LIFE-SUPPORTING ASIA-PACIFIC MARINE ECOSYSTEMS, BIODIVERSITY AND THEIR FUNCTIONING

Edited by
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Potentially toxic algal species, seasonal variability of their quantitative indices and phycotoxin contents in Japanese scallop *Mizuhopecten yessoensis* (Jay, 1857)

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All over the world, a great number of cases of phycotoxin (poisonous substances produced by unicellular algae and cyanobacteria) poisoning are registered annually. A list of toxic and potentially toxic species of the unicellular algae includes more than 300 representatives, so, usually the most mass and dangerous species and phycotoxins produced by them are monitored. In Russia and countries of the Customs Union the unified sanitary-epidemiologic and hygienic requirements to the goods under sanitary-epidemiologic supervision have been set ("Technical Regulations of the Customs Union TR TS 001/2011 for safety of food production"). The admissible levels (AL) of contents of phycotoxins and their derivatives in mollusks are documented: saxitoxin 0.8 mg/kg, amnesic poison of mollusks (domoic acid) 20 mg/kg, for internal organs of crabs 30 mg/kg, diarrheic poison of mollusks (okadaic acid) 0.16 mg/kg.

In Aniva Bay, the colonies of Japanese scallop *Mizuhopecten yessoensis* (Jay, 1857) are located on several restricted sites with silty-sand and pebble-sand grounds that include a touch of dead shells. They are separated by areas with soils unfit for scallop habitat (Salmon Bight, LNG plant area and others). There are three traditional areas with Japanese scallop colonies: along western, northern and eastern coasts of Aniva Bay with a wide depth range from 2 m to 24 m (Galanin et al., 2012a, b).

From the results of earlier studies, composition of the most potentially dangerous toxic species and their seasonal abundance dynamics (Orlova et al., 2007) were determined. The levels of phycotoxins accumulation: saxitoxin (paralytic poison, PSP), ocadaic acid (diarrhetic poison, DSP), domoic acid (amnesic poison, ASP) were revealed as well as the seasonal changes in phycotoxin concentrations in Japanese scallop during the development of mass species of the toxic phytoplankton at the commercial coastal sites in Aniva Bay (Mogilnikova, Konovalova, 2005; Mogilnikova et al., 2007).

Based on these studies, there was revealed a need in conducting regular observations for the annual levels of phytoplankton vegetation including the potentially toxic group of species, seasonal variability of quantitative indices, and determination of phycotoxin contents in sea waters and tissues of Japanese scallop.

The aim of this work was to estimate both a state and seasonal changes in quantitative phytoplankton indices at the sea coastal sites, including the potentially toxic species, and potential danger of the fishery objects in the period of mass development of the toxic phytoplankton by defining a level of phycotoxin contents in Japanese scallop.

Materials and Methods

The surveys were conducted on the six testing areas along the southern and southeastern Sakhalin coasts: in Aniva Bay on “Taranai”, “Prigorodnoe”, “Aracul”, “Yunona”, and “Busse” testing areas and in Mordvinov Bay on “Okhotskoye” testing area. A map of location of the testing sampling areas is presented in the work of Nikulina et al., 2017 published in this book.

Water sampling for chemical analysis, and soil and phytoplankton sampling have been conducted since February through October 2015 in the surface horizon at coastal stations located 1 m away from the shore at 0.5 m depth and additionally – in the surface horizon above the depth of 15–20 m at the sampling sites for Japanese scallop. Water and phytoplankton samples were taken, fixed, concentrated and treated according to the accepted methods (Manual on harmful…, 1995; Radchenko et al., 2010; and others).

In order to study phycotoxins in tissues of Japanese scallop *Mizuhopecten yessoensis* (Jay), the samples were taken near Cape Yunona at the depth of 15–20 m and in Busse Lagoon at the depth of 3.3–5.3 m. The specimens of Japanese scallop sampled near Cape Yunona had commercial sizes of 16.4 –17.9 cm high, those taken from Busse Lagoon were mainly of 10.1–14 cm commercial height, specimens less than 12 cm were single.

Phycotoxin contents in the sea water and Japanese scallop tissues were defined using the method of the immuno-ferment analysis (IFA) on the analyzer IFA ELISA 301 using the test-systems with microtiter plates according to the producer’s method. The number of saxitoxin (PSP-toxin) – paralitic poison was measured using the test-system RIDASCREEN®FASTPSPSC, producer “R-BiopharmAG” (Germany). The detection limit of the saxitoxin test-system was 50 µg/kg. The ocadaic acid (DSP-toxin) – diarrhetic poison was defined using a microtiter plate OkadaicAcid (DSP) ELLISA, MicrotiterPlate. The detection limit via the DSP-toxin test-system was 100 µg/kg. ABRAXIS (USA). The domoic acid (ASP-toxin) – amnesic toxin was defined using ASPPlateKit, BiosenseLaboratoriesAS (Norvey). The sensitivity of definition of the ASP-toxin test-system was 10 µg/kg. Saxitoxin was defined in three specimens and more (3–5) of Japanese scallop, diarrhetic and amnesic toxins in two or three individuals.

Results and Discussion

Phytoplankton

In the study area in 2015 the most frequent and mass representatives of toxic phytoplankton have been found since June through September. On the testing area of the Taranai River mouth in August 2015 the number of potentially-toxic groups of species *Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle, *Pseudo-nitzschia* spp. composed 44.145×10^{3}cell/L under the mass phytoplankton vegetation; in September the species from the *Pseudo-nitzschia* group were recorded under the low abundance.

In the coastal zone of Prigorodnoye testing area in July and August the number of the potentially-toxic species *Pseudo-nitzschia* sp. was temperate (0.412×10^{3}cell/L), and *Dinophysis acuminata* Claparède et Lachmann was observed in July at different sites of Aniva Bay under the low abundance. In September, the species of the group *Pseudo-nitzschia*, *Dinophysis acuminata*, *Ostreopsis siamensis* Johs.Schmidt were recorded under the low abundance.

On the Arakul testing area (mouth), the species of the genera *Pseudo-nitzschia*, *Dinophysis acuminata* and *Prorocentrum micans* Ehrenberg of the potentially-toxic and harmful algae were observed in September.

In the sea waters the main saxitoxin producers are species from the genus *Alexandrium*. The maximum number of its representative (*Alexandrium tamarense* (Lebour) Balech) was found in the surface above horizon the depth of 15–20 m in the channel of Busse Lagoon in June and near Cape Yunona in July (Table 28.1). The species

Table 28.1 Abundance (N×10^{3}cell/L) of groups of phytoplankton species – producers of phycotoxins: saxitoxin (PSP-toxin), diarrheic (DSP-toxin), amnesic (ASP-toxin) in the coastal zone near Cape Yunona (Anuva Bay) and channel of Busse Lagoon at the sampling sites for Japanese scallop in 2015

Group of species	Toxin	Sampling site	June	July	August	September
Alexandrium tamarense + *Alexandrium* spp.	PSP	Busse Lagoon channel	2.88	no	no	no
		Cape Yunona	no	1.52	0.292	no
Prorocentrum minimum + *Prorocentrum balticum* + *Prorocentrum* spp.	DSP	Busse Lagoon channel	1.632	no	no	0.006
		Cape Yunona	no	1.28	0.292	0.127
Dinophysis rotundata + *D. acuminata* + *D. sphaerica*		Busse Lagoon channel	no	0.019	0.095	0.006
		Cape Yunona	no	0.088	0.292	0.007
Pseudo-nitzschia pungens + *P. seriata* + *P.* cf. *delicatissima* + *Pseudo-nitzschia* spp.	ASP	Busse Lagoon channel	no	0.006	no	1.354
		Cape Yunona	no	1.28	2.628	1.196

“no” – samples were not taken.

of this genus occurred since June through August. As a rule, this most numerous species *Alexandrium tamarense* reaches maximum development in Sakhalin waters in the first half of the summer (June–early July). On the Busse testing area the mass development of the species from genera *Pseudo-nitzschia*, *Prorocentrum micans*, *P. minimum* (Pavillard) J.Schiller, and *Dinophysis acuminata* were observed as well in September.

The species-producers of diarrheic toxin (DSP-toxin) of the genus *Prorocentrum* occurred in maximum number in the channel of Busse Lagoon in June, near Cape Yunona in August. Species of the genus *Dinophysis* have been found since July through September (maximum number near Cape Yunona in August).

The species-producers of amnesic toxin (ASP-toxin) of the genus *Pseudo-nitzschia* have been developed since July through September reaching the maximum level of vegetation near Cape Yunona in August and September and in the channel of Busse Lagoon in September.

The vegetation of toxin-producing species found in 2015 agrees with the stated notion about the seasonal features of vegetation of species found in the preceding years.

Phycotoxin contents in sea water patterns and tissues of Japanese scallop

From the results of studies on saxitoxin content in tissues of Japanese scallop there was ascertained the occurrence of toxins in all of the studied samples from Busse Lagoon and coastal zone near Cape Yunona (Table 28.2).

In Busse Lagoon the maximal concentrations of saxitoxin in muscle of Japanese scallop, higher than the admissible level (AL) >80.0 μg/kg, were recorded in July in two of the five tested individuals, and also in August in one individual of Japanese scallop of the tree tested. In September, a decline in saxitoxin concentration in Japanese scallop muscle was noted.

Of the individuals of Japanese scallop collected in the coastal zone near Cape Yunona, the maximal saxitoxin concentrations were found in muscle of one of the three individuals in June (Table 28.2). In August and September, saxitoxin was found only in hepatopancreas of Japanese scallop. This proved the ability of Japanese scallop specimens for autopurification from phycotoxins when occurring in natural habitat. It should be noted that saxitoxin is not destroyed under boiling, so if preparing scallop as a whole with shells, saxitoxin could move from hepatopancreas to other organs (muscle, mantle, broth). Saxitoxin is a persistent toxin to any temperatures both low and high. So, boiling and freezing for several months do not destroy toxin, but even enlarge toxicity of some toxins from this group.

During the study period the amnesic toxin was not defined in water, and in tissues of Japanese scallop its concentrations were minimal.

Since June through September, tissues of Japanese scallop from Busse Lagoon have been tested for diarrheic toxin content (two individuals each month). This poison was not found in scallop muscle. As for diarrheic toxin content, Japanese scallop in Busse Lagoon was not dangerous as a fishery object.

Table 28.2 Results of saxitoxin (PSP-toxin) analysis in tissues of Japanese scallop sampled from Busse Lagoon and near Cape Yunona (Aniva Bay) in 2015

N	Date	Kind of tissue, organ	Concentration of saxitoxin, μg/100g	N	Date	Kind of tissue, organ	Concentration of saxitoxin, μg/100g
		Busse				**Yunona**	
1	June	muscle	52.245	1	June	**muscle***	**>80.00**
2	June	muscle	72.065	2	June	muscle	16.79
3	June	muscle	78.143	3	June	muscle	25.64
4	July	muscle	77.39	4	June	mantle*	58.278
5	July	muscle	67.49	5	June	**hepatopancreas***	**>80.0**
6	July	muscle	63.148	6	August	**muscle**	**>80.0**
7	July	**muscle**	**>80.0**	7	August	muscle	15.221
8	July	**muscle**	**>80.0**	8	August	muscle	28.014
9	August	muscle	63.148	9	August	muscle	30.651
10	August	muscle	64.567	10	August	**hepatopancreas**	**391.14**
11	August	**muscle**	**>80.0**	11	September	muscle	27.521
12	September	muscle	46.198	12	September	muscle	25.988
13	September	muscle	35.154	13	September	muscle	10.962
14	September	muscle	25.182	14	September	**hepatopancreas**	**467.701**

Samples of tissues pointed * belong to one and the same individual of Japanese scallop.

In the period from June to September, tissues of Japanese scallop from coastal waters near Cape Yunona were tested in two individuals for each month. Insignificant concentrations of diarrheic toxin in muscle were revealed in September (0.96 mg/kg) and in hepatopancreas in June (10.99 mg/kg), August (8.15 mg/kg), and September (12.22 mg/kg). The level of concentration of diarrheic toxin did not exceed AL (20 mg/kg).

The studies of sea water for diarrheic toxin showed the occurrence of toxin in minimal concentrations is lower than a detection limit: 0.5–1.23 μg/100 ml.

Thus, despite the fact that saxitoxin-producer species have not been always found in the study area, saxitoxin occurred in tissues of Japanese scallop for the whole period of researches. Because of spotty distribution of the toxic species cells, we sometimes fail to find them. However, saxitoxin occurrence in scallop tissues proves its accumulation in organs and their slow purification from the toxin during the toxic species vegetation.

However, because of samples collected in water near the shore the toxic benthic dinoflagellates *Ostreopsis siamensis* and *Ostreopsis* spp., which are the producers of the palytoxin group, have been revealed. Single individuals of *Ostreopsis* occurred earlier in Aniva Bay.

From the results of earlier studies the development of phytoplankton and toxic phytoplankton, particularly, was revealed to be more active in the water column at isobaths of 5, 10 and more meters. Further there is a need to conduct phytoplankton surveys, including a toxic one, on the shore-sea transects at several horizons.

In 2015, during the study period the development of species producing DSP-toxin was not high. Perhaps, due to that the diarrheic toxin was not found in Japanese scallop from Busse Lagoon and lower than AL near Cape Yunona; in water its content was insignificant.

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