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Morphogenetic and toxin composition variability of *Alexandrium tamarense* (Dinophyceae) from the east coast of Russia

TATIANA Y. ORLOVA[1*], MARINA S. SELINA[1], EMILY L. LILLY[2], DAVID M. KULIS[3] AND DONALD M. ANDERSON[3]

[1]*A. V. Zhirmunsky Institute of Marine Biology, Far East Branch of Russian Academy of Science, Vladivostok, 690041 Russia*
[2]*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02360 USA*
[3]*Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA*

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Twenty-seven clones were established from elongate *Alexandrium* sp. cysts collected in six regions along the Russian Pacific coast. All isolates were identified as *Alexandrium tamarense* via detailed epifluoresence microscopy of thecal plates. Morphological differences of both cultured and wild cells from the study regions mainly occurred in the shape of the cell (length/width ratio), degree of development of the sulcal list, and the shape of the posterior sulcal (S.p.) and second antapical (2′′′′) plates. Cells were divided into two cell types: 'short' (isodiametrical or wide) and 'tall'. Each cell type exhibits specific features of tabulation, mainly the shape of the S.p. and 2′′′′ plates and was dominant in each particular region of the study. The short type, with a wide S.p. and reduced length in the dorsoventral 2′′′′ plates, was characteristic of *A. tamarense* from Primorye and southern Sakhalin Island. The tall cells, i.e., with cell length exceeding width, and having and elongate S.p. and dorsoventrally elongate 2′′′′ plates, prevailed in Avachinskaya Guba Inlet and in the Bering Sea. The differences reported here between the two types are within the range of morphological variability of *A. tamarense sensu* Balech, 1995. The D1–D2 fragment of the large subunit nuclear ribosomal DNA was analyzed for 24 clones. *Alexandrium tamarense* from the Russian Pacific coast compose three genetically distinct populations that correspond to the Japanese temperate Asian, eastern North American, and western North American ribotypes of the '*tamarensis*' complex. The presence and distribution of eastern and western North American ribotypes along the Russian Pacific coast suggest that dispersion to the temperate Asian region occurred long ago via natural currents and processes, and not through human-mediated introductions, as has been proposed. No strict correlation was observed between different morphological types of cells and ribotypes. High-performance liquid chromatography toxin analyses showed that all isolates were toxic and demonstrated variability in toxin content and composition among different populations. These data document the significant and previously uncharacterized risk of shellfish contamination with paralytic shellfish poisoning toxins from blooms of *A. tamarense* in Russian marine waters.

KEY WORDS: *Alexandrium tamarense*, Distribution, Far Eastern seas of Russia, LSU rRNA, Morphology, PSP, Toxicity

INTRODUCTION

The Russian Pacific coast is one of many areas affected by harmful algal blooms (HABs), which have greatly increased in frequency and geographic distribution worldwide over the last several decades. Dinoflagellates of the genus *Alexandrium* (Halim) Balech produce saxitoxin and its derivatives, which cause paralytic shellfish poisoning (PSP), a HAB syndrome that affects many coastal countries (Prakash *et al.* 1971; Anderson 1989; Hallegraeff 1995).

PSP has a long history in Russia, but reports of poisoning and subsequent death are uncommon in coastal areas. The earliest documented case of PSP in Russia was in September 1945 on the west coast of the Bering Sea when six crew members were poisoned and two died (Lebedev 1968). Another incident occurred in Petropavlovsk-Kamchatski in mid-August 1973, when two children died during a toxic red tide of *Alexandrium tamarense* (Lebour) Balech in Avachinskaya Guba Inlet (Kurenkov 1974; Konovalova 1993). The presence of 11-hydroxysaxitoxin in mussels from Avachinskaya Guba Inlet was confirmed in the early 1980s by V. Sova *et al.* (unpublished data) at the Pacific Institute of Bioorganic Chemistry of FEB RAS (V.A. Stonik, personal communication stonik@piboc.dvo.ru).

Because *Alexandrium* spp. are a common the source of saxitoxin, taxonomic investigations of *Alexandrium* were conducted in some areas in the Far Eastern seas of Russia. As a result, six species of *Alexandrium* were found: *A. tamarense, A. acatenella* (Whedon & Kofoid) Balech, *A. ostenfeldii* (Paulsen) Balech & Tangen, *A. insuetum* Balech, *A. pseudogonyaulax* (Biecheler) Horiguchi ex Yuki & Fukuyo, *A. tamutum* Montresor, Beran & John and *A. margalefi* Balech (Kisselev 1959; Konovalova 1989, 1991, 1993, 1998; Selina & Konovalova 2001; Selina & Morozova 2005). Until now, morphological study of *A. tamarense* was restricted to certain areas of the Bering Sea and Kamchatka, where red tides of this species were observed (Konovalova 1991, 1993). There has been no work, however, on the genetics and toxicity of Russian *Alexandrium*. It was unknown whether *A. tamarense* was a single, widespread, homogeneous population or composed of several subpopulations along the Russian Pacific coast. Previous work on the '*tamarensis*' complex [*A. tamarense, A. catenella* (Whedon & Kofoid) Balech and *A. fundyense*

* Corresponding author (torlova@imb.dvo.ru).

Balech] in the North Pacific, including East Asia, suggested that there are several subpopulations that correspond to multiple variants of the temperate Asian ribotype, including the Korean and Japanese temperate Asian (JTA), and western and eastern North American ribotypes (WNA, ENA) (Anderson *et al.* 1994; Scholin & Anderson 1994, 1996). Subsequently, a single isolate from a remote location on the Russian coast, Avachinskaya Guba Inlet, Kamchatka, was analyzed and found to display the WNA ribotype (Scholin 1998).

In the present work, we studied the morphological characteristics of cultured and wild *A. tamarense* from multiple locations along the Russian Pacific coast *via* microscopic examination of thecal plate structure, and tested a series of clones for genetic relatedness using sequences of the D1R–D2C region of the large subunit ribosomal DNA (LSU rDNA). The isolates were also analyzed for toxin composition using high-performance liquid chromatography (HPLC).

MATERIAL AND METHODS

Isolation and cultivation

Surface sediment and water samples were collected between August 2000 and July 2002 from 11 stations in 6 geographic regions along the Russian Pacific coast (Fig. 1, Table 1). Plankton was collected by bottle or a 20-μm mesh net and preserved with Lugol's iodine. Sediment samples were collected by divers using glass or aluminum coring tubes, or by a grab sampler. Sediment samples were stored in the dark at 4°C and used for germination experiments within six months of collection. Sediment processing generally followed the methods of Anderson *et al.* (1982) and Matsuoka *et al.* (1989). Individual cysts were isolated by microcapillary pipette, washed in filtered seawater and placed in separate wells of a 96-well tissue culture plate containing modified f/2 medium (Guillard & Ryther 1962) without silicate. The f/2 medium was modified by adding Na_2SeO_3 to 10^{-8} M and decreasing the concentration of $CuSO_4 \cdot 5H_2O$ to a final concentration of 10^{-8} M. All isolation plates and cultures were incubated at 15°C on a 14 : 10 h light : dark cycle (*c.* 200 μmol photons m^{-2} s^{-1} irradiance provided by cool white fluorescent bulbs. The plates were sealed with tape to avoid evaporation and were monitored for germination for two weeks under a Zeiss inverted microscope, IM 35 (Carl Zeiss, Oberkochen, Germany). Isolations were used to establish clonal cultures from the cyst germinations, and the same media and conditions were used to incubate cultures.

Morphology

For morphological analysis, only newly established cultures within 3–4 weeks of cyst germination were used. One-milliliter aliquots were taken from cultures in the early exponential phase of growth. Thirty-five cells were examined for each clone. The number of examined cells from natural samples was: for Primorye (region I) 15; southern Sakhalin (region II) 42; Kamchatka (region III) 15; Olytorskii Bay (region IV) 15. Natural samples from regions V and VI were not available for morphological analysis. The number of examined cysts was not less than 100 for each location. Dimensions are given as mean ± standard deviation.

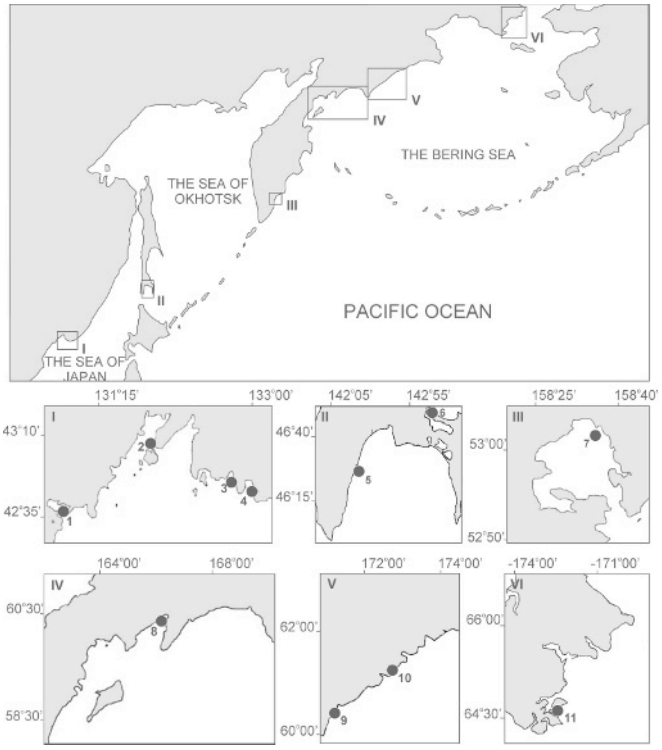


Fig. 1. Map showing the sampling regions and stations on the Russian east coast: (I) Primorye, the Sea of Japan; (II) southern Sakhalin Island, the Sea of Okhotsk; (III) Avachinskaya Guba Inlet, Kamchatka; (IV) Olytorskii Bay, the Bering Sea; (V) west coast of the Bering Sea; (VI) Chukotka, the Bering Sea.

The thecal plate morphology of wild and cultured cells was examined using ultraviolet epifluoresence microscopy after calcofluor white staining (Sigma Chemical Co.) following the method of Fritz & Triemer (1985). Thecae were suitably positioned by rolling them between the slide and coverslip; in some cases, thecae were disassociated using 5% sodium hypochlorite solution. Cells were photographed with a Zeiss Axioscope light microscope (Carl Zeiss, Oberkochen, Germany) with a Zeiss G365 excitation filter and a long-pass 420-emission filter. Terminology used in this work was adopted from Balech (1995).

DNA purification

Approximately 10 ml of a mid-exponentially growing culture were harvested by gentle centrifugation and the supernatant was removed by aspiration. The cell pellet was resuspended in 400 μl of a buffer containing 100 mM Tris-HCL, pH 8.3 (at 25°C), 500 mM KCL, 15 mM MgCl and 0.01% (w/v) gelatin, and transferred to a sterile 1.5-ml microcentrifuge tube. Samples were boiled for 5 minutes to disrupt the cells. Cell debris was removed by centrifugation, and the supernatant stored at −20°C. DNA was purified from this extract with the Qiagen DNeasy Kit.

Table 1. Location on a map (Fig. 1), station, sampling date, latitude, longitude, type of sample and strain code[1]

Geographic region	Station no.	Sampling date	Latitude N	Longitude E	Cysts (C)/ plankton (P)	Strain code[1]
I	1	28 Sep. 2000	42°37′	130°49′	C/P	
Primorye,	2	16 Aug. 2000	43°15′	131°50′	C	ATRU16
the Sea of Japan	3	15 Sep. 2000	42°50′	132°46′	C/P	
	4	20 Sep. 2000	42°45′	133°00′	C/P	ATRU11, ATRU12, ATRU13, ATRU14, ATRU15
II	5	17 Jun. 2001	46°24.9′	142°23.3′	C/P	ATRU17, ATRU18, ATRU20, ATRU24, ATRU25, ATRU26, ATRU30
Southern Sakhalin Island, the Sea of Okhotsk						
	6	08 Jul. 2001	46°52′	143°07′	C	ATRU27, ATRU28
III Avachinskaya Guba Inlet, Kamchatka	7	07 Sep. 2001	53°02′	158°35′	C/P	ATRU21, ATRU22
IV Olytorskii Bay, the Bering Sea	8	20 Jul. 2002	60°13.33′	166°48.22′	C/P	ATRU6/1, ATRU6/2
V	9	21 Jul. 2002	60°21.236′	170°37.513′	C	ATRU1/1, ATRU2/1,
The west coast of the Bering Sea						ATRU3/1, ATRU4/1, ATRU5/1, ATRU5/2
	10	22 Jul. 2002	61°07.46′	172°15.63′	C	ATRU10
VI Chukotka, The Bering Sea	11	26 Jul. 2002	64°38.8′	172°31.3′	C	ATRU9/1

[1] Strain designations currently used in the D.M. Anderson culture collection, Woods Hole Oceanographic Institution, Woods Hole, MA, USA.

Polymerase chain reaction (PCR) amplification of LSU rDNA

Partial LSU rDNAs were amplified from total cellular DNA using PCR (Saiki *et al*. 1988) with the D1R and D2C primers and 1–5-ng template (Scholin & Anderson 1994). A Perkin Elmer Gene Amp PCR system 2400 DNA thermal cycler was programmed for 30 cycles of 94°C, 1 min; 50°C, 1.5 min; 72°C, 1 min, with a 5-second autoextension per cycle at the 72°C step. Products were purified in Qiagen MinElute columns and stored in deionized distilled water at −20°C. The concentration of purified products was determined relative to a DNA mass marker ladder (Life Technologies, low DNA mass ladder).

Restriction digests

All restriction digests followed manufacturer recommendations, using the buffers and bovine serum albumin (BSA) extract supplied with the enzymes *Nsp*I, *Mse*I and *Apa*LI (BioLabs New England, Beverly, MA, USA). Water, buffer, BSA, and the enzyme were prepared in a master mix; 4 µl of this were added to 10-µl aliquots of purified PCR product. Digests were prepared using 2–5 units of restriction enzyme and approximately 0.5–1 µg of PCR product per 12-µl reactions. Reactions proceeded for 24 hours at incubation temperature of 37°C to ensure complete digestion. Products were stored at −20°C.

Gel electrophoresis

Restriction fragment length polymorphism (RFLP) products were resolved on a 3.0% agarose gel of a 3 : 1 mixture of Nusieve and low EEO agarose and 1× Tris-acetate-EDTA buffer, with ethidium bromide incorporated in the gel (Scholin & Anderson 1996). Samples were mixed with 2 µl of loading dye, applied to the gel and run at 75 V for 90 min. Gels were photographed using an MP-4 camera system and 667 print film or the ChemiImager (Alpha Innotech, San Leandro, CA, USA) digital picture system. Sizes of PCR and digestion products were estimated in comparison with mobility size standards (BioMarker Low, Bioventures, Murfreesboro, TN, USA).

Toxin analysis

Separate 15-ml samples of each culture, grown in duplicate, were harvested at mid-exponential growth phase (10^6–10^7 cells l^{-1}), and subjected to centrifugation (3000 × *g*). The pellet was resuspended in 0.05 M acetic acid and extracted by sonification while the samples were cooled in an ice water bath. The extracts were stored at −20°C until analysis by HPLC following the methods of Oshima *et al.* (1989) with modifications as described by Anderson *et al.* (1994).

Abbreviations used throughout this text are: C1,2, C toxins 1 and 2; GTX1,4, gonyautoxins 1 and 4; GTX2,3, gonyautoxins 2 and 3; GTX5, gonyautoxin 5; NEO, neosaxitoxin; dcSTX, decarbamoylsaxitoxin and STX, saxitoxin. As toxins C1,2, GTX1,4 and GTX2,3 are epimeric pair toxins, their toxin values will be grouped together when calculating molar percentage values to account for potential epimerization that might occur after toxin extraction. Potencies of the individual toxins are expressed as saxitoxin equivalents (fg STX equiv. $cell^{-1}$) and were converted from the molar concentrations using specific toxicities for each toxin found in Oshima 1995 (µg STX eq. $umol^{-1}$) as follows: C1, 3.45; C2, 55.0; GTX1, 567.6; GTX2, 205.2; GTX3, 364.3; GTX4, 414.7; GTX5, 36.8; dcGTX2, 87.9; dcGTX3, 215; NEO 527.9; dcSTX, 293.0; STX, 571.1.

RESULTS

Morphological analysis

Twenty-seven clonal cultures of *Alexandrium* established from six study regions along the Russian Pacific coast were investigated. All isolates shared the typical morphological features of *A. tamarense*. Additionally, *A. tamarense* from plankton samples were analyzed. Examination of both cultured and wild cells revealed considerable variation in morphological features (Table 2, Figs 2–58). The length of cells varied between 25 and 47.5 μm and the width between 25 and 45 μm. The length-to-width ratio (L/W) ranged from 0.8 to 1.12. There was also high variability in shapes of apical pore plate (Po), and the first apical (1′), anterior sulcal (S.a.), posterior sulcal (S.p.) and second antapical (2′′′′) plates (Table 2, Figs 5–9, 15–22, 28–33, 37–41). The 1′ in most cases directly contacted the Po plate and contained a small pore (Figs 4–6, 15, 22, 29, 37, 39, 48, 53–55). However, the position of the pore and the mode of connection with Po varied markedly. The S.p. plate often had a connecting pore on the right side linking to the right edge by a groove (Figs 8, 9, 19, 50).

As a rule, cells from the different study regions were characterized by morphological features that prevailed among the cells in that region. The prevailing cells from Primorye (region I) had an isodiametrical form, with weakly developed sulcal lists, a wide 1′ plate, a nearly isodiametric S.p. and a large connecting pore (Table 2, Figs 2–6, 8, 9). In plankton samples from this region, only single cells were observed, but in the cultures, the chains consisted of four to eight cells. The smallest cells (32.3 ± 3.19 μm long, 32.3 ± 2.93 μm wide) were found in the Primorye (Table 2). Cysts in this area were oval, or elongate to oval, but sometimes nearly round (Figs 10, 11), 32–42 μm long, 23–27.5 μm wide. Round cysts germinated and produced the *A. tamarense* form typical for that region.

The prevailing cells from the coastal waters of southern Sakhalin Island (region II) were wide and had a well-developed sulcal list on the left side (Table 2, Figs 12–14). Cells were 32.1 ± 3.58 μm long and 35.3 ± 3.97 μm wide. The S.p. plate was frequently wider than longer (Figs 18, 19, 21). Natural blooms were typically composed of single cells, and sometimes pairs; in culture the chains consisted of four to eight cells. Cysts of *Alexandrium* from this region were 40–62 μm long and 25–30.5 μm wide, mainly elongate or elliptically shaped (Fig. 24).

The largest (43.2 ± 4.08 μm long, 41.1 ± 3.52 μm wide) asymmetric cells were observed in Avachinskaya Guba Inlet (region III) (Table 2, Figs 25–27). The first apical plate (1′) is slim, sometimes with elongate upper and lower corners (angles) (Figs 27, 29). In cultured cells, the anterior portion of the S.a. plate was elevated and penetrated into the epitheca (Fig. 27). The S.p. usually was longer than wider and had a connecting pore, situated right at the margin of the plate without a groove (Figs 28, 30, 31). The 2′′′′ plate was strongly elongate dorsoventrally (Figs 28, 32). Single cells and pairs of cells were observed both in plankton samples and in the cultures. Cysts found in region III were elliptical, 42–50 μm long, 25–28.5 μm wide (Fig. 34).

The tall rounded cells with a noticeable antapical protrusion and a well-developed sulcal list were observed in the Bering Sea (regions IV–VI) (Figs 35, 36). Cells were 37.9 ± 4.06 μm long and 37.0 ± 4.06 μm wide. Morphological features of 1′, S.a., and S.p. plates of cells from the Bering Sea were similar to cells from the Avachinskaya Guba Inlet (region III) (Figs 38–41). But the 2′′′′ was dorsoventrally elongated and intermediate in shape (Figs 40, 41). Only single cells were observed in nature, and pairs of cells were observed in the culture. Cysts were elliptical, 42–50 μm long, 25–28.5 μm wide (Fig. 42).

The above morphological differences between cells from different regions on the Russian Pacific coast occurred both in plankton samples and in cultures, but were, however, less pronounced in cultures. An exception was provided by the ATRU28 and ATRU27 isolates from cysts collected at station 6 in southern Sakhalin Island area (region II), which differed from all other isolates and natural cells in details of the thecal structure. In ATRU28, the shape and morphology of S.a., S.p. and 2′′′′ plates were similar to cells from regions III–VI (Table 2, Figs 44, 45, 49, 50). A specific feature of this clone was that cells without a ventral pore on the 1′ plate were dominant (Figs 45–47); however, 25% of the cells of this clone had a ventral pore (Fig. 48). Clone ATRU27 was characterized by cells morphologically similar to cells from Primorye (region I) (Figs 51–55). Here, however, we observed an unusual feature of the S.p. plate. In most cases, the connecting pore was linked to the ventral margin of plate S.p. with a groove (Figs 56, 57), but in some cases, a large connecting pore was situated in the center of the plate (Fig. 58). These morphological features were not observed in other isolates or in wild cells.

LSU rDNA RFLP data

Twenty-four clones were analyzed by RFLP with each of three enzymes. The RFLP pattern for 13 representative cultures is presented in Fig. 59. Three different RFLP patterns were observed (Table 2, Fig. 59). All isolates from Primorye (region I) displayed the JTA ribotype. The WNA ribotype was found in the Avachinskaya Guba Inlet at Kamchatka (region IV). All isolates from southern Sakhalin Island (region II) and from the Bering Sea (regions IV–VI) displayed the ENA ribotype.

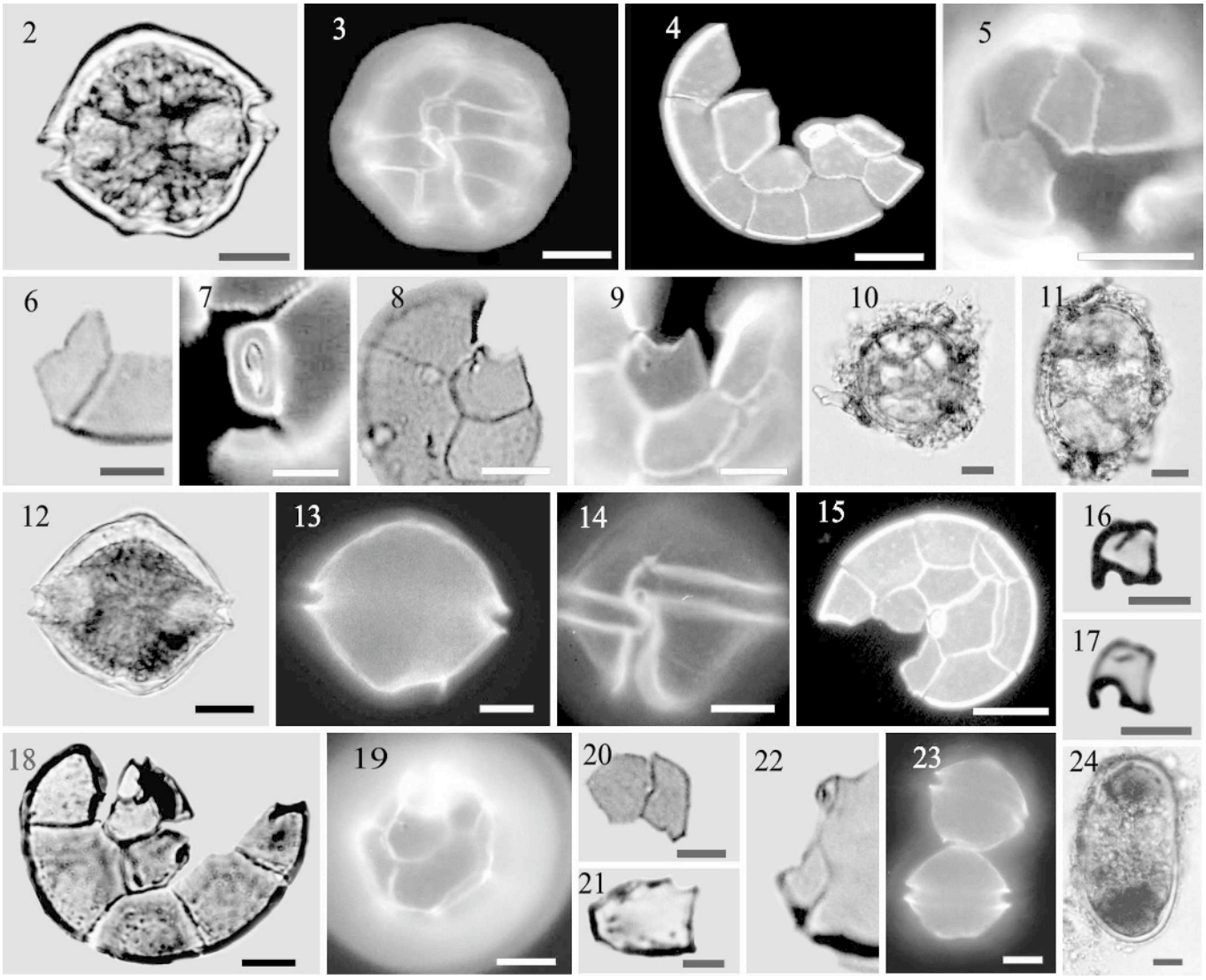
Toxin analysis

All of the isolates were found to contain saxitoxin or its congeners (derivatives) (Table 3, Fig. 60) and within the 25 cultures analyzed, eight distinct toxin composition profiles were observed. For the most part, toxin composition was unique and conserved for the isolates from a particular region, as was toxin concentration and potency. However, there were two exceptions found in cultures from regions II and V. Cultures ATRU17, 18, 20, 25, 26, 27 and 28, from region II, had moderate levels of GTX1,4 and NEO (10 and 12%) and lower amounts of GTX2,3 and STX (3% and 4%). No GTX5 was detected in these seven cultures and all cultures from this region had the highest proportion of C1,2 toxins – on average, 71%. In contrast, isolate ATRU30, also from region II, did not produce any NEO or STX but

Table 2. Morphogenetic characteristics of *A. tamarense* from the Russian Pacific coast

Geographic region	Strain code	Ribotype[1]	Ratio length/width[2] in culture/nature	Ventral pore	Shape of the S.p. plate	Peculiar characteristic	Type of 2'''' plate[3]	Maximum Number cells per chain in culture/nature
I	ATRU11	JTA	≤ 1/0.94	+	almost as wide as long	S.p. with big pore	type B	8/1
Southern Primorye, the Sea of Japan	ATRU12	JTA						
	ATRU13	JTA						
	ATRU14	JTA						
	ATRU15	JTA						
	ATRU16	JTA						
II	ATRU17	ENA	0.91/0.91	+	wide, sometimes almost as wide as long	cell with well-developed sulcal lists	type B	8/2
Southern Sakhalin Island, the Sea of Okhotsk	ATRU18	ENA						
	ATRU19	-						
	ATRU20	ENA						
	ATRU24	ENA						
	ATRU25	-						
	ATRU26	-						
	ATRU27	ENA	≥ 1/-	+	almost as wide as long	connecting pore connected with sulcus	type B	4/-
	ATRU28	-	> 1/-	−/+	longer than wide	s.a. often project into the epitheca	type A	4/-
III	ATRU21	WNA	1.05/1.05	+	longer than wide	cells longer, noticeable asymmetric	type B	2/1
Avachinska ya Guba Inlet, Kamchatka	ATRU22	WNA						
		WNA						
IV	ATRU6/1	ENA	1.03/1.03	+	almost as wide as long or longer than wide	cells round with well-developed sulcal list	type B	2/2
Olytorskii Bay, the Bering Sea	ATRU6/2	ENA						
V	ATRU1/1	ENA	≥ 1/-	+	longer than wide	s.a. sometimes project into the epitheca	type B	2/-
The west coast of the Bering Sea	ATRU2/1	ENA						
	ATRU3/1	ENA						
	ATRU4/1	ENA						
	ATRU5/1	ENA						
	ATRU5/2	ENA						
	ATRU10	ENA						
VI	ATRU9/1	ENA	≥ 1/-	+	longer than wide		type B	2/-
Chukotka, the Bering Sea								

[1] Ribotypes: JTA, Japanese temperate Asian; WNA, western North American; ENA, eastern North American.
[2] Wide = maximum cingular width.
[3] Type of the 2'''' plate: type A, elongated in the dorsoventral direction; type B, short in the dorsoventral direction (Balech 1995); –, data absent.



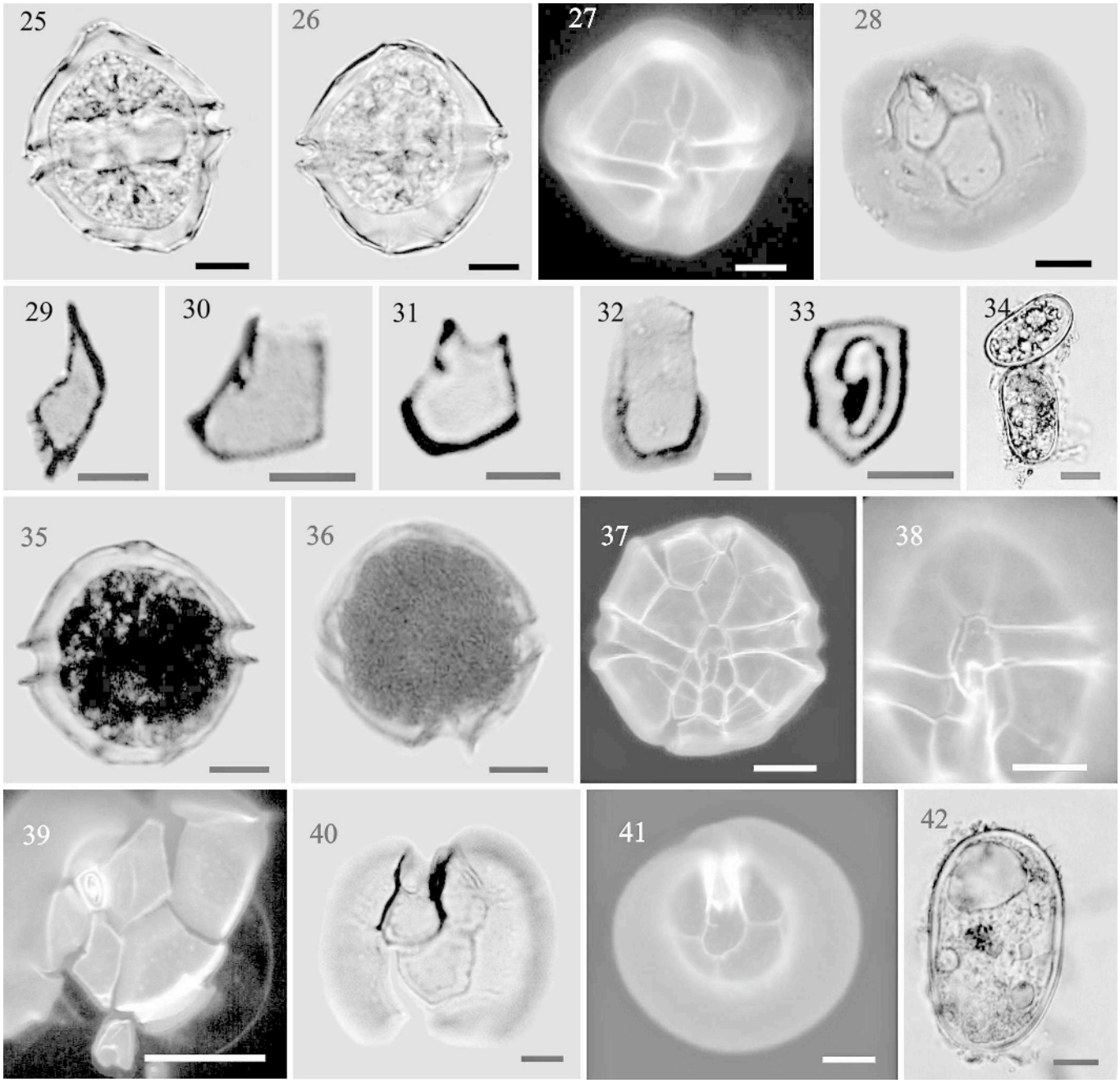
Figs 2–24. *Alexandrium tamarense* from the Pacific coast of Russia. Figs 2, 6, 8, 10–12, 16–18, 20–22, 24, light microscopy (bright field); Figs 3–5, 7, 9, 13–15, 19, 23, light microscopy (epifluorescence using calcofluor white). All Figs wild cells except Figs 3–5, 9, clone ATRU11; Fig. 6, ATRU16; Fig. 15, clone ATRU17; Fig. 19, clone ATRU25. All scale bars = 10 μm except Figs 6, 7, 16, 17 = 5 μm.

Figs 2–11. *Alexandrium tamarense* from Primorye.

Fig. 2. General view of the cell.
Fig. 3. Ventral view, showing the descending cingulum.
Fig. 4. Squashed epithecal plates showing direct contact between Po and 1′ plates.
Fig. 5. First apical (1′) and sixth precingular (6″) plates.
Fig. 6. First apical (1′) plate.
Fig. 7. Po plate.
Fig. 8. Antapical view, showing the isodiametrical S.p. plate with a connecting pore and intermediate-type second antapical (2‴) plate.
Fig. 9. Antapical view of cell showing nearly isodiametrical S.p. and elongated transversely 2‴ plates.
Fig. 10. Elongate cyst.
Fig. 11. Ellipsoidal cyst.

Figs 12–24. *Alexandrium tamarense* from southern Sakhalin Island.

Fig. 12. Ventral view of the cell.
Fig. 13. Dorsal view, showing a well-developed sulcal list.
Fig. 14. Ventral view, showing descending cingulum and a well-developed sulcal list on the left side.
Fig. 15. Plates of epitheca.
Figs 16, 17. Anterior sulcal plates (S.a.) with slanting plica.
Fig. 18. Plates of the hypotheca.
Fig. 19. Antapical view, showing wide posterior sulcal plate (S.p.) and transversely elongate 2‴ plates.
Fig. 20. The first apical (1′) and sixth precingular (6″) plates.
Fig. 21. Wide S.p. plate.
Fig. 22. First apical plate connected with the Po plate by a threadlike prolongation.
Fig. 23. Two-celled chain.
Fig. 24. Ellipsoidal cyst.



Figs 25–42. *Alexandrium tamarense* from the Pacific coast of Russia. Figs 25, 26, 28–36, 40, 42, light microscopy (bright field); Figs 27, 37–39, 41, light microscopy (epifluorescence using calcofluor white). All Figs wild cells except Fig. 27, clone ATRU22; Fig. 37, clone ATRU1/1; Figs 38, 39, clone ATRU5/1; Fig. 41, clone ATB9/1. All scale bars = 10 μm, except Figs 3, 34 = 20 μm and Fig. 33 = 5 μm.

Figs 25–34. *Alexandrium tamarense* from Avachinskaya Guba Inlet, Kamchatka.

Fig. 25. Ventral view of asymmetric cell.
Fig. 26. Dorsal view.
Fig. 27. Ventral view of cell, showing the descending cingulum, 1′ and 6″ plates.
Fig. 28. Antapical view, showing S.p. and dorsoventrally elongate 2″″ plates.
Fig. 29. First apical plate (1′).
Figs 30, 31. Posterior sulcal plate (S.p.) with a connecting pore situated at the margin of the plate.
Fig. 32. Dorsoventrally elongate 2″″ plate.
Fig. 33. Po plate.
Fig. 34. Two ellipsoidal cysts.

Figs 35–42. *Alexandrium tamarense* from the Bering Sea.

Fig. 35. General view of the cell.
Fig. 36. Ventral view of the cell with a well-developed sulcal list.
Fig. 37. Ventral view of the cell.
Fig. 38. Ventral view, showing S.a. penetrating into the epitheca.
Fig. 39. Epithecal plates, showing direct contact between Po and 1′ plates.

did produce 14% GTX5 and, as was the case with the other seven cultures for this region, ATRU30 had a high percentage (65%) of C1,2. Toxins GTX2,3 at 20% and about 1% GTX1,4 completed the toxin spectrum for this culture.

ATRU1/1 established from region V had a toxin profile dominated by toxins C1,2 (58%), which is similar to most of the clones isolated from the Russian Pacific coast. Moderate amounts of GTX1,4 and GTX2,3 (13%) were also detected in this isolate along with 7% NEO, 5% STX, 4% GTX5 and $>$ 1% dcSTX. In cultures ATRU2/1, 3/1, 4/1, 5/1 and 5/2 from region V as well, GTX5 was the dominant toxin at 51 mol% and only trace amounts of C1,2 were detected. In addition to the high percentage of GTX5, the five isolates from the west coast of the Bering Sea also produced significant amounts of dcSTX, 9% on average, and relatively high levels of STX (28%) and NEO (13%). These were the only cultures to produce more than trace amounts of dcSTX.

The remainder of the cultures analyzed in this study were dominated by a high percentage of C1,2 toxins (42–71%). In Primorye (region I) isolates, high levels of toxins GTX5 and NEO (24% and 33% respectively) were found, along with low to trace levels of STX (2%) and GTX2,3 (0.4%). Cultures isolated from Avachinskaya Guba Inlet (region III), ATRU21 and 22, had high molar percentages of GTX2,3 (35%), lower levels of GTX1,4, and GTX5, (6% and 11%) and small amounts of NEO and STX (1% and 0.3% respectively). ATRU6/1 and 6/2, from region IV, produced on average about 35% STX and C1,2. In addition, about 10% or less of NEO, GTX2,3, GTX1,4, GTX5 and trace amounts of dcSTX were seen in these isolates. The ATRU 9/1 isolate from region VI had a toxin profile where C1,2 was found to contribute about 43% of the total molar content. Moderate levels of GTX2,3 and GTX5, 18% and 17% respectively, were also measured followed by 8% GTX1,4 and STX, and 7% NEO.

In terms of toxin concentration (fmol cell^{-1}) and toxicity (fg STX equiv. cell^{-1}), clones ATRU6/1 and 6/2 from region IV produced more toxin per cell, resulting in a higher potency than the other cultures examined. On average, these two clones produced 176 fmol cell^{-1} total toxin, which corresponds to an average toxicity of 55,200 fg STX equiv. cell^{-1}. Toxin content and toxicity of the rest of the isolates were found to be within the typical published range for *A. tamarense* isolates (Maranda *et al.* 1985; Cembella *et al.* 1987, 1988; Kim *et al.* 1993; Anderson *et al.* 1994; Mendez *et al.* 2001; Persich *et al.* 2006) with cellular concentrations ranging from a low of 31.1 fmol in culture ATRU1/1 from region V to a high of 71.3 fmol for cultures ATRU2/1, 3/1, 4/1, 5/1 and 5/2 from the same region. These concentrations result in toxicity values between 6,082 and 19,308 fg STX equiv. cell^{-1}.

DISCUSSION

Morphology

It is well known that *A. tamarense* is one of the most morphologically variable species of the genus *Alexandrium* (Balech 1995). Cultured and wild cells of *A. tamarense* from the different study regions along the Pacific coast of Russia demonstrate high variability of several morphological characteristics. Variations of both general characteristics such as the shape of the cell, L/W ratio, degree development of sulcal list, number of cells per chain and peculiar characteristics such as plates Po, 1′, S.a., S.p. and 2′′′′ were observed. The most conservative feature for both wild and cultured cells was the presence of a ventral pore. This is the most reliable criterion for the distinction between *A. tamarense* and the other two closely related species *A. catenella* and *A. fundyense* (Taylor 1975, 1984; Balech 1985, 1995; Fukuyo 1985; Fukuyo *et al.* 1985). Nevertheless, relatively high numbers of cells lacking the ventral pore were observed for isolate ATRU28 from region II (station 6). This fact, together with the shape of cells and the morphological features of the Po, S.a., S.p. and 2′′′′ plates of this isolate, allows us to assign it to *A. fundyense*. However, Balech (1995) notes that these features were also observed in cultures of *A. tamarense* as well. In addition, 25% of the cells from isolate ATRU28 had a ventral pore. Unfortunately, we had no plankton samples from station 6 (region II). However, morphologically similar cells were found in plankton samples from Avachinskaya Guba Inlet, but unlike the cultured cells of isolate ATRU28, always with a ventral pore. Moreover, cells of isolate ATRU28 were frequently seen in four-celled chains. This feature is not described in the literature for *A. fundyense*, but it is repeatedly observed in cultures of this species by one of the authors of this paper (D. Kulis, personal communication). Thus, this isolate exhibited features consistent with both *A. tamarense* and *A. fundyense*. However, we believe that the morphology of this culture is most consistent with *A. tamarense*.

The simultaneous existence in culture and in wild populations of cells with and without a ventral pore has been described for *A. minutum* (Hansen *et al.* 2003), *A. catenella* (Kim *et al.* 2002), *A. taylori* (Delgado *et al.* 1997), and to a limited extent, *A. fundyense* and *A. tamarense* (Anderson *et al.* 1994). Loeblich & Loeblich III (1975) studied two cultures of *A. tamarense* from the Tamar River, one with a pore and the other without it. Discussing the differences between *A. tamarense* and *A. catenella*, Taylor (1984) noted that in some cases the pore can be absent in the former species and be present in the latter. Thus, our study confirms recent doubt as to the validity of the ventral pore as a specific characteristic (Yoshida & Fukuyo 2000; Kim *et al.* 2002).

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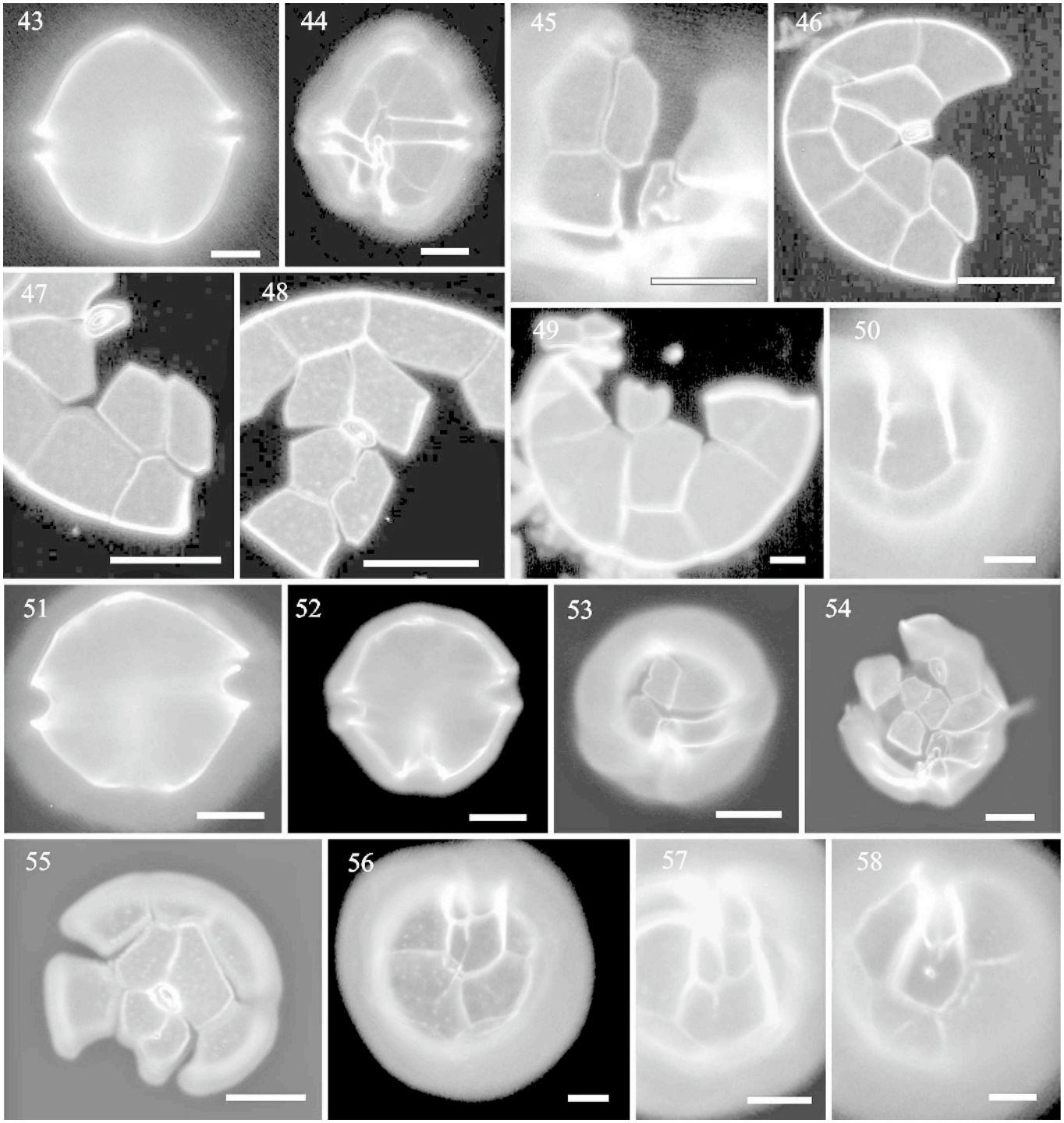
Fig. 40. Antapical views showing S.p. plate and intermediate-type 2′′′′ plate.
Fig. 41. Antapical view, showing elongate S.p. plate.
Fig. 42. Ellipsoidal cyst.



Figs 43–58. *Alexandrium tamarense* from the Russian east coast. Figs 43–58, light microscopy (epifluorescence using calcofluor white). All scale bars = 10 μm.

Figs 43–50. *Alexandrium tamarense* from clone ATRU28.

Fig. 43. General view of the cell.
Fig. 44. Ventral view, showing S.a. plate penetrating into the epitheca.
Fig. 45. Ventral view, showing S.a. plate penetrating into the epitheca and 1′ plate without a pore.
Figs 46, 47. Squashed epithecal plates, showing 1′ plates without a pore.
Fig. 48. S squashed epithecal plates, showing 1′ plate with a pore.
Fig. 49. Hypothecal plates.
Fig. 50. Posterior sulcal plate (S.p.).

Figs 51–58. *Alexandrium tamarense* from clone ATRU27.

Figs 51, 52. General view of cells.
Fig. 53. Ventral view, showing wide 1′ plate.
Fig. 54. Ventral view, showing epithecal plates.

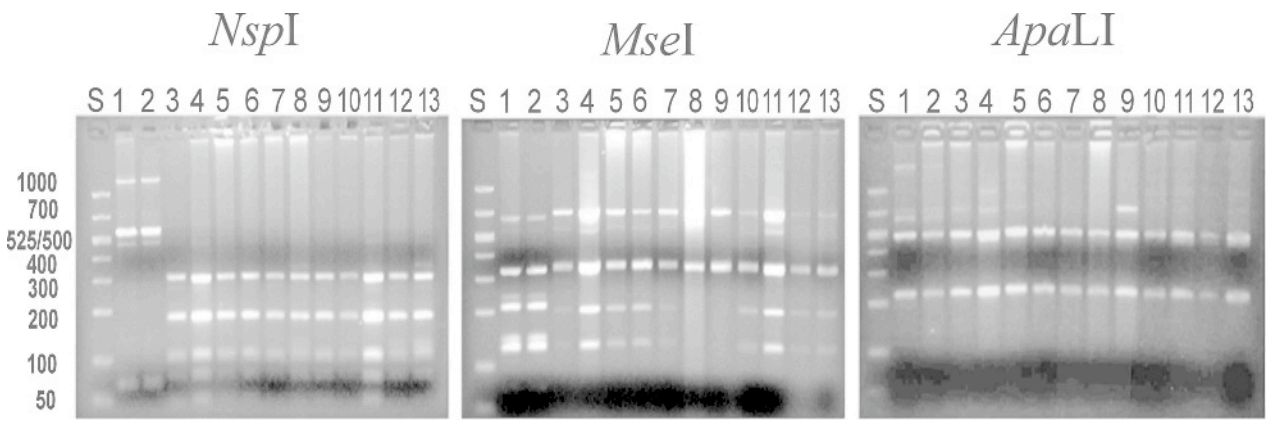


Fig. 59. RFLP patterns of selected *A. tamarense* isolates from Russian waters. Fragments were generated with the enzymes *Nsp*I, *Mse*I, and *Apa*LI. Lane designations are as follows: S, molecular weight standards; 1, ATRU11 (Primorye, station 4); 2, ATRU14 (Primorye, station 2); 3, ATRU17 (southern Sakhalin, station 5); 4, ATRU18 (southern Sakhalin, station 5); 5, ATRU20 (southern Sakhalin, station 5); 6, ATRU24 (southern Sakhalin, station 5); 7, ATRU27 (southern Sakhalin, station 6); 8, ATRU21 (Avachinskaya Guba Inlet, station 7); 9, ATRU22 (Avachinskaya Guba Inlet, station 7); 10, ATRU5/1 (west coast of the Bering Sea, station 6); 11, ATRU6/1 (Olytorskii Bay, station 8); 12, ATRU9/1 (Chukotka, station 11); 13, ATRU10 (west coast of the Bering Sea, station 10).

In cells of the isolate ATRU27, the pore on the S.p. plate is connected with the sulcus. This is not typical of *A. tamarense*, but was observed in *A. tamarense* from Limfjord in Danish waters (Moestrup & Hansen 1988).

One of the criteria for the distinction between *A. tamarense* and *A. catenella* is the number of cells per chain. *Alexandrium tamarense* can form short two- to four-cell chains, as opposed to *A. catenella*, which is known as chain-forming species with eight- and even 16-cell chains being common (Fukuyo *et al.* 1985). Eight-cell chains were observed for isolates of *A. tamarense* from Primorye (ATRU11–ATRU16) and Sakhalin (ATRU17–ATRU26) but only in tissue culture plate wells within 5–10 days after cyst germination. These were short-lived chains that easily broke apart. After transferring these *A. tamarense* isolates to 50-ml tubes for routine culturing, only two- and four-cell chains were observed thereafter. Given the ephemeral nature of the eight-cell chains observed with these *A. tamarense* isolates, we do not feel that this should confound the species designation we have made. On the other hand, the presence of numerous, long-lasting eight- and 16-cell chains in *A. catenella* indicates that this character can be a diagnostic criterion for that species. Overall, the morphology of cultured and wild cells of *A. tamarense* from the Pacific coast of Russia, despite large variation, agrees with *A. tamarense* by Balech (1995).

All cells from field samples and clones can be divided into two cell types: short (isodiametrical or wide) and tall. Each cell type exhibits specific features of tabulation, mainly the shape of the S.p. and 2′′′′ plates, and each was dominant in particular regions of study. The morphological differences between cells from different regions of the Russian Pacific coast occurred both in nature and in cultures, being, however, less pronounced in cultures. Apparently, the shape of S.p. and 2′′′′ plates was related to the general shape of the cell. This relation was clearly visible in wild cells, whereas in cultures it was lost because of the larger variety of forms.

Toxins

Of the eight distinct toxin profiles observed in the 25 cultures examined in this study, four were found within the Bering Sea (regions IV–VI), two from southern Sakhalin (region II), and single profiles were found in cultures from Avachinskaya Guba Inlet (region III) and Primorye (region I) (Table 3, Fig. 60). The toxin composition patterns for isolates from a particular region were unique and conserved save for one culture each established from regions II and V where two profiles emerged that were different from the other cultures analyzed from those areas.

In region V, culture ATRU1/1 had a profile dominated by C1,2 toxins (58%), which is similar to the toxin profile patterns observed in the other five regions. In cultures ATRU2/1, 3/1, 4/1, 5/1 and 5/2, also established from region V, the dominant toxin was GTX5, which accounted for 52% of the total toxin in these cultures on an average mole percentage basis. These cultures only produced trace amounts of toxins C1,2. Another atypical attribute of these five isolates is that they had a relatively high concentration of dcSTX, about 8% on average, whereas only trace amounts of this congener were detected in just three other cultures: ATRU1/1, 6/1 and 6/2. Moreover, ATRU2/1, 3/1, 4/1, 5/1 and 5/2 were devoid of all other gonyautoxins aside from GTX5 and had a high percentage of STX (27%) and moderate amounts of NEO (13%). ATRU1/1, in contrast, had moderate percentages of GTX1,4 and GTX2,3 (13%) and lesser amounts of GTX5 (4%), NEO (7%), STX 5% and only a trace amount of dcSTX (< 1%). Hence, five of the

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Fig. 55. Apical view, showing epithecal plates.
Figs 56, 57. Connection of S.p. plate connecting pore with sulcus.
Fig. 58. S.p. plate with a central connecting pore.

Table 3. HPLC analysis of Russian *Alexandrium* extracts

		Toxin										
Strain		C1	C2	GTX1	GTX2	GTX3	GTX4	GTX5	NEO	dcSTX	STX	TOTAL
ATRU1/1	Average fmol/cell^{-1}	0.54	17.23	0.21	0.16	3.98	3.88	1.18	2.18	0.18	1.57	31.10
	(Std. dev.)	(0.50)	(0.97)	(0.08)	(0.03)	(0.26)	(1.32)	(0.58)	(0.94)	(0.02)	(0.11)	(2.79)
	Average fgSTX/cell^{-1}	1.87	947.34	121.21	32.76	1448.34	1606.93	43.26	1149.21	53.42	896.78	6734.93
	(Std. dev.)	(1.71)	(53.46)	(45.20)	(5.24)	(94.98)	(548.84)	(21.30)	(495.03)	(4.67)	(60.53)	(1039.82)
	Average mol%	1.62	55.96	0.67	0.52	12.85	12.22	3.65	6.82	0.59	5.10	100.00
	(Std. dev.)	(1.38)	(6.62)	(0.20)	(0.11)	(1.10)	(3.07)	(1.46)	(2.33)	(0.06)	(0.60)	
	Average combined epimer pair%[1]	57.58		12.89	13.37			3.65	6.82	0.59	5.10	100.00
	(Std. dev.)	(6.76)		(3.08)	(1.10)			(1.46)	(2.33)	(0.06)	(0.60)	
ATRU2/1	Average fmol/cell^{-1}	0.00	0.08	0.00	0.00	0.00	0.00	36.06	8.95	5.92	18.78	69.78
ATRU3/1	(Std. dev.)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(1.99)	(0.83)	(0.41)	(1.30)	(3.94)
ATRU4/1	Average gSTX/cell^{-1}	0.00	4.47	0.00	0.00	0.00	0.00	1326.83	4722.81	1734.13	10,725.84	18,455.08
ATRU5/1	(Std. dev.)	(0.00)	(0.29)	(0.00)	(0.00)	(0.00)	(0.00)	(73.38)	(438.07)	(119.24)	(740.62)	(1084.96)
ATRU5/2	Average mol%	0.00	0.12	0.00	0.00	0.00	0.00	51.90	12.83	8.43	26.72	100.00
	(Std. dev.)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.73)	(1.14)	(0.35)	(0.74)	
	Average combined epimer pair%[1]	0.12		0.00	0.00			51.90	12.83	8.43	26.72	100.00
	(Std. dev.)	(0.01)		(0.00)	(0.00)			(0.73)	(1.14)	(0.35)	(0.74)	
ATRU6/1	Average fmol/cell^{-1}	0.97	64.72	0.03	0.32	13.74	6.14	8.97	18.48	0.63	57.37	171.37
ATRU6/2	(Std. dev.)	(0.61)	(11.95)	(0.02)	(0.05)	(0.98)	(0.45)	(0.78)	(1.46)	(0.17)	(2.84)	(17.18)
	Average fgSTX/cell^{-1}	3.33	3557.84	15.57	64.91	5006.57	2548.20	330.22	9755.71	185.42	32,760.71	54,641.16
	(Std. dev.)	(2.12)	(657.06)	(11.28)	(9.76)	(357.18)	(187.98)	(28.81)	(770.25)	(51.19)	(1622.45)	(3051.51)
	Average mol%	0.54	37.11	0.01	0.19	8.11	3.62	5.25	10.96	0.37	33.85	100.00
	(Std. dev.)	(0.32)	(2.94)	(0.01)	(0.03)	(0.47)	(0.34)	(0.23)	(1.01)	(0.11)	(1.74)	
	Average combined epimer pair%[1]	37.65		3.63	8.29			5.25	10.96	0.37	33.85	100.00
	(Std. dev.)	(2.97)		(0.34)	(0.48)			(0.23)	(1.01)	(0.11)	(1.74)	
ATRU9/1	Average fmol/cell^{-1}	0.73	19.44	0.00	0.00	8.29	3.60	7.95	3.01	0.00	3.72	46.73
	(Std. dev.)	(0.08)	(1.51)	(0.00)	(0.00)	(0.53)	(0.54)	(1.94)	(0.17)	(0.00)	(0.21)	(4.55)
	Average fgSTX/cell^{-1}	2.52	1068.44	0.00	0.00	3018.74	1491.46	292.42	1590.00	0.00	2127.27	9590.85
	(Std. dev.)	(0.26)	(83.19)	(0.00)	(0.00)	(191.78)	(222.46)	(71.21)	(90.10)	(0.00)	(119.64)	(539.36)
	Average mol%	1.56	41.67	0.00	0.00	17.79	7.66	16.76	6.47	0.00	8.09	100.00
	(Std. dev.)	(0.01)	(0.82)	(0.00)	(0.00)	(0.60)	(0.40)	(2.51)	(0.26)	(0.00)	(1.24)	
	Average combined epimer pair%[1]	43.23		7.66	17.79			16.76	6.47	0.00	8.09	100.00
	(Std. dev.)	(0.82)		(0.40)	(0.60)			(2.51)	(0.26)	(0.00)	(1.24)	
ATRU11	Average fmol/cell^{-1}	0.05	14.78	0.00	0.00	0.15	0.00	7.85	12.56	0.00	0.77	36.22
ATRU12	(Std. dev.)	(0.01)	(1.07)	(0.00)	(0.00)	(0.06)	(0.,00)	(0.75)	(0.75)	(0.00)	(0.15)	(2.32)
ATRU13	Average fgSTX/cell^{-1}	0.18	812.70	0.00	0.00	55.06	1.98	288.97	6631.22	0.00	441.66	8233.33
ATRU14	(Std. dev.)	(0.,04)	(58.61)	(0.00)	(0.00)	(21.88)	(1.98)	(27.52)	(398.13)	(0.00)	(87.75)	(397.30)
ATRU15	Average mol%	0.15	41.41	0.00	0.00	0.38	0.01	23.61	32.65	0.00	1.69	100.00
ATRU16	(Std. dev.)	(0.04)	(0.81)	(0.00)	(0.00)	(0.13)	(0.01)	(0.79)	(0.85)	(0.00)	(0.44)	
	Average combined epimer pair%[1]	41.57		0.01	0.38			23.61	32.65	0.00	1.69	100.00
	(Std. dev.)	(0.84)		(0.01)	(0.13)			(0.79)	(0.85)	(0.00)	(0.44)	
ATRU17	Average fmol/cell^{-1}	0.48	50.80	0.05	0.00	1.81	6.66	0.00	8.22	0.00	2.80	71.02
ATRU18	(Std. dev.)	(0.14)	(8.11)	(0.01)	(0.00)	(0.13)	(0.28)	(0.00)	(0.52)	(0.00)	(0.21)	(9.04)
ATRU20	Average fgSTX/cell^{-1}	1.67	2792.60	29.45	0.00	658.58	2763.78	0.00	4341.10	1.26	1600.02	12,225.69
ATRU25	(Std. dev.)	(0.48)	(446.03)	(4.17)	(0.00)	(45.87)	(115.26)	(0.00)	(276.70)	(1.26)	(119.06)	(835.65)
ATRU26	Average mol%	0.71	70.68	0.07	0.00	2.78	9.40	0.00	12.30	0.02	3.78	100.00
ATRU27	(Std. dev.)	(0.14)	(2.93)	(0.01)	(0.00)	(0.55)	(0.76)	(0.00)	(1.43)	(0.02)	(0.48)	

Table 3. Continued

			Toxin								
ATRU28	Average combined epimer pair%[1]	71.38	9.47	2.78		0.00	12.30	0.02	3.78	100.00	
	(Std. dev.)	(2.95)	(0.76)	(0.55)		(0.00)	(1.43)	(0.02)	(0.48)		
ATRU21	Average fmol/cell^{-1}	0.12	24.15	0.00	0.26	13.73	1.63	5.26	0.35	0.03	0.13	45.66	
ATRU22	(Std. dev.)	(0.01)	(0.94)	(0.00)	(0.03)	(2.27)	(0.09)	(0.23)	(0.07)	(0.03)	(0.10)	(2.16)	
	Average fgSTX/cell^{-1}	0.43	1327.76	0.00	54.24	5003.13	673.96	193.49	184.68	7.79	72.20	7517.67	
	(Std. dev.)	(0.03)	(51.59)	(0.00)	(6.02)	(828.47)	(38.44)	(8.53)	(37.92)	(7.79)	(58.51)	(781.68)	
	Average mol%	0.25	46.76	0.00	0.72	34.27	6.00	10.47	1.22	0.04	0.26	100.00	
	(Std. dev.)	(0.02)	(1.63)	(0.00)	(0.09)	(2.74)	(0.26)	(0.50)	(0.21)	(0.04)	(0.22)		
	Average combined epimer pair%[1]	47.02	6.00	34.99				10.47	1.22	0.04	0.26	100.00	
	(Std. dev.)	(1.63)	(0.26)	(2.74)				(0.50)	(0.21)	(0.04)	(0.22)		
ATRU30	Average fmol/cell^{-1}	0.21	31.88	0.00	0.08	10.68	0.41	6.84	0.00	0.00	0.00	50.10	
	(Std. dev.)	(0.01)	(1.31)	(0.00)	(0.04)	(5.06)	(0.12)	(1.07)	(0.00)	(0.00)	(0.00)	(7.51)	
	Average fgSTX/cell^{-1}	0.72	1752.64	0.00	17.07	3889.55	169.96	251.58	0.00	0.00	0.00	6081.52	
	(Std. dev.)	(0.05)	(72.09)	(0.00)	(8.56)	(1844.84)	(47.99)	(39.56)	(0.00)	(0.00)	(0.00)	(1995.88)	
	Average mol%	0.43	64.71	0.00	0.18	20.25	0.80	13.63	0.00	0.00	0.00	100.00	
	(Std. dev.)	(0.09)	(7.08)	(0.00)	(0.11)	(7.07)	(0.11)	(0.10)	(0.00)	(0.00)	(0.00)		
	Average combined epimer pair%[1]	65.13		0.80	20.43			13.63	0.00	0.00	0.00	100.00	
	(Std. dev.)	(7.08)		(0.11)	(7.07)			(0.10)	(0.00)	(0.00)	(0.00)		

[1] Epimer pair toxins are: C1 + C2, C3 + C4, GTX1 + GTX4 and GTX2 + GTX3.

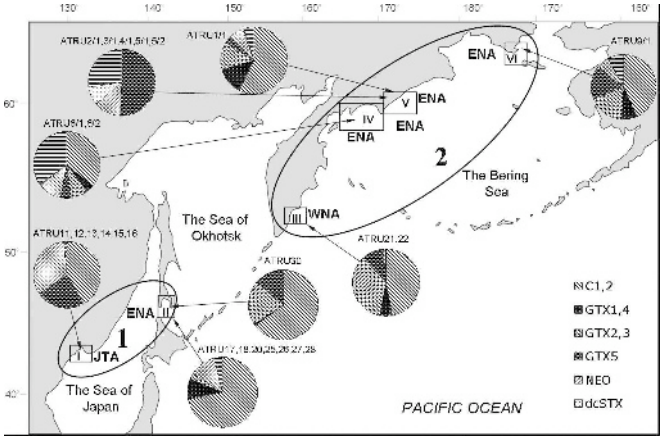

Fig. 60. Morphogenetic and toxin composition characteristics of *Alexandrium tamarense* from the Russian Pacific coast. Morphogroups: 1, 'short cells', 2, 'tall cells'. Ribotypes: JTA, Japanese temperate Asian; ENA, eastern North American, WNA, western North American, isolates; toxin composition, expressed as mol% of the total toxins in isolates.

cultures from region V (ATRU2/1, 3/1, 4/1, 5/1 and 5/2) are most distinct in regard to their toxin composition when compared with all the other cultures analyzed in this study.

In region II, the variation between the toxin profiles of ATRU30 and ATRU17, 18, 20, 25, 26 27 and 28 was not as dramatic as that seen between region V isolates. Here, ATRU30 produced moderate amounts of GTX5 (14%) and did not produce any NEO or STX. The other isolates from this region were found to have around 12% NEO and 4% STX. All of these cultures had high levels of C toxins – 65% for ATRU30 and 71% for the other isolates. In addition, all cultures produced GTX1,4 and GTX2,3 but in significantly differing percentages.

When the entire study region of the Russian Pacific coast is considered, it is evident that there is a heterogeneous population with regard to toxin composition. However, regions II and V cultures notwithstanding, the general trend toward a unique and conserved toxin pattern was witnessed in all the isolates from a specific region, and this aligns well with observations from other studies that suggest that a culture's toxin profile is a relatively stable trait for laboratory isolates from a specific geographic area (Hall 1982; Boyer *et al.* 1987; Cembella *et al.* 1987; Ogata *et al.* 1987; Anderson *et al.* 1994). However, if more cultures were established and tested from the other five regions it is possible that additional deviations from this pattern may exist on the basis of the results from regions II and V.

The majority of the cultures analyzed in this study produced moderate concentrations of saxitoxin and its congeners, resulting in moderate cellular toxicity values compared with other *A. tamarense* cultures analyzed in previous studies (Maranda *et al.* 1985; Cembella *et al.* 1987, 1988; Kim *et al.* 1993; Anderson *et al.* 1994; Mendez *et al.* 2001; Persich *et al.* 2006). The variation in toxin content for isolates within a given region was minimal. Total toxin per cell ranged from a low of 31 fmol cell^{-1} to a high of 71 fmol cell^{-1} in 22 of 25 cultures analyzed. When these cellular concentrations are converted into potency values, they span a range of 6,081 fg STX equiv. cell^{-1} to 12,226 fg STX

equiv. cell^{-1}. However, as the data reveal, the lowest or highest cellular concentration does not necessarily equate to the lowest or highest potency as the toxicity conversion is dependent upon both the potency and concentration of the derivatives produced by an isolate. The potency of the various saxitoxin congeners can vary by nearly two orders of magnitude, with sulfamate toxins such as C1,2 and GTX5 having the lowest potency and carbamate forms like GTX1, NEO and STX having the highest (Hall 1982). Clones ATRU6/1 and 6/2 from the Bering Sea produced almost 2.5 times more toxin (175.8 fmol cell^{-1}) and were 2.8 times more toxic (55,188.6 fg STX equiv. cell^{-1}) than any of the other clones screened in this study. These two cultures are amongst the most toxic *A. tamarense* cultures reported in the literature. Only laboratory cultures from Brazil and the St. Lawrence estuary (Anderson *et al*. 1990; Persich *et al.* 2006) have higher values.

In contrast to the toxin composition data where eight toxin profiles were found, there are only three distinct RFLP patterns (see below) and two morphological phenotypes for all 25 isolates. It is noteworthy that while strains of the same ribotype and morphotype display multiple toxin profiles, isolates with a given toxin profiles are all of the same ribotype and morphotypes.

LSU rDNA RFLP data

Cultures derived from sites along the Russian Pacific coast displayed the three different RFLP profiles defined in Scholin & Anderson (1996). Previously, the WNA ribotype had been found in southern Alaska and one isolate from Kamchatka; some strains from Japan also showed the WNA ribotype while others had the JTA ribotype (Scholin *et al*. 1995; Scholin & Anderson 1996). Our finding of the JTA ribotype in Primorye correlates well with its presence in Japan, given the geographic closeness of these regions and the heavy shipping traffic between Primorye and ports in Japan. However, given this exchange, it is curious that none of our isolates from Primorye displayed the WNA ribotype that is also found in Japan (Scholin & Anderson 1996).

Scholin & Anderson (1996) had originally theorized that the WNA ribotype present in Japan's Ofunato Bay had been introduced from North American ports. Subsequently, when a single isolate from Kamchatka was discovered with the WNA ribotype, a new theory was needed in which the WNA ribotype spread throughout the rim of the northern Pacific Ocean via natural dispersal, moving from Alaska to Kamchatka to Japan. Here, we have confirmed that the single strain from Kamchatka showing the WNA ribotype is representative of the *Alexandrium* populations of Kamchatka, as all of the isolates we tested from this region also displayed this pattern. We also have documented, however, a population of the ENA ribotype on Sakhalin Island, located between Kamchatka and northern Japan, where we would expect to have uncovered either the WNA ribotype or a mixture of this ribotype and the JTA ribotype.

The isolates from Sakhalin are not the only ones in the region to display the ENA ribotype, as this was previously found in Korea (Lilly 2003), and we have shown that it is present in the Bering Sea. Similarly to the WNA ribotype in Japan, the ENA ribotype was thought to have been introduced by anthropogenic means into Korea. However, with its presence in the Bering Sea and the Sea of Okhotsk, we feel that a better explanation is that the distributional patterns of *Alexandrium* in the northern Pacific Ocean may have been caused by natural current patterns.

It is possible that the ENA population of *A. tamarense* is distributed throughout the Arctic, a supposition that we are presently exploring. *Alexandrium tamarense* is known for its presence in Norwegian and Barents seas and the northern coast of Alaska (Okolodkov 2005). A continuous population spanning the Arctic Ocean would have southern representatives in areas where the ENA ribotype has been documented, including the Bering Sea, the Sea of Okhotsk, Korea (Lilly 2003), eastern North America (Scholin & Anderson 1994; Scholin *et al*. 1995), and the Orkney Islands (Medlin *et al.* 1998).

The presence of the WNA ribotype around the Pacific Rim can be explained by divergence of *A. tamarense* complex cells in western North America. This population may have spread along the coastline through Alaska, and then moved southward to Kamchatka and Japan where this ribotype augmented the ENA ribotype. The main hydrographic similarity between the Bering Sea and the Sea of Okhotsk is their relative isolation, and this feature may have preserved populations of the ENA ribotype in these regions.

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