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Prediction of on-site depuration of paralytic shellfish poisoning toxins accumulated in the scallop *Patinopecten yessoensis* of Ofunato Bay, Japan

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Abstract In this paper, we present a decay equation for paralytic shellfish poisoning (PSP) toxins using long-term field data spanning more than 10 years in order to predict the detoxification period for the scallop *Patinopecten yessoensis* in Ofunato Bay, Japan. From the data, we obtained the date of maximum toxicity in the digestive gland (DG) of the scallop and then the date of detoxification. Next, we performed linear regression analysis between $\log_e$-toxicities and days after the maximum toxicity level for each study year. Toxicity declined at a rate of 1.5 %/day, and a period of 3–9 months was required for the scallop to achieve a toxicity level of 20 mouse units (MU)/g DG tissue, which is a critical level for determining monitoring frequency and area of scallop toxicity. We then estimated the number of days needed to reach 20 MU/g DG tissue (t_{20}) using the equations obtained by the above-mentioned analysis, and we performed another linear regression analysis between the $\log_e$ (maximum toxicity) and t_{20} for each year. The difference between the actual and predicted detoxification time ranged from −16 to 18 days. We conclude that these equations can be used to predict the depuration of PSP toxins from scallops in Ofunato Bay.

Keywords Detoxification rate equation · Dinoflagellate · Scallop detoxification · Paralytic shellfish poisoning

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Introduction

Bivalves exposed to blooms producing paralytic shellfish poisoning (PSP) toxins vary markedly in their ability to detoxify these accumulated toxins [1]. A field survey along the Pacific coast of northern Japan revealed that Yesso giant scallops *Patinopecten yessoensis* retained high levels of PSP toxins for a longer duration than the ascidians *Halocynthia roretzi*, blue mussels *Mytilus galloprovincialis*, or Pacific oysters *Crassostrea gigas* [2]. Scallop toxicity and the abundance of toxic dinoflagellates have been monitored in Ofunato Bay, Iwate Prefecture [3–7], since the occurrence of shellfish poisoning in 1961. While studies have shown accumulated PSP toxins in *P. yessoensis* through ingestion of toxic *Alexandrium tamarense* and *Alexandrium catenella* [4, 8, 9], our previous study showed little depletion of the high levels of these toxins in the scallop [4]. After the Great East Japan Earthquake in 2011, accumulation of PSP toxins through the ingestion of *A. tamarense* was reported in the mussel *M. galloprovincialis*, the scallop *P. yessoensis*, and other species in Ofunato Bay [10, 11]; in 2013, a study found that a period of 9 months was needed for PSP toxin levels in the scallops to decrease from the maximum to 20 MU/g digestive gland (DG) tissue [11]. In light of these issues, scallop farming is being replaced by oyster farming, owing to the lower toxicity levels and higher detoxification rates in oysters. In fact, in 2013, the production volume and production value of scallops in Ofunato Bay were 90 tons and 25 million yen, respectively, a reduction of 82 % (407 tons and 113 million yen) compared to those in 2009 [12, 13].

Elucidation of the detoxification kinetics of PSP toxins in the scallop will facilitate prediction of the detoxification period required to reach a level of 20 MU/g DG tissue and subsequent cost mitigation measures for scallop

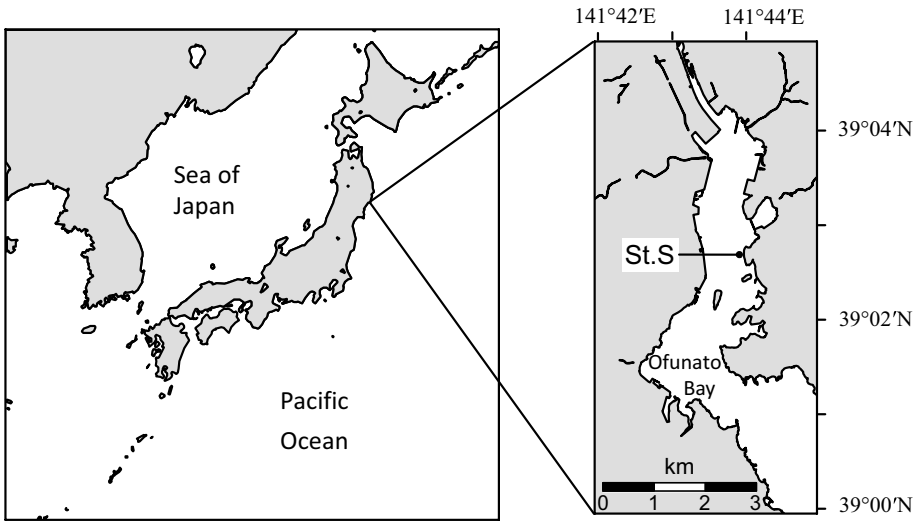

Fig. 1 Map showing the sampling station (St. S) in Ofunato Bay, Iwate Prefecture, Japan

farming. The production of scallops is more intensive than that of other bivalves in Japan, and determination of detoxification rates for scallops would be useful for local fishery cooperatives and fishermen. This information would allow them to calculate the length of closures of areas contaminated with PSP toxins. People engaged in scallop farming could then negotiate with their customers regarding shipment quantities after detoxification and would be able to achieve stable income by planning annual production levels.

It is well known that there are significant differences in detoxification rates among shellfish species [14], which may be affected by interspecific differences in detoxification capacity, such as metabolism (e.g., excretion) of toxins [1]. The rate of detoxification (percentage loss of toxins per day) has been calculated by fitting a negative exponential function of bivalve toxicity levels against time from data derived from laboratory or field studies [1]. Bricelj and Shumway [1] determined a detoxification rate of 1–4 %/day in the scallop *P. yessoensis* affected by the dinoflagellate *A. tamarense* in a field survey on the basis of Japanese reports [2, 4, 15, 16].

Toxins in the scallops accumulated by the ingestion of *A. tamarense* in feeding experiments likely undergo metabolic processes into derivatives that are undetectable by high-performance liquid chromatography (HPLC). For example, during a no-feeding period in a feeding experiment, some of the toxin supplied (the sum of the amount of toxins in the scallop and that released into the environmental water) seemed to disappear, and most of the supplied toxin was recovered thereafter [17]. This result indicates that decomposition of toxins in the scallop is unlikely, and suggests that the decrease in accumulated toxins is due to excretion of the toxins into the ambient seawater. It is probably for this reason that, to the best of our knowledge, no study has yet determined the detoxification rate of PSP toxins in scallops after ingestion of *A. tamarense* in the field.

In this work, we performed linear regression analysis between the $\log_e$-toxicities and number days after maximum toxic level in the scallop *P. yessoensis* in order to predict the duration of detoxification using long-term field data (1978–1988) for PSP toxins in Ofunato Bay. Our findings suggest that the equation obtained by the above-mentioned linear regression line is useful to predict the duration of scallop detoxification reaching under the waring level in Ofunato Bay.

Materials and methods

Sample collection and toxicity assay

A sampling station (23 m depth) was installed at the Shizu station (39.04°N, 141.73°E) in Ofunato Bay from May 1978 through December 1988 (Fig. 1). Water samples were collected one to four times per month at depth intervals of 2 m from the surface to the bottom (12 layers) for monitoring cell numbers of *Alexandrium* spp. A 500-ml sample of seawater was concentrated by gravity filtration using an 8-μm membrane filter (Millipore, type SC; EMD Millipore, Darmstadt, Germany). The concentrate was collected in aliquots of 20 or 40 ml, and each 1-ml portion was used for microscopic counting of *Alexandrium* spp. The *Alexandrium* spp. observed during the survey were identified as *A. tamarense* and *A. catenella* [4–6, 8]. Previous research found that the toxicity of *A. catenella* cells collected from Ofunato Bay as detected by mouse assay was significantly

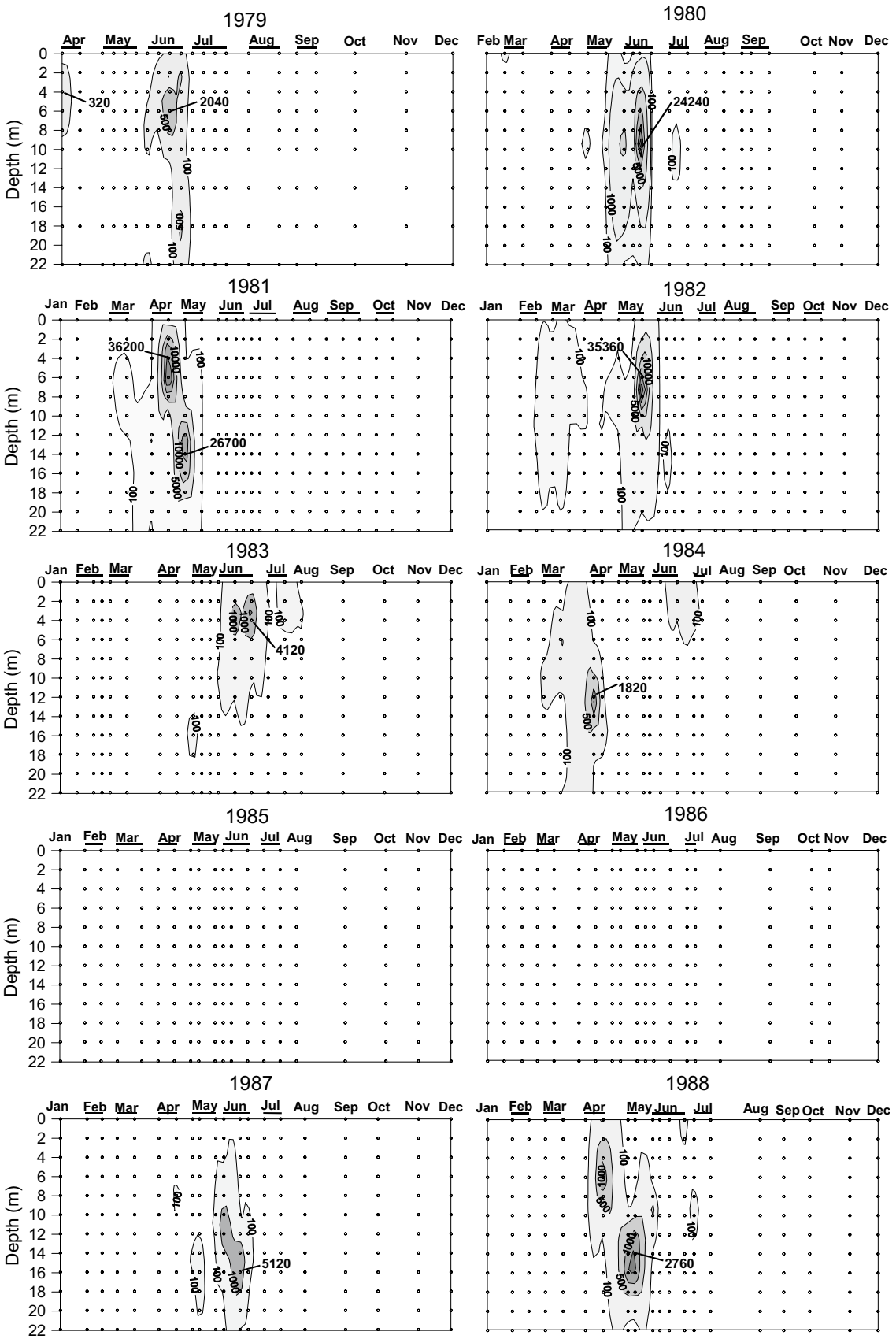

Fig. 2 Temporal change in vertical distribution of *Alexandrium tamarense* at St. S from 1979 to 1988. Cell numbers of *A. tamarense* indicate ≥100 cells/l

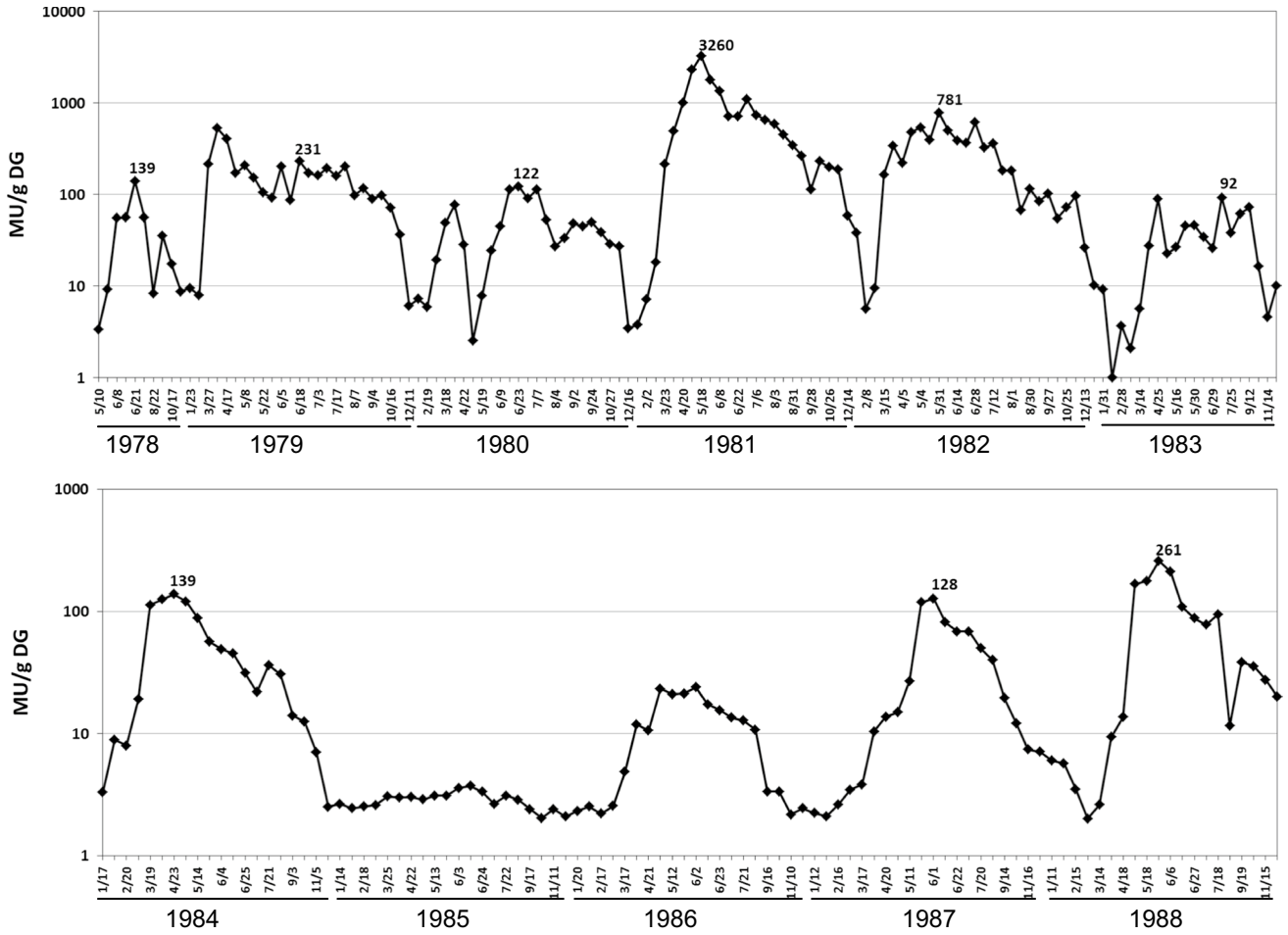

Fig. 3 Toxicity of digestive gland (DG) tissue in terms of mouse unit (MU)/g DG wet tissue weight in the scallop *P. yessoensis*. Maximum toxicity levels are shown for each year (in 1979, the final peak was adopted as the maximum)

lower (<0.005 MU/10^4 cells) than that of *A. tamarense* (0.5–2.6 MU/10^4 cells) [8]. In Ofunato Bay, *A. catenella* has been found to occur at the surface of the water column [4], where no cultured scallops were placed in this study. Therefore, the cell numbers of *A. tamarense* were adopted, although successful monitoring of *A. tamarense* was not possible in 1978 and 1979 (at depths of 12, 16, and 20 m). The nontoxic scallop specimens were transplanted to the station (at a depth of approximately 10 m) from February to March each year where *A. tamarense* had been reported to be most dense [4, 5].

Five scallop specimens that had been hung at a depth pf approximately 10 m were collected for toxicity tests within a few days before or after seawater sampling. The digestive glands were excised, combined, and measured for PSP toxins according to the Association of Official Analytical Chemists (AOAC) method at Ofunato Public Health Center (Iwate Prefecture). Field data in which the maximum toxicity during a year was <50 MU/g were excluded from data analysis, as this level is too low to trace changes in toxicity.

Data analysis

The time course of toxicity in each year was traced from the date of maximum toxicity to the date on which the toxicity reached a level below 20 MU/g. Twenty mouse units per gram of DG tissue is the critical level for the Iwate Prefecture monitoring program for PSP. Sampling frequency must be increased when toxicity in scallops exceeds this level [18]. We performed linear regression analysis between $\log_e$-toxicity and estimated days of decline to 20 MU/g DG tissue from the date showing the maximum toxicity (t_{20}) on the basis of parametric Pearson's correlation coefficient and non-parametric Spearman's correlation coefficient tests using the Statcel 2 software program (OMS Publishing, Tokyo, Japan). Normality was verified when necessary.

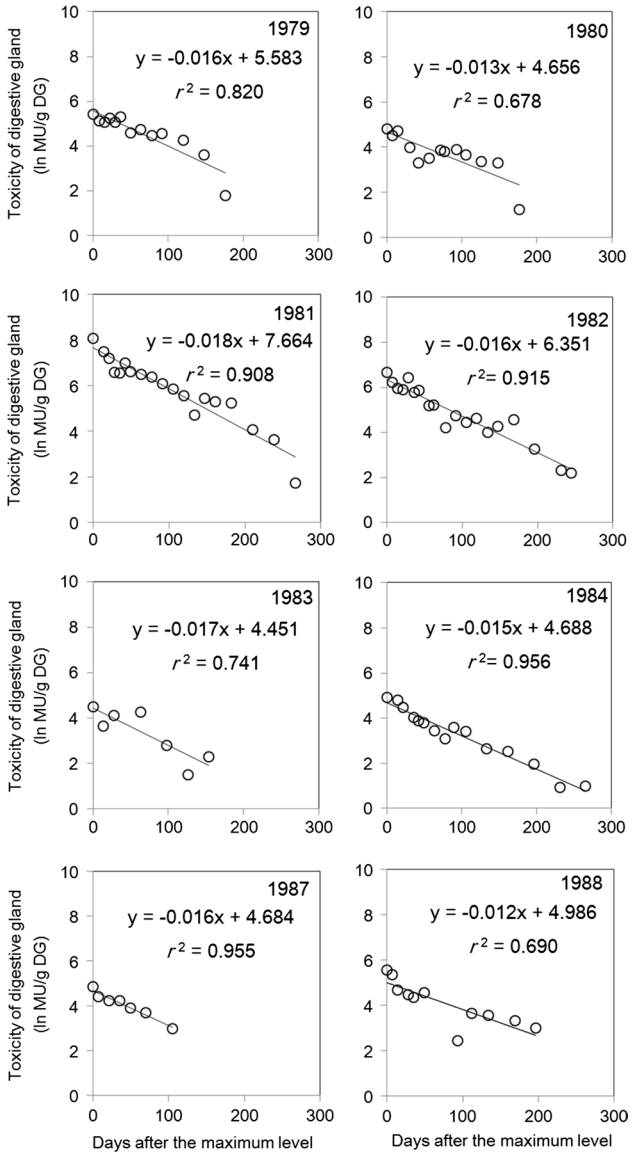

Fig. 4 The relationship between toxicity in digestive gland tissue (MU/g DG wet tissue weight) in the scallop *P. yessoensis* and number of days in detoxification period after maximum toxic level. Scallops were infected with the dinoflagellate *Alexandrium tamarense* in Ofunato Bay during eight sampling years. Linear regressions and their coefficients are shown

Results

Temporal change in vertical distribution of *A. tamarense*

Figure 2 displays contour graphs showing temporal changes in vertical distribution of *A. tamarense* from 1979 through 1988. Most of *A. tamarense* were distributed at depths of 4–16 m (excluding 1985 and 1986, when <100 cells/l were obtained) (Fig. 2). *A. tamarense* reached maximum abundance in April through June of each year. The peak bloom was followed by a sharp decrease. The maximum cell number (36,200 cells/l) was observed at a depth of 4 m in April 1981.

When *A. tamarense* appeared in high densities in Ofunato Bay from 1980 to 1984 (Fig. 2), environmental factors such as water temperature, salinity, concentration of chlorophyll *a*, and inorganic nutrient [nitrate (NO_3^-) and phosphate (PO_4^{3-})] concentrations were within ranges of 5–10 °C, 33.0–33.7, 0.7–8.5 μg/l, 0.3–5.9 μM, and 0.1–0.7 μM, respectively [5].

Preparation of the data set

Maximum toxicity of *P. yessoensis* was observed from April through July each year and ranged from 92 to 3260 MU/g DG tissue (excluding 1985 and 1986, when low toxicity levels were obtained) (Fig. 3). In most years, we observed a rapid decline in toxicity after maximum toxicity was attained (Fig. 3). The average (±SD) reduction in toxicity over 1 week was 23.8 ± 9.1 %. In 1979, two toxicity peaks appeared, in May and June, after the first peak (528 MU/g) in April, and we selected June 18 as the date of maximum toxicity (231 MU/g DG tissue) for this year. For the endpoint of the dataset, we selected the date showing a toxicity value < 20 MU/g DG tissue each year. We obtained 6–19 data pairs for $\log_e$-toxicity and days after the maximum toxic level in each of nine sampling years (1978–1984, 1987, and 1988).

Determination of significance for linear regression lines

There was a significant reciprocal relationship between $\log_e$-toxicity and number of days in the detoxification period after maximum toxic level in all study years except 1978 ($P < 0.05$) (Fig. 4; Table 1). Plots were abnormally distributed to some extent in 1979 and 1980, and Spearman's rank test was performed for these data showing significant correlations ($P < 0.05$) (Fig. 4; Table 1). No significant correlation was observed in 1978 (data not shown).

The coefficients obtained were high for all study years. In addition, the detoxification rate was almost constant among all study years (1.5 ± 0.2 %/day) (Fig. 4; Table 2). The actual period required for detoxification (t_{20}) from the date of maximum toxicity varied from 88 to 260 days (Table 3). There was a significant relationship between t_{20} (Fig. 5) and the $\log_e$ (maximum toxicity for each year), and the fitted linear equation was given by:

$$y = 0.020x + 2.464\,(r^2 = 0.949,\ P < 0.01)$$

where y is the $\log_e$ (maximum toxicity for each year) and x is detoxification time (t_{20}).

Table 1 Relationship between $\log_e$-toxicity and number of days in the detoxification period after maximum toxic level in all study years except 1978

Years	Number of data pairs (n)	Pearson's correlation coefficient (r)	Spearman's rank correlation coefficient (r_s)	Significance (P)
1978	6	−0.790	–	NS
1979	13	–	−0.918	*
1980	13	–	−0.808	**
1981	19	−0.953	–	***
1982	19	−0.957	–	***
1983	7	−0.861	–	*
1984	15	−0.978	–	***
1987	7	−0.977	–	***
1988	11	−0.830	–	**

NS not significant

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

*** $P < 0.001$

Table 2 Rates of detoxification in digestive gland tissue of the scallop *P. yessoensis* for each study year as determined by linear regression analysis with fitted linear equations

Years	a		Coefficient of determination (r^2)	Significance of regression coefficient (P)
	Regression coefficient	Detoxification rate (%/day)		
1979	−0.0158	1.58	0.82	**
1980	−0.0133	1.33	0.68	**
1981	−0.0180	1.80	0.91	**
1982	−0.0162	1.62	0.92	**
1983	−0.0165	1.65	0.74	*
1984	−0.0147	1.47	0.96	**
1987	−0.0157	1.57	0.95	**
1988	−0.0118	1.18	0.69	**

* $P \leq 0.05$

** $P \leq 0.01$

Table 3 Actual and predicted detoxification time indicating number of days required to achieve toxicity level of 20 MU/g digestive gland tissue from maximum toxicity for each sampling year

Years	Equation used: $y = ax + b$			Equation used: $y = 0.020x + 2.464$	$x_a - x_p$ Difference (days)
	a	b	x_a (actual days) $y = \ln(20)$	x_p (predicted days) $y = \ln$ (maximum toxicity)	
1979	−0.0158	5.583	164	146	18
1980	−0.0133	4.656	125	115	10
1981	−0.0180	7.664	260	276	−16
1982	−0.0162	6.351	207	206	1
1983	−0.0165	4.451	88	101	−13
1984	−0.0147	4.688	115	121	−6
1987	−0.0157	4.684	107	117	−10
1988	−0.0118	4.986	169	152	17

From this equation, the predicted detoxification time (x_p) varied from 101 to 276 days (Table 3; Fig. 5). The difference between actual and predicted detoxification duration ranged from −16 to 18 days (Table 3).

Discussion

Retoxification based on the presence of *A. tamarense* after maximum toxicity levels were reached in scallops

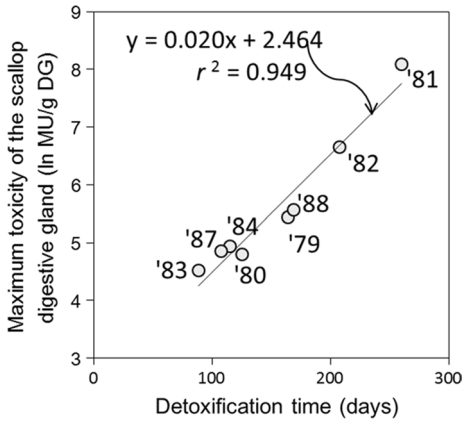

Fig. 5 Relationship between number of days in detoxification period required to achieve 20 MU/g digestive gland (DG) tissue from maximum toxic level (t_{20}) and $\log_e$-maximum toxicity for each sampling year. Detoxification periods for the scallop *P. yessoensis* infected with the dinoflagellate *Alexandrium tamarense* in Ofunato Bay, Japan, from1979 through 1988 (excluding 1985 and 1986) were used

was hardly observed when the abundance of *A. tamarense* decreased to < 100 cells/l after 2–5 weeks from the date on which maximum toxicity was observed (Figs. 2, 3).

Bricelj and Shumway [1] reported that a period of 3.75–15 months was necessary for detoxification of *A. tamarense*-contaminated Yesso giant scallops *P. yessoensis* to levels below the regulatory limit (0.8 μg STXeq or 4 MU/g DG tissue). In the present study, detoxification of PSP from maximum toxicity levels required approximately 3–9 months (Table 3). In Ofunato Bay, PSP toxin levels of >2000 MU/g DG tissue were observed in 1981, 1989, 1993 and 1998 [5, 6, 10]. Using our linear equation, it took approximately 9 months for toxin levels to drop to 20 MU/g DG tissue. This is consistent with our findings that the scallops in Ofunato Bay retained PSP toxin levels over 20 MU/g DG tissue for 3–9 months.

Our observed rates of detoxification (1.5 ± 0.2 %/day; Table 2; Fig. 4) for the scallops are also in agreement with the findings of Bricelj and Shumway [1], who calculated detoxification rates of 1–4 %/day (DG tissue). However, our rates are lower than those reported for the Pacific oyster *C. gigas* (38 %/day for DG and 31 %/day for whole tissue) [19, 20], the short-necked clam *Tapes japonica* (8 %/day for whole tissue) [21], the blue mussel *M. galloprovincialis* (*M. edulis*) (14 %/day for DG and 11 %/day for whole tissues) [1, 22], and the ascidian *H. roretzi* (3 %/day for hepatopancreas tissue) [2]. Thus, the DG of the scallop retains *A. tamarense*-induced PSP toxins longer than the DG of the oyster and the blue mussel and the hepatopancreas of the ascidian [2]. In feeding experiments with *A. tamarense*, PSP toxicity was retained longer in whole tissues of the king scallop *Pecten maximus* and the blue mussel than in those of the Pacific oyster or the short-necked clam *Ruditapes philippinarum* [23]. Oshima et al. [2] suggested that when frequent sampling is not practical, the scallop can provide a good index for predicting past contamination with PSP toxins.

Toxicity levels in the scallop declined rapidly after maximum toxicity was reached (Fig. 3), as observed previously by other researchers [2, 4, 22]. However, the rate of toxicity reduction 1 week after the maximum value (24 %) was lower in the present study than that observed by Suzuki et al. (47 %) [22]. In addition, maximum toxicity in our study was higher than that observed by Suzuki et al. (70 MU/g DG tissue) [22]. These differences suggest that lower maximum toxicity levels lead to more rapid detoxification. Therefore, linear equations developed using a time-dependent decay of toxicity values should be modified by the maximum toxicity values when estimating detoxification.

In conclusion, the linear regression equation obtained in the present study is useful for predicting the duration of detoxification in scallops in Ofunato Bay. Because the biphasic detoxification model (e.g., initial rapid decline and subsequent exponential decline in toxins) is widely accepted in describing the detoxification kinetic patterns in bivalves, further investigation incorporating this model will be needed to establish a more accurate and reasonable method for predicting detoxification in scallops.

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