallops also accumulated a large amount of pyropheophorbide-a. The amount determined with high-performance liquid chromatography was 300 to 530 μg per 1 g of the homogenate of the midgut glands. The dose of pyropheophorbide is estimated to be 0.99 to 2.3 mg per a mouse with 20-g body weight. It is sufficient to cause the photosensitivity in a mouse. Moreover, comparative mouse tests showed that the onset of the symptoms needs light and a substantial amount of pyropheophorbide. Therefore, we defined that the symptoms were photosensitivity caused by pyropheophorbide. Scallops, which are suspension-feeding bivalves, also accumulate a large amount of pyropheophorbide in the midgut glands depending on the habitat environment.

Keywords Diarrhetic shellfish poisoning · Midgut gland · Mouse Bioassay · Photosensitivity · Pyropheophorbide · Scallop · Suspension feeder

Introduction

The midgut glands of abalones or turban shells are eaten raw or cooked in some areas of Japan.

Eating midgut glands that are darker green than normal in early spring can induce food poisoning, the main symptom of which is dermatitis. The poisoning is classified as a photosensitivity disease because exposure to light is needed for the onset of the symptoms. Hashimoto *et al.* showed that the photosensitivity is induced by pyropheophorbide, which is a metabolic product of chlorophyll [1-4]. Pheophorbide, which is another metabolic product of chlorophyll, is also harmful [5, 6, 17].

Because pyropheophorbide and pheophorbide are photosensitizers, they and light combine to generate singlet oxygen from ground-state molecular oxygen. It is thought that the damage of cellular structures by the singlet oxygen is a cause of the inducement of symptoms [7-9].

The feeding habit of abalones or turban shells is the reason why they accumulate pyropheophorbide in spring. They are herbivorous gastropods that ingest seaweeds, and they directly take in chlorophyll from seaweeds. The increased amount of pyropheophorbide in the gastropods in spring reflects the increase in the amount of chlorophyll in seaweeds, which grow most rapidly in spring.

Suspension feeders such as scallops or oysters can also accumulate pyropheophorbide or pheophorbide because they eat phytoplankton, zooplankton that feed on phytoplankton, or detritus.

Pyropheophorbide or pheophorbide has usually been detected in bivalves [10]; however, this has received little attention since no cases of photosensitivity disease caused by edible bivalves have been reported.

In accordance with Food Sanitation Laws in Japan, we tested cultured scallops *Patinopecten yessoensis* for diarrhetic shellfish poisoning (DSP) with a mouse bioassay and found unusual symptoms and behaviors in some mice that had received intraperitoneal injections of extracts of the midgut glands of the scallops. A positive DSP test is defined as death within 24 h after injection. The scallop extract-injected mice were all alive after 24 h and the scallops therefore passed the DSP test. However, the symptoms developed in the mice had never been seen before in DSP testing. The symptoms included piloerection, substantial eye discharge, swelling of the ears and head, and ear scratching. One of the mice ran around in the cage intermittently. The symptoms were similar to gastropod photosensitivity disease [1, 3, 5] and we inferred that the mice were photosensitized by pyropheophorbide or related substances in the midgut glands of the scallops. Also, the filtrates after acetone extraction appeared to be abundant in chlorophyll, since they were dark green, whereas a normal filtrate is brownish.

In this study we measured pyropheophorbide, pheophorbide, and other pheopigments in scallop samples with high-performance liquid chromatography (HPLC), and performed comparative mouse bioassays to confirm the requirement of light for the onset of the symptoms. It

became clear that scallops, which are suspension feeders, accumulate pyropheophorbide in early spring, in the same manner as abalones or turban shells, which are herbivorous gastropods. We also measured pheopigments in other types of bivalves to compare with scallops.

Materials and methods

Chemicals

HPLC-grade solvents (acetone and methanol), chlorophyll-a, pheophytin-a, pyropheophytin-a, and chlorophyllid-a were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

Pheophorbide-a and pyropheophorbide-a were obtained from Frontier Scientific, Inc. (Logan, Utah, USA). All other chemicals used were of analytical reagent grade from Wako Pure Chemical Industries and were used without further purification.

Samples

The experiments were conducted by using the remainders of the homogenates of the midgut glands that were used in DSP inspections. The bivalves for the inspections were commonly brought alive to our research institute under refrigeration. About 1 kg of shucked bivalves were prepared from them and the entire weight was measured accurately. The midgut glands were then cut from the

bodies, and the whole was weighed to determine the weight ratio of the midgut glands to the whole bodies before being homogenized with a homogenizer (AM-10, NIHONSEIKI KAISHA LTD., Tokyo, Japan). The ratio was needed to calculate the amount of the homogenate that was used for a DSP mouse bioassay. The tested homogenates were kept at -80 °C for the verification of the results of the inspections. We used the frozen homogenates and the data of the weight ratio in this study.

The photosensitivity disease-like symptoms in mice were observed in our administrative DSP inspections of scallops. Figure 1 shows their fishing area and the regular sampling points, Oshamanbe and Otoshibe. In the area, we were monitoring DSP levels in the scallops sampled every two weeks during the scallop fishing season. Therefore, the series of tested samples including the targets were used to elucidate the fluctuation of the contents of chlorophyll-a and the pheopigments with HPLC. Two samples, Otoshibe, February 20, 2006 and Oshamanbe, March 7, 2006, in them were used as high-pyropheophorbide samples in our mouse bioassays. The Oshamanbe sample, March 7, 2006 was also used in the comparison analysis between bivalve species described below.

We also measured the contents of each pigment in several bivalve species with HPLC to compare composition of pigments between them. We conduct additional inspection of edible bivalves. These samples were used for the comparison analysis because their samplings in Hokkaido markets were not periodic but the samples have variety. The midgut gland homogenate

of scallop caught in Abuta (Fig1) area on June 26, 2006, was used as a low-pyropheophorbide sample in our mouse bioassays.

Preparation of Measurement Solutions

Measurement solutions for HPLC analysis to elucidate fluctuation of contents of chlorophyll-a and three pheopigments: pheophorbide-a, pyropheophorbide-a, and pheophytin-a, in scallops were prepared as follows: one gram of the scallop homogenate was shaken for 1 min with 20 ml of 85% acetone in a 50-ml centrifuge tube, and then centrifuged for 10 min at $1,600\times g$ (CF16RX, Hitachi Koki Co., Ltd., Tokyo, Japan). The supernatant fluid was transferred to a 50-ml measuring flask. The precipitate was extracted again with the same volume of 85% acetone with a homogenizer (Ultra Turrax T25-basic, IKA works Inc., Wilmington, North Carolina, USA) and the second supernatant was added to the measuring flask. The extraction was then repeated with 8 ml of 85% acetone. The volume of the extract solution was adjusted to 50 ml with 85% acetone and small portions were kept at -80°C until measurement with HPLC.

Measurement solutions for HPLC analysis to compare composition of pigments between bivalve species were prepared as follows: one gram of homogenate of midgut glands was homogenized for 1 min with 8 ml of 85% acetone in a 50-ml centrifuge tube with a homogenizer, and then centrifuged for 10 min at $1,600 \times g$. The supernatant fluid was transferred to a 10-ml

measuring flask. The precipitate was shaken for 1 min with the 1 ml of 85% acetone and the supernatant was added to the measuring flask after centrifugation. The volume of the extract solution was adjusted to 10 ml with 85% acetone. The solutions were also used to measure pyropheophytin-a and chlorophyllid-a. The HPLC measurement was performed immediately after the extraction. The sample solution of which concentration of any one of the pigments was out of the range of the calibration curve was diluted with 85% acetone.

Measurement of Pigments with HPLC

Chlorophyll-a, pheophorbide-a, pyropheophorbide-a, pheophytin-a, pyropheophytin-a, and chlorophyllid-a in midgut glands of scallops were measured with HPLC. The system consisted of a pump (LC7100, Hitachi High-Technologies Co., Tokyo, Japan), an autosampler (L7200, Hitachi), an oven (L7300, Hitachi), and a photodiode array detector (L7455, Hitachi). Slit width of the detector was set at 4 nm and the spectrum bandwidth was automatically selected for the slit width. The absorbance spectrum was recorded between 350- and 730-nm wavelength and the sampling interval was 400-ms. Pyropheophorbide-a and pheophorbide-a was monitored at 650-nm wavelength for quantitation. Other pigments were quantified by monitoring at 410-nm wavelength for increasing sensitivity. Chromatographic separations of the pigments were performed under the conditions of Gauthier-Jaques *et al.* [11], modified column length, column temperature, and

monitored wavelengths. They were as follows: Column: Inertsil ODS2 (150 x 4.6-mm i.d.) with 5- μ m particle size (GL Sciences Inc., Tokyo, Japan); column oven temperature: 40°C; injection volume: 20 μ l; flow rate: 1.0 ml/min; mobile phase: solvent A was 1 mol/l aqueous ammonium acetate-methanol (1:4, v:v), solvent B was acetone-methanol (1:4, v:v); a linear gradient profile: 100% A to 100% B in 15 min and held at 100% B for a further 25 min; column equilibration time, 10 min. Peaks in the chromatograms were identified by comparing their retention times and absorbance spectra between 350- and 730-nm wavelength with those of the standard pigments. The concentrations were estimated using the calibration curve of standard solutions in the range of 0.3 to 20 μ g/ml. Chlorophyllid-a was only qualitatively analyzed because the concentration of that solution we purchased was too low to be used for our quantitative analysis. The quantitation limit of each pigment was 0.3 μ g/ml. It corresponds to 15 μ g/g of homogenate of scallops or to 3 μ g/g of homogenate of other bivalves.

Methods of Mouse Bioassays

The comparative mouse bioassays were performed according to the official DSP method in Japan [12, 13]. Acetone extract equivalent to the weight of midgut gland in 20 g of whole body homogenate weight was injected into male ddY mice (16- to 20-g body weight, 4 weeks old).

The first experiment was designed to confirm the necessity of light for the development of

symptoms. One ml of extract from the pyropheophorbide-rich homogenate (Otoshibe, February 20, 2006) suspended in saline containing 1% Tween 60 was intraperitoneally injected into two mice per cage. One ml of saline containing 1% Tween 60 was also injected into two mice per cage to estimate effect of the surfactant-saline in mice. The cages were illuminated at approximately 5,000-6,000 lx with a desk fluorescent lamp. Illuminance was measured with a luxmeter (TOPCON IM-2D, Tokyo Optical Co. Ltd., Tokyo, Japan). Six hours after beginning the exposure, the illuminance was set to normal (65-400 lx) for DSP inspection with only the room light. The room light was turned off 3 h after the light change, as the room was automatically kept on an 11 h dark: 13 h light cycle. Matching cages with injected mice were shielded from light throughout the assay. The condition of the mice was observed at 3, 6, and 24 h after the injection.

The second experiment was designed to confirm the necessity of pyropheophorbide for the onset of symptoms. One ml of extract from the high-pyropheophorbide homogenate (Oshamanbe, March 7, 2006) or from the low-pyropheophorbide homogenate (Abuta, June 26, 2006) suspended in saline containing 1% Tween 60 was intraperitoneally injected into two mice per cage. One ml of saline containing 1% Tween 60 was also injected into two mice per cage to estimate effect of the surfactant-saline in mice. The mice were exposed to strong light as described above. The condition of the mice was observed at 3, 6, and 24 h after the injection.

Results

HPLC Separation and Absorbance Spectra of Pigments

Figure 2 shows the reversed-phase HPLC profiles of six standard pigments and of the extract from the midgut glands of scallops and on ODS. The standard pigments of chlorophyllid-a, pheophorbide-a, pyropheophorbide-a, chlorophyll-a, pheophytin-a and pyropheophytin-a were eluted in this order from the column with good separation within 30 min after sample injection under the conditions employed (Figs. 2a and 2b). The same elution order with a longer elution time on reversed-phase columns has been observed in plant extracts [11]. The retention times of five peaks detected in the extract from the midgut glands of scallops (Fig. 2c) were in close agreement with those of standard pigments. The absorbance spectrum of the predominant component was the same as that of standard pyropheophorbide-a (Fig. 3).

Figure 2 shows the reversed-phase HPLC profiles of the extract from the midgut glands of scallops and of six standard pigments on ODS. The peak at 10.15 min in the chromatogram of a standard preparation mixture was pheophorbide-a, at 12.17 min was pyropheophorbide-a, at 19.55 min was chlorophyll-a, at 24.32 min was pheophytin-a, and at 28.77 min was pyropheophytin-a (Fig. 2a). The peak at 5.90 was chlorophyllid-a (Fig. 2b). The retention times of five peaks detected in the extract from the midgut glands of scallops (Fig. 2c) were in close agreement with those of standard

pigments. The absorbance spectrum of the predominant component was the same as that of standard pyropheophorbide-a (Fig. 3).

Pyropheophorbide Content in the Midgut Glands of Scallops

Figure 4 shows the amount of chlorophyll-a, pyropheophorbide-a, pheophorbide-a, and pheophytin-a in homogenates of the midgut glands of cultured Japanese scallops which had been sampled in Funka Bay from January 24 to April 26, 2006. The total content of the four pigments in the Otoshibe samples started to increase at the beginning of February 2006 and reached a peak (637 μg per 1-g homogenate) on February 20, 2006. After the peak, the amounts of pigment began to decrease and fell to a level as low as at the start of the sampling, on April 26, 2006. Pigment content of the Oshamanbe samples did not peak as clearly as the Otoshibe samples, and the pigment content began to increase approximately 2 weeks later than in the Otoshibe area. The sample on March 7, 2006 had the highest total pigment content (399 μg per 1-g homogenate), but the highest value was approximately 40% lower than in the Otoshibe samples.

Pyropheophorbide-a, which is known to be photosensitizer, accounted for 68-92% of the total pigment content during the pigment-increasing phase, whereas pheophorbide-a, which is similarly harmful, accounted for only 3-4% of the total pigment content. Pheophytin-a and chlorophyll-a respectively accounted for 6-21% and 2-12% of the total pigment content in the same period. The

extracts which induced the apparent symptoms of photosensitive reaction in mice were from scallops collected in both fishing areas on February 21 2006, i.e., in the high pyropheophorbide period.

Results of Mouse Bioassays

In the bioassay for the light requirement for the symptoms, mice that were injected with the pyropheophorbide-rich extract (Otoshibe, 510 $\mu\text{g/g}$ of the homogenate) and exposed to light were dead at 3 h after the injection, whereas the mice in all the other groups were alive with no symptoms after 24 h.

In the bioassay for pyropheophorbide requirement for the symptoms, all of the mice that received extract from the high-pyropheophorbide homogenate (Oshamanbe, 340 $\mu\text{g/g}$ of the homogenate), or from the low-pyropheophorbide homogenate (Abuta, 36 $\mu\text{g/g}$ of the homogenate), or saline only (control group) were still alive after 6 h under the light, but the mice in the high-pyropheophorbide group had symptoms of swelling of the ears(Fig. 5c) and head (Fig. 5d), and ear scratching. They were dead at 24 h after the injection, whereas the mice in the other groups were alive with no symptoms after 24 h.

Pyropheophorbide Content in the Midgut Glands of Other Edible Bivalves

The pigment contents of non-scallop bivalves collected in Hokkaido were also analyzed. Table 1 shows the amount of each of the pigments in the homogenate of the midgut glands. Non-scallop bivalves also contained pyropheophorbide but, with the exception of two surf clams *Pseudocardium sachalinense*, the amounts were less than in the scallops. The surf clams fished in Otsu area, off the Pacific coast of Eastern Hokkaido on February 5, 2007, had approximately 300 µg of total pigment per 1 g of midgut gland homogenate, but the pyropheophorbide content was only 88 µg. The surf clams collected in Tomakomai, off the Pacific coast of Eastern Hokkaido on February 20, 2007 contained almost the same amount of pyropheophorbide as the Otsu clams. Non-scallop bivalves, with the two surf clams, caused no symptoms in mice in the DSP inspections. Additionally, Pyropheophorbide content of scallops collected in early spring was high, approximately 300 to 500 µg/g, but the content in summer was 36 to 100 µg per 1 g of midgut gland homogenate and the samples induced no symptoms in mice in the DSP inspections.

Discussion

Our study shows that the midgut gland of the scallop *Patinopecten yessoensis*, which is a suspension feeder, can potentially cause photosensitivity disease in mice. This is similar to the midgut glands of abalones or turban shells, which are herbivorous gastropods. We believe that the

untypical symptoms originally observed in the DSP tests were of photosensitivity disease caused by pyropheophorbide in midgut glands, but we have no direct evidence of this, as we did not measure the concentration of pyropheophorbide in the blood of the mice. However, the HPLC measurements revealed that photosensitivity disease symptoms in mice were induced by extracts from scallops collected in the high pyropheophorbide period. The pyropheophorbide content was 300 to 530 μg per 1 g of the homogenate of midgut glands of scallops. The weight of the homogenate used for the assay corresponded to 3.28-4.28 g per injection; therefore, the applied dose of pyropheophorbide per mouse with 20 g body weight was 0.99-2.3 mg in the calculation. It has been reported that the lethal dose of pyropheophorbide in mice exposed to strong light (10,000 lx) is approximately 3.3 to 5.0 mg per 100 g body weight by injection into the abdominal cavity [14-17], which equates to 0.66 to 1.0 mg per 20 g body weight. Therefore, the content of pyropheophorbide in our bioassay which we suspect caused the onset of photosensitivity was enough to induce death in a mouse. Additionally, from the comparative mouse bioassays, it is clear that a substantial amount of pyropheophorbide and exposure to light are necessary for the onset of the symptoms.

However, not all mice that were injected high-pyropheophorbide extract showed strong photosensitivity in the actual DSP inspections, for example, Otoshibe or Oshamanbe sample, March 7, 2006 (Fig4, marked “b”, only eye discharge). Meanwhile, the content of

pyropheophorbide of Oshamanbe sample, February 20, 2006 was almost equal to that of above-mentioned Oshamanbe sample, March 7, 2006, but swelling of the ears and head and ear scratching were observed in mice injected it (Fig4, marked “a”). We attribute the failure to cause symptoms to differences in illuminance due to the location of the mouse cage. As a result of measuring the illuminance in our cage rack, almost all the cages were indirectly illuminated by approximately 65-400 lx with fluorescent lights on the ceiling, but there were some locations at the end of the rack where part of a cage was exposed to light directly, approximately 600-1100 lx. The results above suggest that pyropheophorbide in the midgut glands of scallops induced photosensitization in mice.

The total content of four pigments in our samples had a peak in late February to early March in Funka Bay. The increase would reflect a concurrent increase in diatoms in the culture area. The cultured scallops in Funka Bay are strongly influenced by the diatom bloom, which occurs near the sea surface, because the scallops are cultured hanging between 10 and 25 m below the sea surface. The diatom bloom in this area commonly occurs between late February and mid-March [18, 19]. Baba *et al.* and Kawano *et al.* reported an increased amount of chlorophyll in the seawater at different points in Funka Bay between February and March 2006 [20, 21]. The origin of pyropheophorbide we detected in the midgut glands of scallops might be chlorophyll in diatoms ingested by scallops, if the chlorophyll is metabolized into mainly pyropheophorbide in the

metabolic pathway of scallops. Alternatively, the pyropheophorbide might be derived mainly from metabolites of phytoplankton feeders converging on the bloom, or feces and remains that increase there. In our study, we do not have data about the origin of the pyropheophorbide detected in the midgut glands of scallops. To clarify the origin, we need to research metabolic pathways of chlorophyll in scallops and composition of pyropheophorbide and the related substance in the suspended matters that scallops ingest.

We also measured pigments in some non-scallop bivalves collected in Hokkaido.

Pyropheophorbide was detected in the almost all of the bivalves measured, but it was present in insufficient quantity to cause photosensitivity disease in mice. The sample numbers were insufficient for us to deny a risk of pyropheophorbide in edible bivalves causing photosensitivity to humans; however, we think that eating edible bivalves have a low risk of photosensitivity disease if we correctly precook them or eat them in normal quantities. The midgut glands of large bivalves like scallops or surf clams are generally removed before eating. If the problematic scallops which we detected are eaten, approximately 270 to 630 g of the whole body with the midgut glands needs to be consumed to induce photosensitization, since the lowest effective level of pheophorbide and pyropheophorbide is 25 mg/person/day [5]. Small bivalves such as littleneck clams or short-necked clams are eaten with the midgut glands, but the risk of photosensitization is also low because the midgut glands are small and they are usually not eaten in large quantities. Therefore, we think that

an intake of edible bivalves with normal behavior causes little photosensitivity disease to humans. However, there is reason to be concerned photosensitization caused by oysters. Oysters are more likely to cause photosensitization because they have comparatively larger midgut glands than littleneck clams or short-necked clams and are often eaten raw with the midgut glands. Some people eat a lot of oysters at restaurants that serve oysters in an all-you-can-eat style. Moreover, oysters may be also influenced by the diatom bloom strongly because they are cultured using hanging method like scallops in Japan. Our measured values of pigments in Pacific oysters *Crassostrea gigas* were low, but the samples may have been collected out of the diatom blooming season. Therefore, we need more data of the content of pyropheophorbide in oysters. It is necessary to attend to amount of pyropheophorbide in oysters fished during a diatom bloom if oysters tend to accumulate pyropheophorbide in midgut glands as high as scallops.

To secure food safety, we need more data of the content of pyropheophorbide in edible bivalves during diatom blooms. We have ever paid little attention to pyropheophorbide of edible bivalves in food-safety inspections, because we have assumed that only herbivorous gastropods such as abalones cause photosensitivity disease. However, our study shows that photosensitivity was caused in mice by pyropheophorbide accumulated in the midgut gland of some scallops. We think that pyropheophorbide in most bivalves living in their native habitat is generally too little to induce photosensitivity in animals. Actually, the amounts of pyropheophorbide in our non-scallop

bivalve samples were low and those of scallops were also not always high. However, it may be rapidly accumulated in bivalves for some reason. Our study suggests that a diatom bloom is one of the factors for the accumulation. Artificial change of habitat environment of bivalves, such as hanging culture, may also accelerate the accumulation. To secure food safety, we need to confirm whether the pyropheophorbide in edible bivalves increases to the harmful level or not during diatom blooms.

Acknowledgments We thank the staff members of Department of Biological Science in Hokkaido Institute of Public Health for their supports and advice. We also thank the food sanitation division staffs of all health and welfare offices in Hokkaido, and the staffs of Abashiri, Abashiri Tobu, Hiyama, and Iburi Fisheries Technical Guidance Office, for sampling and dispatching samples.

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Table 1 Contents of chlorophyll and pheopigments in midgut glands of edible bivalves

Bivalves		Content ($\mu\text{g/g}$ of homogenate)					
Fishing area	Fishing date	Total	Chlo a	Pheid a	pyrPheid a	Phe a	pyrPhe a
<i>Sakhalin surf clam Pseudocardium sachalinense</i>							
Tomakomai	5-Aug-06	9	-	-	9	-	-
Nemuro	19-Sep-06	12	-	<3	12	-	-
Ootsu	5-Feb-07	294	77	8	88	93	28
Tomakomai	20-Feb-07	120	7	3	78	22	10
<i>Japanese littleneck Ruditapes philippinarum</i>							
Notsuke	26-Jun-06	8	-	-	5	3	<3
Akkeshi	19-Feb-07	38	3	-	18	10	7
<i>Japanese oyster Crassostrea gigas</i>							
Mori	11-Nov-06	7	-	-	7	-	-
Lake Saroma	9-Jan-07	4	4	-	-	-	-
Lake Saroma	3-Feb-07	<3	<3	-	<3	-	-
<i>Japanese scallop Patinopecten yessoensis</i>							
Osyamanbe	7-Mar-06	509	<3	17	420	29	43
Abuta	26-Jun-06	39	<3	<3	36	-	3
Tokoro	26-Jun-06	110	3	-	100	4	3
Abashiri	21-Aug-06	54	3	-	51	<3	<3

Chlo a: chlorophyll-a, Pheid a: pheophorbide-a, pyrPheid a: pyropheophorbide-a, Phe: pheophytin-a, pyrPhe a: pyropheophytin-a. '-': below the limit of detection. We also analyzed chlorophyllid-a, but it was not detected in any sample (below the limit of detection). Limits of quantitation were 3 $\mu\text{g/g}$ of homogenate. Homogenates used for the measurements of the pigments were kept at -80°C for validation of results of inspections for DSP. Only the scallops fished in Osyamanbe on Mar. 7, 2006, caused photosensitivity-like symptoms in the inspection for DSP.

Figure legends

Fig. 1 The periodical sampling points for the administrative monitoring of accumulation of shellfish toxins in the scallops. Oshamanbe Point: lat. 42° 33' N. /long. 140° 30' E. Otoshibe Point: lat. 42° 12' N. /long. 140° 27' E.

Fig. 2 HPLC chromatograms of standard pigments (a and b) and of the extract from the midgut glands of scallops (c) recorded at 650 nm wavelength with photodiode-array detector. Peak identification: 1, pheophorbide-a; 2, pyropheophorbide-a; 3, chlorophyll-a; 4, pheophytin-a; 5, pyropheophytin-a; 6, chlorophyllide. HPLC conditions as given in text.

Fig. 3 Comparison between the absorbance spectrum of a standard preparation and that of a sample with photodiode-array detector. The spectrum of the peak with the same retention time as pyropheophorbide-a in the 650-nm chromatogram of the extract from scallop midgut glands was similar to that of pyropheophorbide-a. The number in parentheses on the vertical axis is the scale of the absorbance for the scallops sample.

Fig. 4 Contents of chlorophyll and three pheopigments in homogenates of midgut glands of scallops sampled for inspections of diarrhetic shellfish poisoning in Funka Bay from January 24 to April 26, 2006. Phe: pheophytin-a, Chlo: chlorophyll-a, Pheid: pheophorbide-a, pyrPheid: pyropheophorbide-a. Digit in column indicates content (μg) of pyropheophorbide-a per 1 g of the homogenate. a: Mice intraperitoneally injected with the extract from this sample showed unusual symptoms: piloerection, substantial eye discharge, swelling of the ears and head, and ear scratching,

in the DSP inspection. b: A few mice intraperitoneal injected with the extract from this sample showed only substantial eye discharge in the DSP inspection.

Fig. 5 Symptoms in the mice injected with the extract from high-pyropheophorbide homogenate under intense light. a: a control mouse, b: substantial eye discharge, c: swelling of the ears, d: swelling of the head.

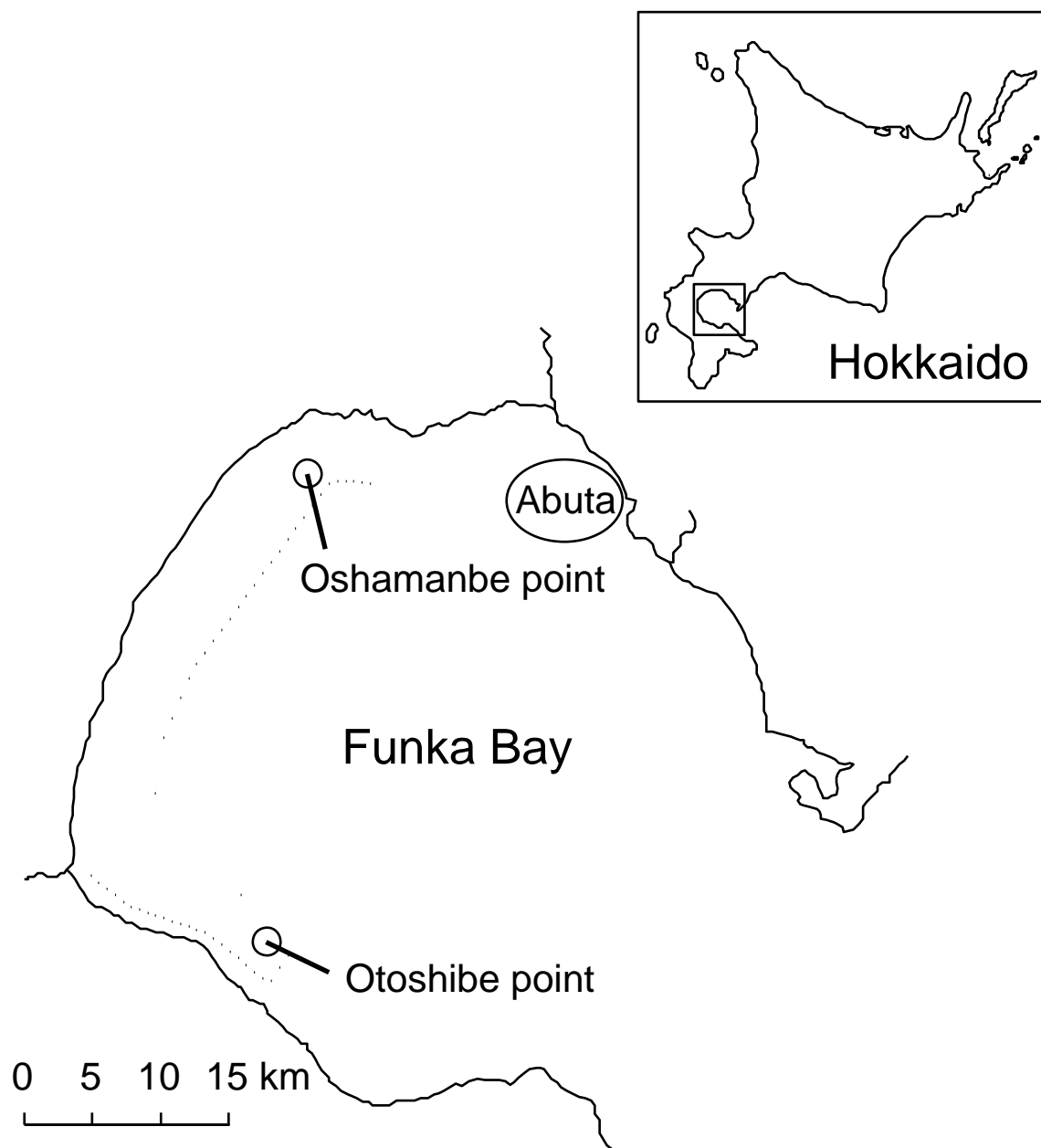


Fig. 1

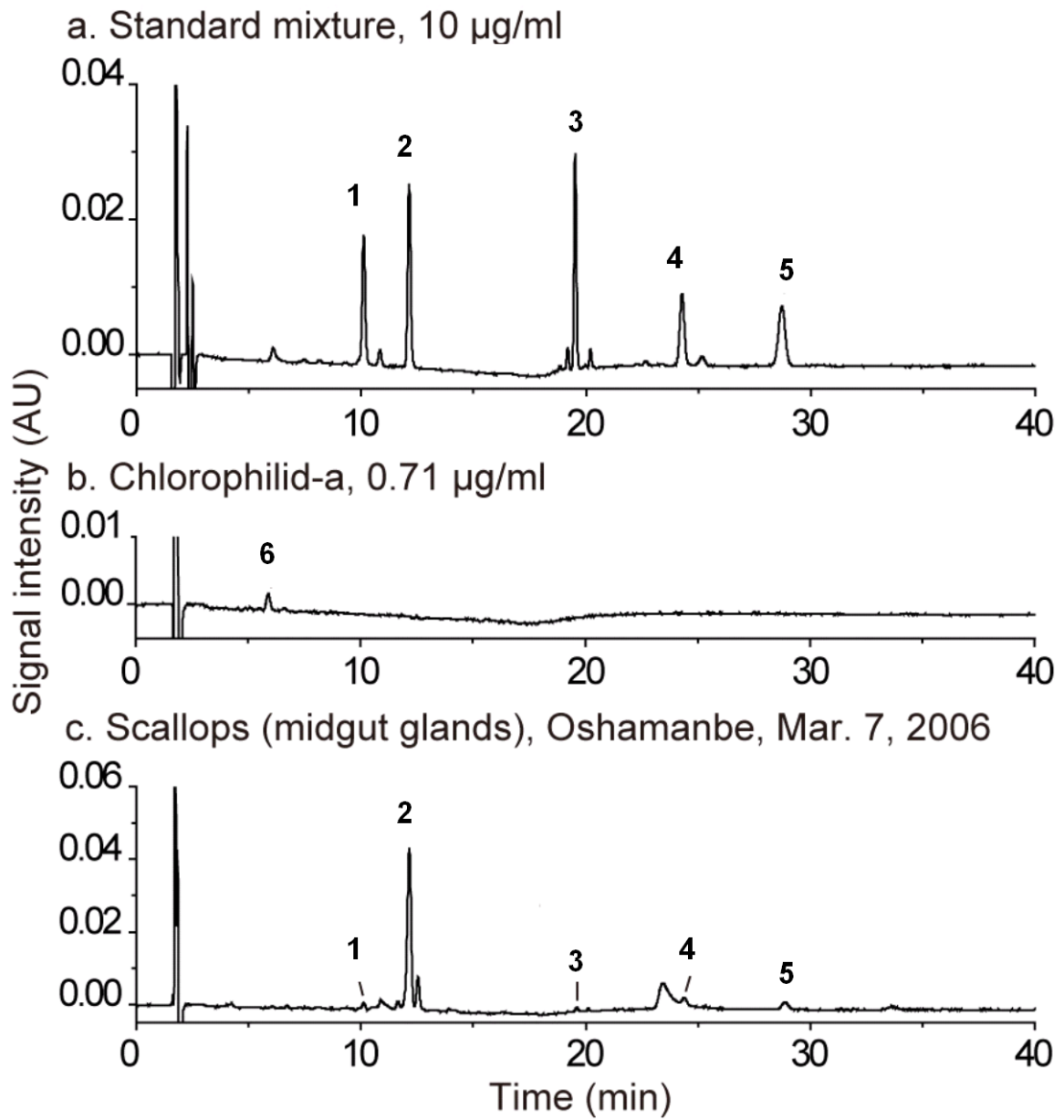


Fig. 2

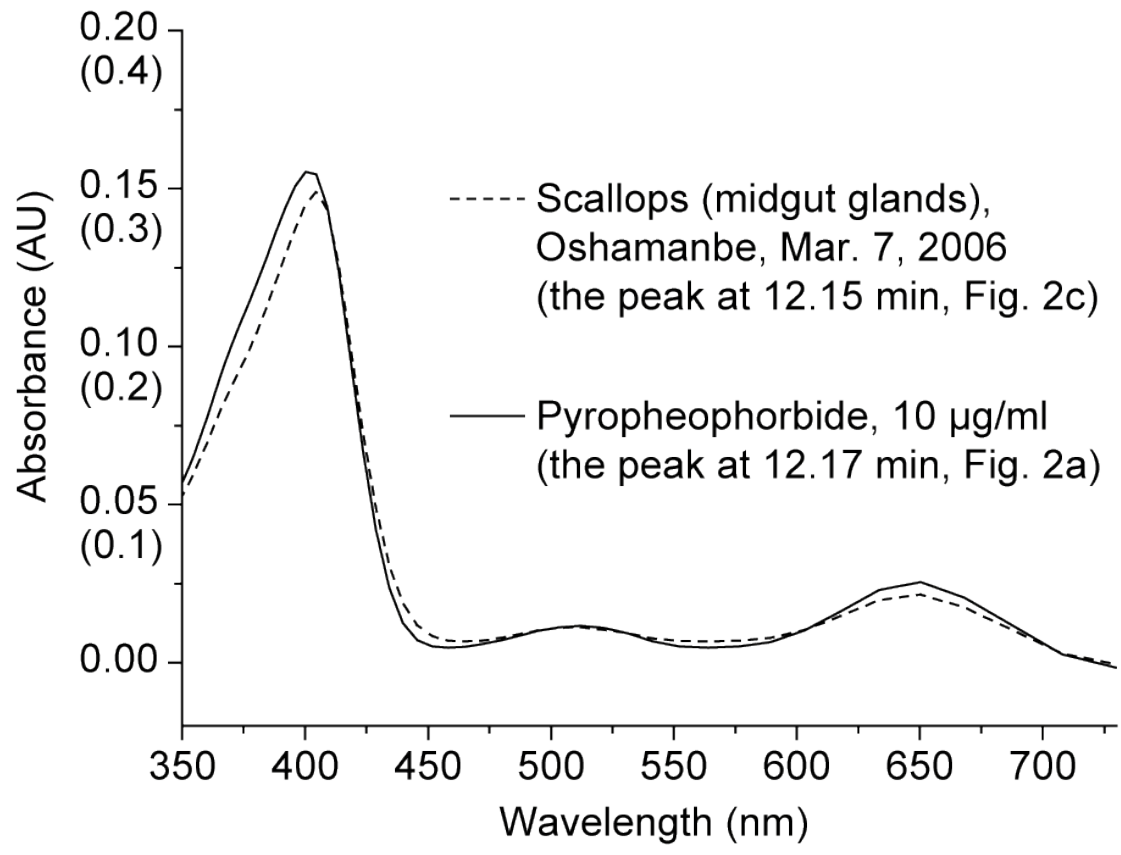


Fig. 3

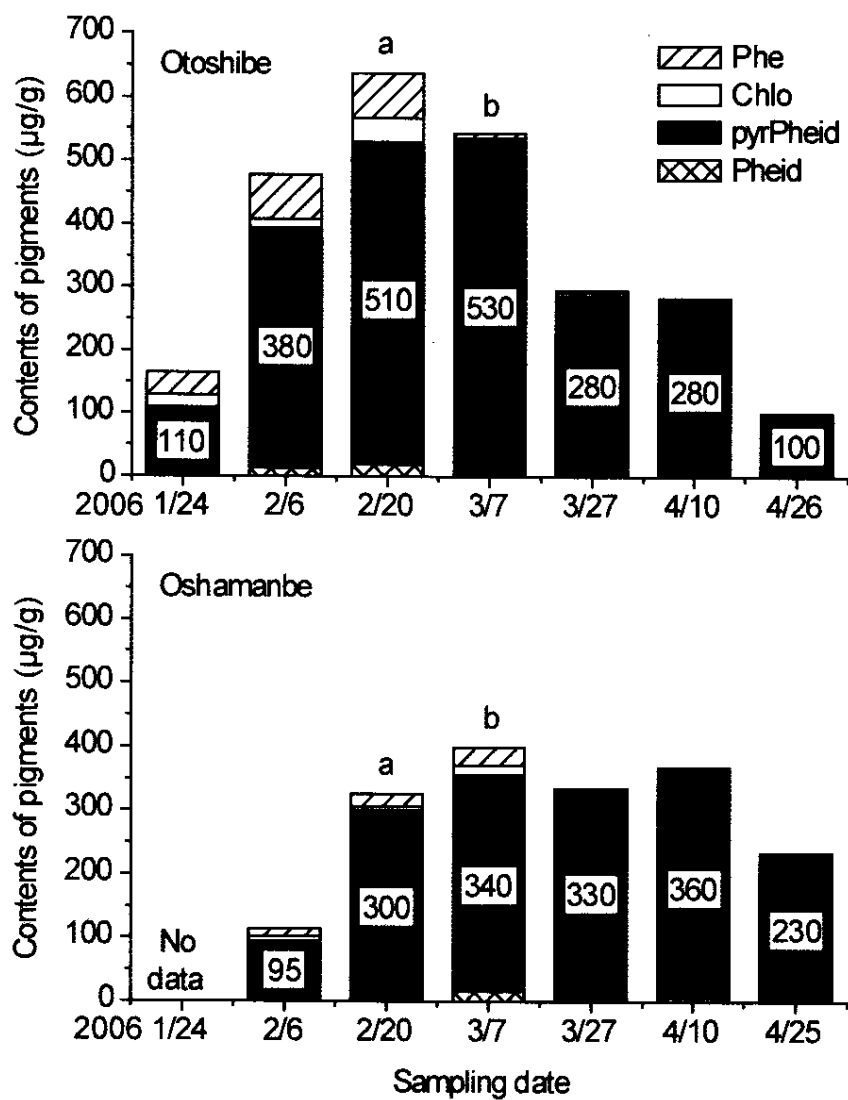


Fig. 4

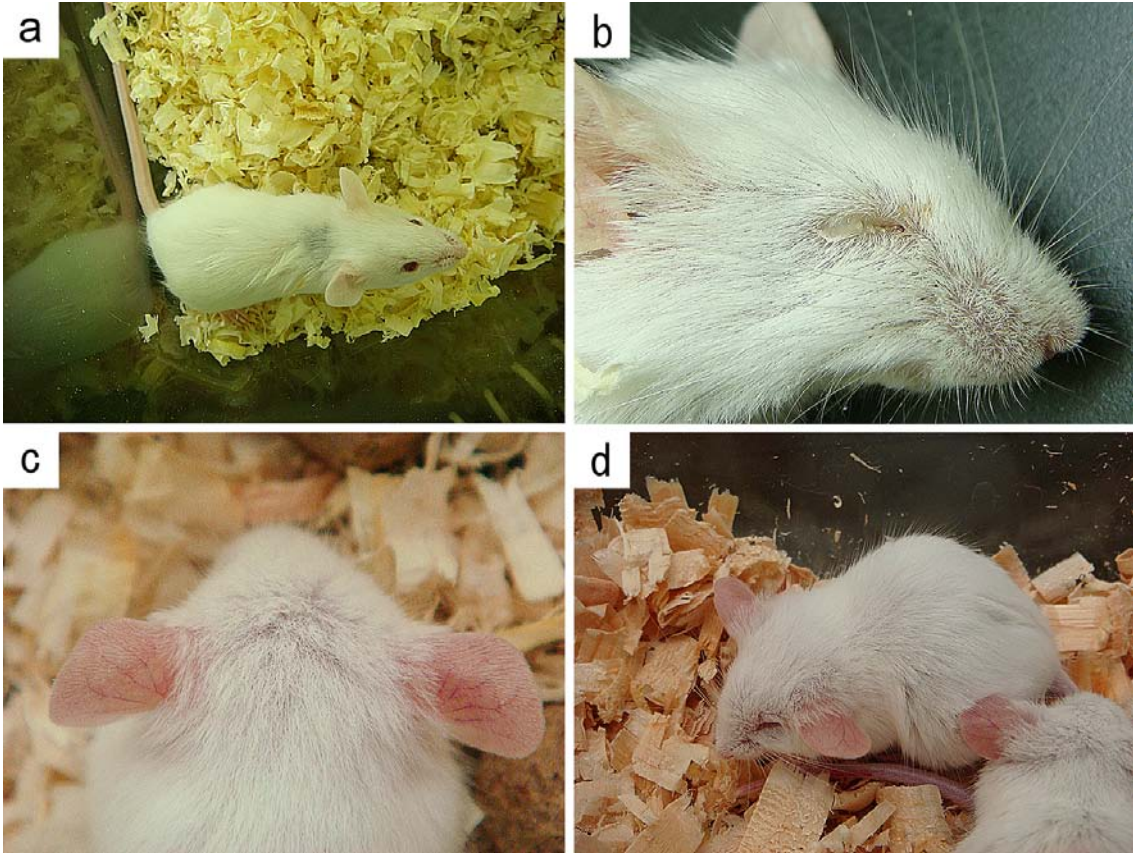


Fig. 5