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Article

Contamination of Sediments and Histological Alterations in Barfin Plaice *Pleuronectes pinnifasciatus* from Amursky Bay (Peter the Great Bay, East Sea/Sea of Japan)

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Abstract : In August-September 2001, 15 samples of bottom sediments were collected in the inner, middle and open parts of Amursky Bay near Vladivostok, Russia, and barfin plaice *Pleuronectes pinnifasciatus* was sampled from the inner and the middle locations of the bay. In the sediments from all three sites, elevated concentrations of several heavy metals, i.e. Zn (102-115 μg/g dry weight), Ni (70-73 μg/g), and Cu (27-35 μg/g) were discovered. The contents of oil hydrocarbons were very close to or slightly higher than the maximal normal environmental background level, 100 μg/g dry weight. The sediments contained negligible amounts of hexachlorocyclohexane, while DDT concentrations were quite high (1.7-16.3 ng/g dry weight). Generally, there were no substantial differences in the pollution levels of the locations studied, and our results resembled those reported for Amursky Bay in the 1990s. Surprisingly, in 2001 "fresh" DDT comprised 70-85% of the total DDT content in sediment from all the locations studied. In fish liver, total DDTs concentrations were 212.8 and 122.54 ng/g wet weight for the inner and the middle locations, respectively, and "fresh" DDT comprised 35 and 64% of DDTs, respectively. These results provide evidence of recent input of DDT from an unknown source into the ecosystem of Amursky Bay. Histopathological changes revealed in the plaice liver (vacuolization of hepatocytes, coagulative necrosis of hepatocytes, inflammatory reaction, and necrosis of epithelial cells of bile ducts) are probably connected with an intensive metabolism of DDT in the fish organism. No histological and histomorphometric differences were found in the state of the interrenal tissue. Similar condition of the liver and the interrenal tissue in barfin plaice sampled from the inner and the middle locations of Amursky Bay may be explained by the absence of great differences in the pollution levels of these sites.

Key words : chlorinated pesticides, DDT, hexachlorocyclohexane, heavy metals, monitoring, fish histopathology, liver, interrenal tissue.

1. Introduction

Amursky Bay is one of the secondary bays of Peter the Great Bay, which is the largest bay in the northwestern part of the Sea of Japan (Fig. 1). It is considered one of the richest and most productive regions in the seas of the Far East. For instance, ichthyofauna of Peter the Great Bay is extremely rich and comprises over 300 of salt- and fresh-water fish species (Sokolovskaya *et al.*, 1998). Amursky Bay is an important area for the spawning, nursery, and migration of commercially valuable fish species including pacific salmon, which use several small rivers on the west coast for spawning. On the other hand, the basin of Amursky Bay is the most developed area in the Primorye Region. In this area, there are large cities such as Vladivostok (the largest seaport in the Russian Far East with a population of over 630 thousand people) and Ussuiysk (161 thousand people), and one of the largest

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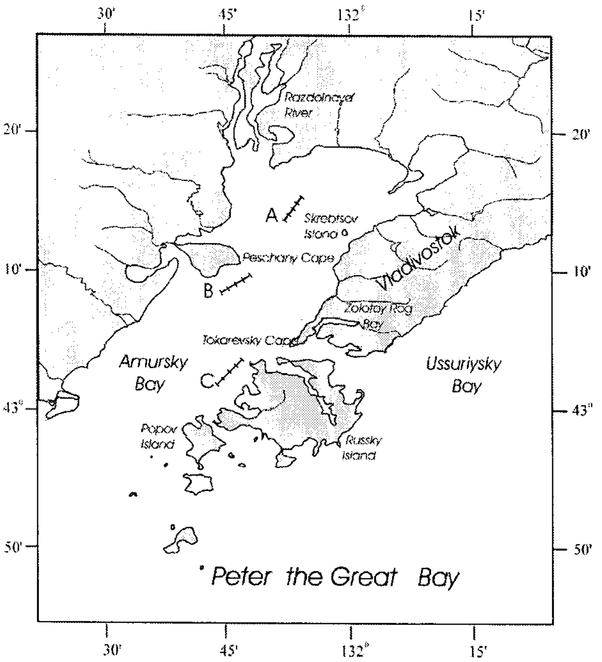

Fig. 1. Sampling sites (A, B, and C) in Amursky Bay (Peter the Great Bay, East Sea/Sea of Japan).

recreational zones in the Far East. Numerous mining enterprises exploit different kinds of minerals in the basins of the rivers entering the bay. Intensive farming has been developed in the basin of the Razdolnaya River, which feeds into the bay in the north. This river is the largest (after the Tumen River) of the rivers of southern Primorye.

Economic development of the region from the 1960s through the 1990s was not accompanied by construction of sufficiently powerful and effective treatment facilities, and the coastal waters of Amursky Bay were used as a receptacle for what is practically untreated sewage. Industrial and domestic wastewater of Vladivostok and its suburbs, marine transportation, agricultural effluent and untreated waste from Ussuriysk feeding into the bay, together with the waters of the Razdolnaya River, and input of pollutants through air and rain-storms are considered among the major sources of contamination in the bay.

The state of the marine environment and biota in Amursky Bay has always attracted particular interest. In the middle of the 1970s, both individual researchers and state programs of environmental monitoring have begun to investigate the levels of anthropogenic contaminants in the water, sediment and biota of the bay as well as the ecological consequences of pollution. The data of these investigations conducted since the 1970s through the 1990s have been reported elsewhere and reviewed (Vaschenko, 2000; Vaschenko and Pitruk, 2001). Amursky Bay (especially its innermost zone and the southeastern zone adjacent to Vladivostok) were considered one of the most polluted areas of Peter the Great Bay.

Of the variety of contaminants, heavy metals and chlorinated hydrocarbons have been of the highest priority because in terms of their potential toxicity and bioaccumulation in all trophic levels. From the 1980s-1990s, the distributions of these compounds in the bottom sediments of Amursky Bay have been studied, and their concentrations in the common mussel species have been detected (Khristoforova *et al.*, 1993; Tkalin *et al.*, 1993, 1996, 1997, 1998, 2000; Shulkin and Kavun, 1995). Several locations with high concentrations of heavy metals of anthropogenic origin (copper, lead and zinc) have been found, and the chlorinated pesticides DDT and hexachlorocyclohexane (HCH) have been registered at almost all stations studied.

At the same time, it should be noted that there is only limited data on the effects of elevated levels of contaminants in sediment and/or in organisms on the health of the animals inhabiting the bottom of Amursky Bay. This information includes data on two common species of bottom invertebrates, the sea urchin *Strongylocentrotus intermedius* and the scallop *Mizuhopecten yessoensis* (Vaschenko *et al.*, 1993, 2000), and provides evidence of the negative effects of heavy metal pollution on the health of these animals. Until now, no data has been available regarding the effects of the chlorinated pesticides on the condition of the marine organisms in Amursky Bay.

The aims of the present study were: 1) to determine the concentrations of heavy metals, oil hydrocarbons and chlorinated pesticides (DDT and HCH) in the surface sediment from different areas of Amursky Bay, and to compare our results with previously published data; 2) to determine the concentrations of DDT and HCH in the liver of barfin plaice *Pleuronectes pinnifasciatus*; 3) to describe the morphology of the liver and the interrenal tissue of the plaice. Our interest in this species is explained by several causes: First, the plaices belong to the bottom-feeding fish species, which are in direct contact with contaminated sediment. Second, *P. pinnifasciatus* is a common and relatively sedentary species in Peter the Great Bay, which does not migrate as far as those of other flounders inhabiting the bay (Lindberg and Fedorov, 1993). Third, hepatic lesions in bottom fish exposed to xenobiotics under both experimental and field conditions are well described and may be used as both indicators of fish health and biomarkers of pollution (Hinton *et al.*, 1992;

Stehr *et al.*, 1998). Finally, it was interesting to compare the morphology of the interrenal tissue in the plaices from different locations of the bay. This tissue located in the head kidney produces sex steroid hormones and plays an important role in fish adaptation in terms of different stress-factors including contamination (Hontela *et al.*, 1997; Girard *et al.*, 1998).

2. Materials and methods

Field sampling

Sampling was performed in August and early September 2001 at three sites in Amursky Bay (Fig. 1). Site A (43°13'600"N-43°14'948"N, 131°50'88"E-131°52'677"E) was located in the inner part of the bay near Skrebtsov Island, site B (43°09'26"N-43°10'953"N, 131°46'78"E-131°47'940"E) was in the middle part of the bay near Peschany Cape, and site C (43°03'102"N-43°04'200"N, 131°46'997"E-131°48'410"E) was situated in the open part of the bay near Russky Island. In August, barfin plaices were collected by otter trawling from sites A and B. Trawling lines were located in subtidal, sedimentary depositional zones. Fish with body lengths of 23 to 30 cm were taken for chemical and histological analysis of the liver (n = 25 for each site). Young fish with body lengths of less than 15 cm (n = 8 for each site) were taken for the interrenal tissue histological analysis.

Just after catching the fish, the liver and kidneys containing the interrenal tissue were dissected and preserved in 10% formalin in water for histological analysis. Pieces of liver from 15 fish were combined in a sample within each site (A and B), frozen and stored at –25°C before chemical analysis.

In September, bottom sediment samples were collected from all three sites (A, B and C). Stations (5 per site) for sediment collection were situated along trawling lines at about 500 m distance from each other. Surface sediment (2 cm layer) was taken using a special plastic grab developed by one of the authors, P.M. Zhadan. Sediment samples of about 100 g in weight were frozen and stored at –25°C before chemical analysis.

Histological analysis

Preserved pieces of the liver and the interrenal tissue were washed with water for 24 h, dehydrated in a graded ethanol series, and embedded in paraffin wax using a routine protocol. Histological sections (5 μm thickness) were stained with haematoxylin and eosin and examined under a light microscope. Classification of lesions in the plaice liver was based on generally accepted diagnostic criteria used in other flatfish species including a description of degenerative, preneoplastic and neoplastic changes (Myers *et al.*, 1987; Hinton *et al.*, 1992; Moore and Stegeman, 1994; Vetthaak and Wester, 1996). The greatest importance was paid to revealing the following alterations in the plaice liver: neoplasms, foci of cellular alterations, spongiosis hepatis, hydropic vacuolization, coagulative necrosis, foci of regeneration, biliary hyperplasia, and cholangiofibrosis, which are well-established as histopathological biomarkers of contaminant effects in fish and show strong evidence of a contaminant-associated etiology based on previous field and laboratory studies (Hinton *et al.*, 1992).

Morphology of the interrenal tissue was examined. For histomorphometric analysis, the diameters of the nuclei in the interrenal cells (n = 100 for each histological slide) were measured, and mean volume of the interrenal cell nuclei for the fish from each location was calculated.

Chemical analysis

In fish liver, the contents of *p,p'*-DDT, and its metabolites *p,p'*-DDD and *p,p'*-DDE as well as α-, β- and γ-isomers of HCH were determined using gas-liquid chromatography (Methodical directions…, 1979). 5 g samples were homogenized and extracted on an ultrasonic bath (twice for 10 min) and extracted with a mixture (1:1) of *n*-hexane and dichloromethane. Elimination of the fat components of the samples with sulfuric acid and separation of organochlorine compounds from accompanying admixtures on a column containing silica gel were made. The purified extracts were concentrated and then analyzed using a Shimadzu GC-16A gas chromotograph with an ECCD-15 electron-capturing detector and a KKK (OV-17) 25 m × 0.22 mm chromatographic column. Chromatographic conditions were as follows: injector temperature 230°C; column temperature 250°C; argon (90%) and methane (10%) mixture as gas-carrier; pressure 2 kg/cm^2; effluent flow rate 0.5 μl/min; injection volume 1 m*l*. Calibration has been made using the government standard for specimens of pesticides.

Sediments were dried at 80°C and analyzed for such contents as heavy metals, oil hydrocarbons and chlorinated hydrocarbons (*p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE, α- and γ-isomers of HCH). Concentrations of heavy metals (Fe, Mn, Zn, Cu, Ni, Co, Pb, and Cr) were determined by flame atomic-absorption spectrophotometry according to the method 3050 (U.S. EPA: Iskandar and Keeney, 1974). Dry sediment samples were sieved through a sieve with

0.5 mm pores. 0.5 g samples were introduced in teflon reactors and digested with a mixture of 0.5 m*l* $HClO_4$, 1 m*l* HNO_3, and 3 m*l* HCl at 50°C for 1 h. After cooling, 2 m*l* HNO_3 and 3 m*l* HF were added, and samples were heated at 80°C for 3 h. After drying, 1 m*l* HNO_3 and 50 m*l* of twice-distilled water were added, and samples were boiled for 2 h. Then twice-distilled water was added to a final volume of 15 m*l*. The relative errors of determination were 3-5%.

Oil hydrocarbons were extracted using an alkaline solution of ethanol; from this solution, oil hydrocarbons were extracted with *n*-hexane. Separation of hydrocarbons from accompanying admixtures on a column with Al_2O_3 was made. After hexane evaporation, hydrocarbons were diluted in CCl_4, and their content was determined by the routine method of IR-spectrophotometry according to the standard technique of the Russian Hydrometeorological Service (Oradovsky, 1979). Chlorinated hydrocarbons were extracted using an acetone and *n*-hexane mixture (1:1). Then extracts were purified with a mixture of H_2SO_4, a water solution of Na_2SO_3 and tetrabutylammoniumsulphate. Purified extracts were concentrated and then analyzed by the method of gas chromatography according to the standard technique of the Russian Hydrometeorological Service (Oradovsky, 1979). A Russian gas chromatograph LCM-8 with glass packed column (1 m × 3 mm, SE-30 stationary phase, oven temperature 220°C, detector temperature 250°C) was used for the analysis.

3. Results and discussion

Sediment analysis

Bottom sediments sampled from three sites in Amursky Bay were represented mainly by silt, sometimes with an admixture of fine-grain sand. No significant differences in levels of heavy metal contamination of the sediments between sites have been revealed (Table 1). In general, spatial distribution of heavy metals was rather homogeneous, and the coefficient of variation of the concentrations of each metal at different sites did not exceed 20%. Some exceptions were provided by site B where more heterogeneous distribution of Co, Pb and Cr were observed (variation coefficient values were 22.3, 24.5 and 38.1%, respectively).

The concentrations of heavy metals (except Pb) in the sediments of all surveyed sites in Amursky Bay were higher than the background level in the open part of Peter the Great Bay near Reyneke Island (Shulkin *et al.*, 2002: Table 1). The concentrations of several metals of anthropogenic origin (Zn, Ni and Cu) were very close to or higher than the concentrations, which can cause adverse biological effects (Long *et al.*, 1995: Table 1).

The distributions of heavy metals in the surface sediment of Peter the Great Bay including Amursky Bay have been studied previously (Tkalin *et al.*, 1993, 1996, 1998; Shulkin and Kavun, 1995; Shulkin *et al.*, 2002). The authors concluded that in the early 1990s the concentrations of heavy metals in the coastal area of the bay adjacent to Vladivostok and Nakhodka cities were comparable to those found in the coastal sediments of heavily industrialized areas of the northwestern Pacific. Comparison of data obtained in September 2001 (Table 1) with data obtained in August 1994 (Tkalin *et al.*, 1996: Table 2) showed that mean concentrations of Fe, Zn and Cu were similar, while Mn, Cr and Pb contents in 2001 were 1.5-3 times lower, and Ni and Co contents were about 2 times higher.

The distribution of organic contaminants in the surface bottom sediments sampled in Amursky Bay in 2001 was characterized by substantial variability, and the coefficient of variation reached 88% in some cases. At the majority of stations, the concentrations of oil hydrocarbons were very close to or slightly higher than the maximal normal environmental background level of 100 μg/g dry weight (Sanders *et al.*, 1980). Mean concentrations of oil hydrocarbons in the surface sediments from sites A and B were greater compared with site C, but significant

Table 1. Content of iron (mg/g dry weight) and other heavy metals (μg/g dry weight) in bottom sediments from Amursky Bay, September 2001.

Site	Fe	Mn	Zn	Ni	Cu	Co	Cr	Pb
A	42.8±0.8	180.3±7.7	115.5±5.6	73.0±4.3	34.3±3.2	29.0±2.1	28.5±2.9	8.4±0.5
B	42.3±5.7	175.8±9.2	102.0±10.6	70.5±6.6	26.8±2.8	25.8±2.9	24.5±4.7	8.0±0.9
C	38.8±2.3	214.5±17.2	106.5±6.3	71.3±2.8	35.5±1.9	26.0±1.6	30.8±1.4	9.1±0.3
BV	15-20	-	35-45	5.0	8-9	7.5	-	8-9
CBE	-	-	150	20.9	34	-	81	46.7

Note: Means ± SE (n = 5) are presented. BV - background values (data from Shulkin *et al.*, 2002); CBE - concentrations causing biological effects (data from Long *et al.*, 1995).

Table 2. Mean concentrations of iron (mg/g dry weight) and other heavy metals (μg/g dry weight) in the surface (2-cm) layer of the sediment in different areas of Peter the Great Bay (data from Tkalin *et al.*, 1996).

Area	Fe	Mn	Zn	Cr	Cu	Pb	Ni	Co
Zolotoy Rog Bay	36.2	226	362	119	181	214	26	10.0
Nakhodka Bay	42.8	579	163	84	31	100	44	13.3
Amursky Bay	44.1	321	121	87	25	28	33	12.1
Ussuriysky Bay	20.3	190	60	48	15	23	15	5.9
Strelok Bay	2.06	199	54	41	8	9	13	5.4

Table 3. Content of organic compounds in bottom sediments from Amursky Bay, September 2001.

Site	Oil hydrocarbons, μg/g dry weight	ΣHCH, ng/g dry weight	DDT, ng/g dry weight	ΣDDD+DDE, ng/g dry weight	ΣDDT, ng/g dry weight
A	118 ± 7.3*	0.34 ± 0.05	5.7 ± 2.3	1.3 ± 0.18	7.0 ± 2.4
	90 – 130	0.2 – 0.5	1.9 – 14.3	1.0 – 2.0	3.0 – 16.3
B	118 ± 13.2	0.24 ± 0.02	1.9 ± 0.6	0.8 ± 0.09	2.7 ± 0.5
	90 – 160	0.2 – 0.3	1.0 – 4.3	0.5 – 1.0	1.7 – 4.8
C	70 ± 17.8	0.35 ± 0.15	4.6 ± 1.2	0.8 ± 0.09	5.4 ± 1.2
	40 – 120	0.2 – 0.8	2.2 – 7.7	0.5 – 0.9	2.7 – 8.6

*Difference between means from sites A and C is significant according to Student's *t*-test ($p<0.05$).
Note: Numerator is mean ± SE (n = 5); denominator is range of concentrations. Σ HCH - total content of α- and γ-isomers of hexachlorocyclohexane, Σ DDT - total content of DDT and its metabolites (DDD and DDE).

Table 4. Concentrations of DDT and its metabolites (Σ DDT), α- and γ-isomers of hexachlorocyclohexane (Σ HCH isomers) and oil hydrocarbons in bottom sediments of different areas in Peter the Great Bay.

Area	Oil hydrocarbons, mg/g dry weight*	ΣHCH isomers, ng/g dry weight**	ΣDDT, ng/g dry weight**
Zolotoy Rog Bay and Bosfor Vostochny Strait	5.4-16.7	<0.2-5.5 (1.66)	0.8-22.7 (9.01)
Amursky Bay	0.03-2.72	<0.2-1.3 (0.58)	4.4-14.8 (7.59)
Ussuriysky Bay	0.03-0.25	<0.2-1.1 (0.32)	4.4-9.1 (6.01)

*Belan, 2001 (data of 1986-1989 and 1994, range of concentrations is presented).
**Tkalin, 1996 (data of 1994, the ranges of concentrations and average values (in parentheses) are given.

differences were detected between sites A and C only (Table 3).

In previous surveys conducted in 1986-1989 and in 1994, a wider range of hydrocarbons concentrations in the sediments of Amursky Bay was found (Belan, 2001: Table 4). The highest hydrocarbons concentrations were registered in the area near the Tokarevsky Cape, where dredged ground from the heavily contaminated Zolotoy Rog Bay was discarded (a dumping site). This area was not studied in the present study.

Despite bans on its production and usage in a number of developed countries, the chlorinated pesticides DDT and lindane (γ-HCH) are still widely used in tropical and temperate Asia. These chlorinated hydrocarbons are persistent in the marine environment of the Pacific Ocean and its marginal seas (Tanabe *et al.*, 1984; Iwata *et al.*, 1994; Guruge and Tanabe, 2001). In September 2001, the sediment collected at three sites in Amursky Bay contained very low amounts of HCH; the highest registered value was 0.8 ng/g dry weight (Table 3). There were no significant differences in both the total content of HCH isomers (HCHs) and the concentrations of α- and γ-isomers between sites. In order to determine lindane lifetime in the environment and organisms, the α-HCH/γ-HCH ratio (α/γ factor) is usually used. These values were 1.4, 1.4 and 2.5 for sites A, B and C, respectively. High α/γ factors (>1) suggest an input of lindane in the sediment of Amursky Bay in the past.

The results of our study are close to those obtained in 1994 and in 1996 (Tkalin, 1996; Tkalin *et al.*, 1997). In 1994, average concentrations of the sum of α- and γ-isomers HCH in the sediments of Amursky Bay were lower than in heavily contaminated Zolotoy Rog Bay and slightly higher than in Ussuriysky Bay (Table 4). The highest HCH concentrations were found near the south-eastern coast of the bay, at the stations near Tokarevsky Cape and Burny Cape - 1.45 and 5.61 ng/g dry weight, respectively (Tkalin *et al.*, 1997). In 1998, Tkalin and coauthors (2000) using more accurate and sensitive techniques could determine the content of β-isomer of HCH and found that this isomer accounted for a significant proportion of the total HCHs in both the sediment and the two mussel species from Amursky Bay. For example, β-isomer comprised about 55% of the HCHs content in the sediment found near Skrebtsov Island (4.41 ng/g dry weight). The concentrations of HCHs in the sediment of the bay varied between <0.2 and 4.47 ng/g dry weight, i.e. close to the data reported previously (Tkalin *et al.*, 1997) and with the results of present work. High values of α/γ factor (>1) detected both in previous studies and our surveys are evidence of a lindane discharge in the past. Nevertheless, according to the results of the 1998 survey (Tkalin *et al.*, 2000), maximal concentrations of HCHs were found in soft tissues of the mussels *Modiolus kurilensis* and *Crenomytilus grayanus* sampled in the southern part of the bay (16.25 and 2.29 ng/g dry weight, respectively). This fact as well as the values of α/γ factor less than 1 (0.35 and 0.18, respectively) allowed the authors to suggest a more recent input of HCH into the marine environment.

In 2001, the total concentration of DDT and its metabolites (DDTs) in bottom sediments collected at three sites in Amursky Bay varied from 1.7 to 16.3 ng/g dry weight (Table 3). No significant differences between sites were revealed. The wider range and the higher mean of DDT concentrations were found at site A. It is interesting that at all three locations, DDT comprised a significant proportion of total DDTs (81.4, 70.4 and 85.2% at sites A, B, and C, respectively). The ratio of concentrations of DDT to its metabolite DDE is usually used for determination of the DDT lifetime in the environment and organisms. The values of DDT/DDE for bottom sediments from Amursky Bay were 7.3, 4.3 and 11.5 for sites A, B and C, respectively. These high values suggest a recent input of DDT into the marine environment.

In previous surveys of 1994, 1996 and 1998 (Tkalin, 1996; Tkalin *et al.*, 1997, 2000), similar concentrations of DDTs have been found. Generally, the level of DDT contamination in the sediment around Amursky Bay was lower than that in heavily contaminated Zolotoy Rog Bay and slightly higher than that in Ussuriysky Bay (Table 4). Comparing our data with previous ones, we noted that in 1994 and 1996 "fresh" DDT predominated, and DDT/DDE ratio range was about 3-15. In 1998, DDD and DDE comprised more than 90% of the total DDTs found in the sediments of Amursky Bay (Tkalin *et al.*, 2000). However, quite a high proportion of DDT (13%) was registered in the mussel *M. kurilenssis* from one of the stations (Tokarevsky Lighthouse) in the southern part of the bay. The results of 2001 provide evidence of a significant input of "fresh" DDT into the marine environment, which took place in recent years.

The sources of "fresh" DDT and HCH in the coastal area near Vladivostok are not clear. These pesticides are officially banned for usage in Russia. Previous investigations have found no indication of a significant effect of the Razdolnaya River discharge on the pesticides distribution both in sediment or mussels from Amursky Bay (Tkalin, 1996; Tkalin *et al.*, 1997, 2000). The authors suggested an unofficial use of DDT and HCH for pest control on ships, in hotels or for domestic purposes. Atmospheric input of the toxicants was also not excluded.

Fish analysis

Total content of HCH-isomers in the liver of the place *P. pinnifasciatus* was low (Table 5). A significant proportion of HCHs was presented by β-isomer (62 and 50% for fish from sites A and B respectively). This finding corresponds to previous results for two mussel species, *M. kurilensis* and *C. grayanus* (Tkalin *et al.*, 2000), as well as to the data of other authors on the accumulation of β-isomer of HCH over time and when moving along the trophic chain (Tanabe *et al.*, 1984). Neither HCH concentrations nor high values of α/γ factor (2.2 and 1.7 for sites A and B, respectively) show recent input of lindane into the plaices inhabiting Amursky Bay.

Table 5. Contents of organochlorine compounds (ng/g wet weight) in the liver of barfin plaice, *Pleuronectes pinnifasciatus*, from Amursky Bay, August 2001.

Site	α-HCH	β-HCH	γ-HCH	ΣHCH	DDT	DDD	DDE	ΣDDT
A	12.0	27.90	5.40	45.3	74.9	58.9	79.0	212.8
B	5.49	8.62	3.27	17.38	78.43	37.25	6.86	122.54

Table 6. Prevalence (%) of pathological changes in the liver of barfin plaice, *Pleuronectes pinnifasciatus*, from Amursky Bay, August 2001.

Site	Hepatocyte vacuolization	Hepatocyte necrosis	Inflammation	Necrosis of bile duct epithelial cells
A	48	8	24	0
B	36	9	16	23

The concentrations of HCH in the liver of the plaice *P. pinnifasciatus* from Amursky Bay were about 10 times less than in the plaices *Platichthys stellatus* and *Pleuronectes obscurus* sampled in the summer of 2001 in the southwestern part of Peter the Great Bay near the Tumen River mouth (Syasina, 2003). The highest HCHs content (340.3 ng/g wet weight) was registered in the liver of *P. stellatus* caught in the lower reaches of the Tumen River. Despite the predominance of β-HCH (84%), the value of α/γ factor was as low as 0.7. This fact indicates that the Tumen River discharge is a source of lindane input in the near-mouth area. Previously, the Tumen River run-off was found to be an important factor determining HCH content in the sediments of the coastal zone of Peter the Great Bay adjacent to the river mouth (Shulkin *et al.*, 2001; Tkalin, 2001). In 1997, the range of HCHs concentrations in the sediments of this area was <0.2-5.8 (mean 2.07) ng/g dry weight. These values are higher than those in other areas of Peter the Great Bay (Tables 3 and 4).

Total content of DDT and its metabolites in the liver of *P. pinnifasciatus* from Amursky Bay was several times greater than the content of HCHs (Table 5). The highest DDTs concentration was found in the plaices from site A, but the proportion of "fresh" DDT was about two times higher in the fish from site B. For the plaices from site A, the DDT/DDE ratio was close to 1 (0.95) while for that of the fish from site B, this value was as high as 11.4. These findings are not in close agreement with the results of the sediment chemical analysis, which demonstrated a higher content of "fresh" DDT at site A. However, the plaices from both sites contained significant amounts of DDT, which comprised 35 and 64% of total DDTs at sites A and B, respectively. This data as well as the results of chemical analysis of bottom sediment samples suggest a recent input of DDT into the ecosystem of Amursky Bay.

In the summer of 2001, the contents of DDT and its metabolites were examined in the liver of several fish species sampled in the Tumen River mouth and the adjacent areas of Peter the Great Bay (Syasina, 2003). The concentrations of DDTs varied from 12 to 40.8 ng/g wet weight. These values are substantially lower than those found in barfin plaice from Amursky Bay. In 1996-1997, the contents of this pesticide in the sediment found near the mouth area were also lower than in other areas of Peter the Great Bay (Shulkin *et al.*, 2001; Tkalin, 2001). The authors concluded that the Tumen River was not a major source of DDT input into the ecosystem of the southwestern part of Peter the Great Bay.

The liver of barfin plaice *P. pinnifasciatus* sampled from Amursky Bay was examined for the presence of histopathological changes, which are known to be the biomarkers of the toxic effects of contaminants on fish organisms. Only a few of the variety of histopathological markers of marine environment pollution (Hinton *et al.*, 1992; Stehr *et al.*, 1998) were revealed in the plaices from sites A and B, i.e. vacuolization of hepatocytes, coagulative necrosis of hepatocytes, necrosis of epithelial cells of bile ducts, and inflammatory reactions (Table 6). The liver neoplasms as well as foci of cellular alterations were not found. In fish from both sites, the vacuolization of hepatocytes was more common than were other pathologies. Only diffuse vacuolization affecting the whole hepatic parenchyma was encountered. Another common pathological alteration observed in the plaice liver was inflammation, the small loci with limphocytic infiltration. On the whole, the liver morphology as well as the occurrence of histopathological alterations were similar in fish from both sites. The only difference was that 23% of the plaices from site B had the pathology of the bile ducts, which was not detected in the fish from site A. The epithelial cells lining the bile ducts contained pyknotic nuclei and were subjected to degeneration. Neither signs of parasitism nor the parasitic organisms themselves were found.

A similar condition in the liver of barfin plaice *P. pinnifasciatus* sampled from sites A and B in Amursky Bay may apparently be related to the absence of great differences in the contamination level between sites. We suggest that necrosis of bile duct epithelial cells found only in the plaices from site B might be caused by the high content of "fresh" DDT and its intensive metabolism in the fish liver.

In our study, the first data on the liver pathology in barfin plaice *P. pinnifasciatus* are presented. Previously, liver morphology in several flounder species from the coastal zone of Peter the Great Bay including the black plaice *P. obscurus* from the polluted innermost part of Amursky Bay has been studied (Syasina *et al.*, 2000, 2001). A wider spectrum and a higher occurrence of histopathological alterations in the liver of *P. obscurus* compared to *P. pinnifasciatus* have been found. These differences seem to be species-dependent.

Morphological and histomorphometric analysis of the steroidogenic interrenal tissue in barfin plaice *P. pinnifasciatus* revealed no differences in the tissue morphology and in the volumes of the interrenal cell nuclei between fish from sites A and B (13.7 ± 0.9 and $13.3 \pm 0.6\ \mu m^3$, respectively). This data also suggests a similar health state of plaices from both locations. Previously, comparative study of the morphology of the interrenal tissue in two flounder species, *P. obscurus* and *P. pinnifasciatus*, sampled in 2000 at two stations in the coastal zone of Amursky Bay adjacent to Vladivostok city has shown only inter-species differences (Durkina, unpublished data). All this data suggests that the absence of the stress-response of the interrenal tissue in fish from different locations in Amursky Bay may be related to the chronic effect of the contaminated environment on the fish organism.

4. Conclusion

In 2001, the bottom sediment collected at three sites in Amursky Bay, which were located in the inner, middle and open parts of the bay, contained elevated concentrations of heavy metals, oil hydrocarbons and chlorinated hydrocarbons, DDT and HCH. The concentrations of the majority of pollutants measured were very close to or higher than the concentrations causing adverse biological effects (Long *et al.*, 1995). Comparing our results with those previously reported (Tkalin, 1996; Tkalin *et al.*, 1996, 1997, 1998, 2000), we concluded that, in general, since the 1990s the level of sediment contamination has not decreased substantially. Moreover, in 2001, "fresh" DDT comprised a significant proportion of total DDTs (more than 70%), and DDT/DDE ratios were high (4.3-11.5). These values are higher than those detected in the 1990s. The liver of barfin plaice *P. pinnifasciatus* caught in the inner and the middle parts of the bay contained high amounts of total DDTs (212.8 and 122.54 ng/g wet weight, respectively) as well as "fresh" DDT (74.9 and 78.9 ng/g wet weight, respectively). These results provide evidence for a recent input of DDT from an unknown source into the ecosystem of Amursky Bay. Histological examination of the liver of barfin plaice showed the presence of histopathological changes, which are known to be the biomarkers of toxic effects of contaminants on the fish organism, i.e. vacuolization of hepatocytes, coagulative necrosis of hepatocytes, inflammatory reaction, and necrosis of epithelial cells of bile ducts. These lesions are probably connected to the intensive metabolism of DDT in the fish liver. A similar condition of the liver and the interrenal tissue in fish from different locations may be apparently related to similar pollution levels.

The data obtained confirmed the urgency of further monitoring of the marine environment and the health condition of the fish inhabiting Amursky Bay.

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References

Belan, T.A. 2001. Some features of abundance and species composition of benthos under conditions of pollution. Abstract of Ph. D. Thesis, Far Eastern Regional Hydrometeorological Research Institute, Vladivostok, Russia. 26 p. (In Russian).

Girard, C., J.C. Brodeur, and A. Hontela. 1998. Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test *in vivo*. *Can. J. Fish. Aquat. Sci.*, 55, 438-450.

Guruge, K.S. and S. Tanabe. 2001. Contamination by persistent organochlorines and butyltin compounds in the west coast of Sri Lanka. *Mar. Pollut. Bull.*, 42, 179-186.

Hinton, D.E., P.C. Baumann, G.R. Gardner, W.E. Hawkins, J.D. Hendricks, R.A. Murchelano, and M.S. Okihiro. 1992. Histopathological biomarkers. p. 155-209. In: *Biomarkers. Biochemical, physiological, and histological markers of anthropogenic stress.* ed. by R.J. Huggett, R.A. Kimerle, P.M. Mehrle, H.L. Bergman. Lewis Publishers, Boca Raton, Fl.

Hontela, A., C. Daniel, and J.B. Rasmussen. 1997. Structural and functional impairment of the hypothalamo-pituitary-interrenal axis in fish exposed to bleached kraft mill effluent in the St Maurice River, Québec. *Ecotoxicology*, 6, 1-12.

Iskandar, I.K. and D.R. Keeney. 1974. Concentration of heavy metals in sediment cores from selected Wisconsin Lakes. *Environ. Sci. Technol.*, 8(2), 165-170.

Iwata, H., S. Tanabe, N. Sakai, A. Nishimura, and R. Tatsukawa. 1994. Geographical distribution of persistent organochlorines in air, water and sediments from Asia and Oceania, and their implications for global distribution from lower latitudes. *Environ. Pollut.*, 85, 15-33.

Khristoforova, N.K., V.M. Shulkin, V.Ya. Kavun, and E.N. Chernova. 1993. Heavy metals in commercially important and cultured mollusks of Peter the Great Bay. Dalnauka, Vladivostok, Russia, 296 p. (In Russian).

Lindberg, G.U. and V.V. Fedorov. 1993. Fishes of the Sea of Japan and neighboring waters of the Sea of Okhotsk and Yellow Sea, vol. 2. Nauka, St.-Petersburg, Russia, 272 p. (In Russian).

Long, E.R., D.D. MacDonald, S.L. Smith, and F.D. Calder. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Management.*, 19, 81-97.

Methods of chemical analysis of organochlorine pesticides. 1979. p. 10-18. In: *Methods of chemical analysis of marine environment samples directions on determination of organochlorine pesticides and polychlorobiphenils in case of their joint presence in the environmental objects and biomaterial.* Vyschaya shkola, Moscow (In Russian).

Moore, M.J. and J.J. Stegeman. 1994. Hepatic neoplasms in winter flounder *Pleuronectes americanus* from Boston Harbor, Massachusetts, USA. *Dis. Aquat. Org.*, 20, 33-48.

Myers, M.S., L.D. Rhodes, and B.B. McCain. 1987. Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions, and other idiopathic hepatic conditions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. *J. Nath. Cancer Inst.*, 78, 333-361.

Oradovsky S.G., ed. 1979. Manual of analysis methods of pollutants in bottom sediments, No. 43. Gidrometeoizdat, Moscow, 39 p. (In Russian).

Sanders, H.L., J.F. Grassle, G.R. Hampsan, L.S. Morse, S. Garner-Price, and C.J. Jones. 1980. Anatomy of an oil spill: long-term effects from the grounding of the barge Florida off West Falmouth, Massachusetts. *J. Mar. Res.*, 38, 256-380.

Shulkin, V.M. and V.Ya. Kavun. 1995. The use of marine bivalves in heavy metal monitoring near Vladivostok, Russia. *Mar. Pollut. Bull.*, 31, 330-333.

Shulkin, V.M., A.V. Moschenko, and T.S. Lishavskaya. 2001. Pollution level and factors determining contaminant contents in the bottom sediments of the Russian part of the Tumen River mouth area. p. 83-95. In: *The state of environment and biota of the southwestern part of Peter the Great Bay and the Tumen River mouth*, vol. 1, ed. by V.L. Kasyanov., M.A. Vaschenko, D.L. Pitruk. Dalnauka, Vladivostok, Russia.

Shulkin, V.M., V.Ya. Kavun, A.V. Tkalin, and B.J. Presley. 2002. The influence of metal concentration in bottom sediments on metal accumulation by mytilids *Crenomytilus grayanus* and *Modiolus kurilensis*. *Russian J. Mar. Biol.*, 28, 53-60.

Sokolovskaya, T.G., A.S. Sokolovsky, and Ye.I. Sobolevsky. 1998. List of fishes of Peter the Great Bay (Sea of Japan). *Vopr. Ikhtiol.*, 38, 5-15 (In Russian).

Stehr, C.M., L.L. Johnson, and M.S. Myers. 1998. Hydropic vacuolation in the liver of three species of fish from the U.S. West Coast: lesion description and risk assessment associated with contaminant exposure. *Dis. Aquat. Org.*, 32, 119-135.

Syasina, I.G. 2003. Organochlorine pesticides in fish and mollusks from lower reaches of the Tumen River and adjacent area of Peter the Great Bay (Sea of Japan). *Biologiya morya (Russian J. Mar. Biol.)*, 29(1), 34-40 (In Russian).

Syasina, I.G., L.L. Arbuzova, E.A. Zhadko, and A.S. Sokolovsky. 2000. Histomorphological changes in organs of the plaice *Pleuronectes obscurus* from the polluted part of Amursky Bay. *Russian J. Mar. Biol.*, 26, 276-282.

Syasina, I.G., A.S. Sokolovsky, and T.G. Sokolovskaya. 2001. Pathology of fishes from the Tumen River mouth and adjacent sea area. p. 138-173 In: *The state of environment and biota of the southwestern part of Peter the Great Bay and the Tumen River mouth*, vol. 2, ed. by V.L. Kasyanov., M.A. Vaschenko, D.L. Pitruk. Dalnauka, Vladivostok, Russia.

Tanabe, S., H. Tanaka, and R. Tatsukawa. 1984. Polychlorbiphenils, DDT and hexachlorocyclohexane isomers in the Western North Pacific ecosystem. *Arch. Environ. Contam. Toxicol.*, 13, 731-738.

Tkalin, A.V. 1996. Chlorinated hydrocarbons in coastal bottom sediments of the Japan Sea. *Environ. Pollut.* 91, 183-185.

Tkalin, A.V. 2001. Contamination of the marine environment in the area of the Tumen River mouth. p. 20-26. In: *The state of environment and biota of the southwestern part of Peter the Great Bay and the Tumen River mouth*, vol. 2, ed. by V.L. Kasyanov., M.A. Vaschenko, D.L. Pitruk. Dalnauka, Vladivostok, Russia.

Tkalin, A.V., T.A. Belan, and E.N. Shapovalov. 1993. The state of the marine environment near Vladivostok, Russia. *Mar. Pollut. Bull.*, 26, 418-422.

Tkalin, A.V., B.J. Presley, and P.N. Boothe. 1996. Spatial and temporal variations of trace metals in bottom sediments of Peter the Great Bay, the Sea of Japan. *Environ. Pollut.*, 92, 73-78.

Tkalin, A.V., T.S. Lishavskaya, and J.W. Hills. 1997. Organochlorine pesticides in mussels and bottom sediments from Peter the Great Bay near Vladivostok. *Ocean Res.*, 19, 115-119.

Tkalin, A.V., T.S. Lishavskaya, and V.M. Shulkin. 1998. Radionuclides and trace metals in mussels and bottom sediments around Vladivostok, Russia. *Mar. Pollut. Bull.*, 36, 551-554.

Tkalin, A.V., D.P. Samsonov, T.S. Lishavskaya, and G.V. Chernik. 2000. New data on organochlorine distributions in the marine environment near Vladivostok. *Mar. Pollut. Bull.*, 40, 879-881.

Vaschenko, M.A. 2000. Pollution in Peter the Great Bay, Sea of Japan, and its biological consequences. *Russian J. Mar. Biol.*, 26, 155-166.

Vaschenko, M.A., P.M. Zhadan, E.M. Karaseva, and O.N.

Luk'yanova. 1993. Disturbance of reproductive function in the sea urchin *Strongylocentrotus intermedius* in the polluted areas of Peter the Great Bay (Sea of Japan). *Russian J. Mar. Biol.*, 19, 57-66.

Vaschenko, M.A., L.N. Luchsheva, N.N. Bel'cheva, I.G. Syasina, and A.V. Silina. 2000. Assessment of the state of the Japanese scallop *Mizuhopecten yessoensis* in Alekseeva Bight (Peter the Great Bay, East Sea/Sea of Japan) based on morphological and biochemical parameters. *Russian J. Mar. Biol.*, 26, 342-349.

Vaschenko, M.A. and D.L. Pitruk. 2001. Comparative assessment of the condition of the marine environment and biota in various parts of Peter the Great Bay including the Tumen River mouth area. p. 3-18. In: *The state of environment and biota of the southwestern part of Peter the Great Bay and the Tumen River mouth*, vol. 3, ed. by V.L. Kasyanov., M.A. Vaschenko, D.L. Pitruk. Dalnauka, Vladivostok, Russia.

Vethaak, A.D. and P.W. Wester. 1996. Diseases of flounder *Platichthys flesus* in Dutch coastal and estuarine waters, with particular reference to environmental stress factors. II. Liver histopathology. *Dis. Aquat. Org.*, 26, 99-116.

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