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Paralytic phycotoxin uptake by scallops (*Pecten maximus*)

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Abstract

It is difficult to apply sanitary standards for mollusc contamination by paralytic shellfish poisoning to animals not consumed whole. This problem is illustrated by the 1990 embargo on a Japanese shipment of frozen scallop muscle and gonad: the sanitary threshold applied to digestive gland (in Japan) does not guarantee that muscle and especially gonad are toxin-free. Accordingly, we performed experimental contaminations of scallops (*Pecten maximus*) from Port-en-Bessin (Normandy, France) using a toxic Japanese strain of *Alexandrium tamarense*. During the contamination/decontamination experiment on different tissues (digestive gland, muscle, gonad), extracts were obtained using cold acetic acid 0.1 N to maintain the perfect integrity of the toxin profile. These initial trials indicated that with a daily concentration of 2×10^6 cells.l^{-1} a maximum toxicity equivalent to 850 µg.100 g^{-1} of digestive gland meat was attained in 2 weeks. Concerning toxin profiles, it would appear that gonad can selectively accumulate gonyautoxins, particularly GTX2/GTX3, even though the relative amount of uptake always follows the order hepatopancreas > gonad > muscle. Moreover, studies of Japanese scallops imported in 1990 showed that 80% of the gonad and muscle samples included all or part of the kidneys and excretory organs, whose relationship with gonad toxicity need further clarification. Finally, the toxin profile of the different organs during decontamination revealed an inversion of the relative proportions of GTX2/GTX3 epimers, as noted by other authors. Confirmation of selective biological conversion between organs will require further study.

Keywords: *Alexandrium tamarense*, *Pecten maximus*, *Patinopecten yessoensis*, phycotoxins, bioaccumulation, toxin profiles.

Bioaccumulation de phycotoxines paralysantes par la coquille Saint-Jacques (Pecten maximus).

Résumé

Les normes sanitaires appliquées à la contamination des bivalves par le poison paralysant des coquillages (PSP) sont difficilement applicables aux coquillages non consommés entiers. C'est le cas des coquilles Saint-Jacques, et l'embargo appliqué en 1990 sur les « containers » japonais contenant des noix et corails congelés, en est un exemple: le seuil sanitaire appliqué à la glande digestive (au Japon) ne garantit pas pour autant que le muscle, et surtout la gonade, soient indemnes de toxines. Pour cette raison nous avons pratiqué des contaminations expérimentales de coquilles (*Pecten maximus*) provenant de Port-en-Bessin (Normandie) par une souche japonaise toxique d'*Alexandrium tamarense*. Lors de l'expérience de bioaccumulation/épuration sur différents tissus (glande digestive, muscle, gonade) les extraits ont été réalisés par l'acide acétique 0,1 N à froid, ceci afin de garder une intégrité parfaite au profil toxinique. D'après ces premiers essais, il apparaît qu'avec une concentration journalière de 2.10^6 cellules.l^{-1} un maximum de toxicité équivalent à 850 µg.100 g^{-1} de chair de glande digestive est atteint en 15 jours. En ce qui concerne les profils toxiniques, il apparaît, d'une part, que la gonade peut accumuler sélectivement des gonyautoxines, en particulier GTX2 et GTX3, même si l'importance de la bioaccumulation suit toujours l'ordre: hépatopancréas > gonade > muscle.

D'autre part, les observations réalisées sur les coquilles japonaises importées en 1990 montrent que 80 % des échantillons gonades et muscles comportaient également les reins ou une partie des reins, organes d'élimination dont la relation avec la toxicité des gonades sera à vérifier. Enfin, le profil toxinique des différents organes en décontamination révèle une inversion des proportions relatives des épimères GTX2/GTX3, comme cela a été observé par d'autres auteurs. Une bioconversion sélective entre organes serait à confirmer ultérieurement.

Mots-clés : *Alexandrium tamarense*, *Pecten maximus*, *Patinopecten yessoensis*, phycotoxines, bioaccumulation, profils toxiniques.

INTRODUCTION

With the exception of two doubtful cases of paralytic shellfish poisoning (PSP) in 1907 and 1911 in the north (Field, 1922), no toxic event of this type was reported in France until 1987. Beginning in 1988, red tides caused by a slightly toxic microalga, *Alexandrium minutum*, were associated with notable contamination of mussels and oysters in North Brittany (Erard-Le Denn, 1991; Frémy and Ledoux, 1991; Frémy *et al.*, 1989; Nezan and Piclet, 1989; Nezan and Ledoux, 1989).

In addition to these recent, geographically limited events, another risk to French consumers is the exportation of shellfish contaminated by paralytic phycotoxins. In 1976, Spanish mussels contaminated by the dinoflagellate *Gymnodinium catenatum* were responsible for an outbreak of poisoning in all European countries receiving shipments: 38 cases in Italy, 19 in West Germany, 23 in Switzerland and 33 in France (including 17 at Marseille) (Estrada *et al.*, 1984; Lüthy *et al.*, 1978). In this event, the sanitary standards in effect in various European countries (threshold of 80 µg of saxitoxin.100 g^{-1} of shellfish meat) could be applied on the basis of mouse-test results (AOAC method, 1985). Estimation of toxicity was based on total mussel meat since the animals were consumed whole. However, when only certain organs of a shellfish are consumed (Pectinidae), the strict application of regulations is less relevant, particularly for imported products. This was the case in May 1990 when an embargo was ordered by the direction générale de l'alimentation (DGA), a French food control agency, against 44 containers of frozen scallops from Japan. Analyses performed by the CNEVA [1] and IFREMER [2] laboratories on batches of muscle and gonad samples showed paralytic phycotoxin concentrations close to or above the sanitary threshold.

The monitoring system in Japan is based on assessment of the toxicity of the digestive gland, considered to be the tissue concentrating the most toxin, and not on toxicity in each separate tissue. The monitoring system used for PSP in Hokkaido stipulates that scallop harvesting and processing are authorized when toxicity level in digestive gland decreases down to 150 mouse units.g^{-1}. (Hokkaido prefectural government, pers. comm.). Several studies have demonstrated low toxicity in *Patinopecten yessoensis* muscle, whereas the digestive gland or the mantle were highly contaminated (Maruyama *et al.*, 1983; Asakawa *et al.*, 1987; Shimizu and Yoshioka, 1980). Moreover, results concerning gonad toxicity are rather dissimilar, apparently because of the intrinsic toxicity of this organ and the possible existence of renal residues. The kidneys, which are anatomically adjacent to the gonad, have an elevated toxic potential (Oshima, pers. comm.). Unfortunately, these different studies do not take into account the biotransformation of toxins during contamination, their possible transfer between organs during depuration, storage conditions, possible interruption of refrigeration during preparation for shipment, etc.

In this context, we first carried out a study based on experimental contamination of molluscs by a toxicogenic strain of *Alexandrium tamarense* (Lassus *et al.*, 1989). These initial results encouraged us to undertake the studies reported here. After slight modification of the extraction protocol, we investigated (i) the influence of the kidneys in some batches of imported products on toxic concentrations; and (ii) the use of a toxicogenic strain of *A. tamarense* to assess toxin accumulation by different scallop organs (digestive gland, muscle, gonad).

METHODS

Kidney study

A phycotoxin salubrity monitoring system was set up by the DGA for batches of scallops (*Patinopecten yessoensis*) imported from Japan. These batches were composed either of gonad and muscle or gonad alone. During analyses of samples by the IFREMER laboratories in La Rochelle and Ouistreham, it was noted that kidneys were often partially destroyed or absent.

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For this reason, kidneys were sampled in several batches to determine their degree of integrity and their toxin concentration.

Experimental study

The toxic strain of *A. tamarense* was kindly provided by Dr. Oshima (University of Tohoku, Japan). Cultures were performed in 10−1 Pyrex flasks containing 6 to 8 l of natural aerated seawater (salinity = 30 $^{0}/_{00}$) filtered on Millipore membrane 0.22 µm and enriched in Provasoli E. S. medium (Provasoli, 1966). A cell concentration of 20×10^6 l^{-1} was thus obtained at 16°C in 10 days, with a 12/12 hours photoperiod and illumination of 50 ± 4 $\mu E.m^{-2}.s^{-1}$. The same medium and culture conditions were used for a non-toxic chlorophycea, *Tetraselmis suecica*, given to shellfish as food during the decontamination phase. The cell densities introduced each day into the experimental tanks containing the shellfish were respectively 2×10^6 cells l^{-1} for *A. tamarense* and 5×10^6 cells l^{-1} for *T. suecica*.

The molluscs (mature adults with 10 cm shells) were collected along the Normandy coasts (Port-en-Bessin). An acclimatization period of 5 to 8 days at 16°C preceded each experiment in order to eliminate weak individuals. Each experimental shellfish was placed alone in a tank containing 4 l of seawater which was changed daily before each introduction of algal culture. The contamination and decontamination phases lasted respectively 15 and 20 days.

Animals sampled during the experiment (two shellfishes each time) were dissected, and the tissues (gonad, digestive gland, muscle) were analyzed separately (four replicates) to assess their toxin concentration.

Chemical analyses

To evaluate the toxicity of separate tissues, cold acetic acid (CH_3COOH 0.1 M) extraction was performed to ensure the integrity of the toxin profile per organ and to detect toxin transfers more readily. One millilitre of acid extract was then transferred into an ultrafiltration system (MPS-1, Amicon Corp., Danvers, MA, USA) and centrifuged. Finally, 10 µl of supernatant was injected into the high-performance liquid chromatography (HPLC) system. The method used was that of Sullivan and Wekell (1984, 1986): paralytic phycotoxins were separated on a poly-(styrene-divinylbenzene) column (PRP-1, Hamilton and Co., Reno, NV, USA) using hexane and heptane sulfonates as ion pairing reagents. The HPLC system (655 A-12 LC pump, Merck L-5000 LC gradient controller) was equipped with a postcolumn reactor (Kratos URS-051) and a spectrofluorometer (Merck F-1050) with a xenon excitation source operating at 340 nm in excitation and at 400 nm in emission. The HPLC column was maintained at 35°C, and postcolumn reaction reaction with the periodic acid took place in a 1-ml reaction chamber at 90°C. Chromatograms were read on an integrator (Merck D 2000).

Paralytic phycotoxins were detected against a standard kindly provided by Dr. S. Hall (Food and Drug Administration, Washington, DC, USA), and the relative percentages of toxins were estimated by comparison with the maximum concentration obtained in 15 days.

RESULTS

The kidneys in samples of Japanese scallops did not show a similar state of integrity. Most muscle and gonad batches included both kidneys, whereas the kidneys in those containing gonad alone were either deteriorated or absent, probably as a result of handling methods before packing (*table* 1).

Table 1. – Presence and state of kidneys in muscle and gonad samples of *Patinopecten yessoensis* imported from Japan in May 1990.

	Batch types	
	Mixed (muscle + gonad)	Gonad alone
Presence of both kidneys	207	1
A single kidney	75	36
Fragments	62	84
Absence of kidney	5	124
Number of batches examined	349	245

During HPLC analyses of the different sample types (*table* 2), neosaxitoxin proved to be the major toxin in kidneys. There was a significant difference in neosaxitoxin concentrations between kidney fragments from gonads packed separately and whole kidneys in the gonad and muscle batches, the latter being 2 to 6 times as toxic and above the salubrity threshold.

These results could not be compared with the toxin profile (unknown in this case) of the contaminating algae. For this reason, we undertook an experimental contamination of scallops using the above-mentioned Japanese toxicogenic strain of *Alexandrium*.

The relative concentrations in the tissues studied (digestive gland, gonad, muscle) are indicated respectively in *figures* 1 *a*, *b* and *c*.

The accumulation of paralytic phycotoxins in digestive gland was low at day 7 of contamination and then increased sharply, reaching its maximum at day 15, with a predominance of GTX4 and GTX1. During depuration, these toxins decreased until

Table 2. – Concentrations of the different paralytic toxins ($\mu g.g^{-1}$) analyzed in kidneys of imported Japanese scallops. C = kidney fragments found in gonads packed separately; M = whole kidneys in gonad + muscle samples; ND = not detected. NEO = Neosaxitoxin, STX = Saxitoxin, GTX = Gonyautoxin, B = Paralytic toxins B.

Toxins							Kidney state
B2	GTX1	GTX2	GTX3	GTX4	NEO	STX	
2.24	ND	0.03	0.01	ND	2.43	0.03	C
ND	0.13	0.30	0.14	0.19	0.82	0.18	C
0.77	ND	ND	ND	ND	0.75	0.30	C
0.08	ND	0.01	0.01	ND	0.98	0.02	C
0.15	ND	0.23	0.15	ND	2.14	0.27	M
0.28	0.07	0.30	0.18	0.19	5.40	0.69	M
0.10	0.11	0.30	0.17	0.32	5.29	0.25	M
0.24	0.09	0.29	0.16	0.26	5.96	0.74	M
0.75	ND	0.17	0.10	ND	3.52	0.53	M
0.88	ND	0.22	0.11	ND	4.00	0.64	M
0.99	ND	0.26	0.13	ND	5.10	0.75	M

day 28 and then increased again at day 35, as did GTX3 and GTX2.

The study of gonad toxin profiles shows two interesting points: the predominance of GTX2 and GTX3 and the existence of higher toxin concentrations during decontamination than those obtained at the end of the contamination phase. Moreover, there was gradual increase in saxitoxin (STX) concentrations during depuration.

Finally, the toxin composition of muscle confirmed a low contamination (*table* 3) observable only at day 15 of the contamination phase, but with a dominance of GTX2/GTX3 as compared to that of GTX2 during the decontamination phase.

The study of total toxin quantities accumulated in each organ (expressed in equivalent STX) (*table* 3) confirms the regularity of the relative percentages and indicates both the strong uptake capacity of digestive gland and the high concentrations found in gonad during depuration.

DISCUSSION AND CONCLUSION

As indicated in our previous study (Lassus *et al.*, 1989), warm acid extraction tends to convert the toxins present into more active components (C toxins → GTX1 to GTX4). The present experiment performed with cold extraction conserved the normal true toxin profile in each tissue. During experimentation, GTX2 and GTX3 accounted for most of the gonyautoxins found in muscle and gonad. In terms of overall toxicity, the toxic concentration of digestive gland was 9 times as high as that of gonad and nearly 200 times as high as that of muscle (*table* 3).

However, increased gonad toxin content during depuration was unclear. As the toxin in digestive gland at day 35 was drastically higher than at day 28, it is likely that the overall toxin profile of the gonad was influenced by this increase. Moreover, it is difficult to account for the increased toxin content of gonad by any other means than a toxin transfer between organs, inasmuch as the other gonyautoxins (GTX1/GTX4) were not present.

C-toxin fluorescence, as revealed by the method of Sullivan and Wekell, was detected in the chromatograms but is not considered in our figures since the peak obtained did not distinguish between compounds C1 to C4 and was not equal to the sum of these different toxins. However, in our initial study (Lassus *et al.*, 1989), C toxins represented 21% of total fluorescence at the end of the contamination phase and only 2% at the end of the decontamination phase. In the present study, the C-toxin peak represented 69 and 20% of total fluorescence at day 15 of contamination for digestive gland and gonad respectively (fluorescence barely detectable or undetectable in muscle), and 45 and 11 % at the end of the decontamination phase. It is thus likely that the C toxins found in total meat represented the highest uptake of these compounds in gonad and digestive gland, although warm extraction probably converted part of these compounds into GTX2 and GTX3 in our initial study. In the present study, the decrease in C toxins in gonad suggests biological hydrolysis of these compounds.

It also appears that the preparation of the scallops had an effect on toxin retention and that the kidneys stored more toxic compounds such as neosaxitoxin. Therefore, the comparative analysis of experimentally and naturally contamined scallop tissues would be of interest.

The results presented here and in our initial study (Lassus *et al.*, 1989) enabled us to point out the influence of the extraction technique on the nature of the toxins present in the shellfish. It is apparent that the AOAC test, requiring extraction by a strong and warm acid, is a logical choice only in the case of

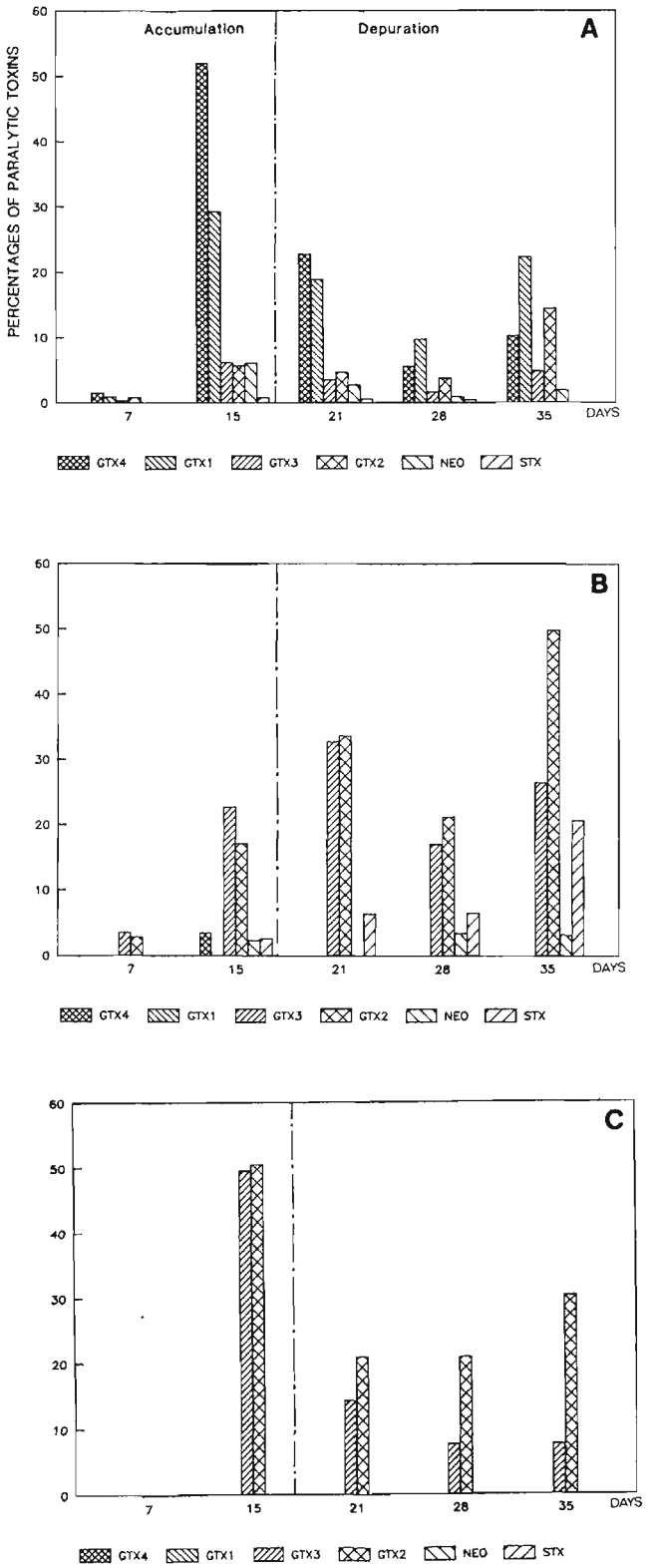

Figure 1. – Relative percentages of paralytic toxins in digestive gland (A), gonad (B) and muscle (C) of contaminated pectinids. Results expressed by comparison with a contamination considered maximal at day 15 of the experiment (end of feeding with the *Alexandrium* culture).

Table 3. – Toxin quantities expressed in µg equivalent saxitoxin per 100 g of meat during contamination and decontamination phases (ND = not detected).

Day of experiment	Digestive gland	Gonad (+ Kidneys)	Adductor muscle
Day 7	29.13	11.54	ND
Day 15	885.48	96.10	5.05
Day 21	464.87	135.23	1.70
Day 28	184.34	93.36	1.28
Day 35	424.52	191.26	1.66

purely "public health" objectives. However, extraction by a weak and cold acid is the only technique which preserves the toxin profile of a product. Moreover, this approach is increasingly accepted by teams working on the analysis of paralytic phycotoxins.

REFERENCES

Asakawa M., M. Takagi, A. Iida, K. Oishi, 1987. Studies on the conversion of Paralytic Shellfish Poison (PSP) components by biochemical reducing agents. *Esei Kagaku*, **33**, 50-55.

Bricelj V. M., J. H. Lee, A. D. Cembella, D. M. Anderson, 1990. Uptake of *Alexandrium fundyense* by *Mytilus edulis* and *Mercenaria mercenaria* under controlled conditions. *In*: Toxic Marine Phytoplankton. Graneli E. *et al.* eds., Elsevier, New York, 269-274.

Erard-Le Denn E., 1991. Recent occurrence of red tide dinoflagellate *Alexandrium minutum* Halim from the northwestern coasts of France. *In*: Recent approaches on Red tides. NFRDA, Park J. S., H. G. Kim eds., 85-98.

Estrada M., F. J. Sanchez, S. Fraga, 1984. *Gymnodinium catenatum* Graham en las rias gallegas (NO de España). *Inv. Pesq.*, **48**, 31-40.

Field I. A., 1922. Biology and economic value of the sea mussel *Mytilus edulis*. *Bull. U. S. Bur. Fish.*, **38**, 127-259.

Frémy J. M., M. Ledoux, 1991. Application de la CLHP à l'étude d'efflorescences d'*Alexandrium* sp. *Rev. Int. Océan. Méd.*, **101/104**, 197-204.

Frémy J. M., M. Ledoux, E. Nezan, C. Piclet, H. Belveze, 1989. Évolution de la présence de toxines paralysantes dans les coquillages lors de l'épisode toxique en Aber Wrac'h (1988). *Toxicorama*, **1**, 23-28.

Lassus P., J.-M. Frémy, M. Ledoux, M. Bardouil, M. Bohec, 1989. Patterns of experimental contaminations by *Protogonyaulax tamarensis* in some French commercial shellfish. *Toxicon*, **27**, 1313-1321.

Ledoux M., 1991. Optimisation d'un dosage par CLHP des phycotoxines paralysantes; application à l'étude de la contamination de fruits de mer. Mémoire Diplôme Ing., CNAM, Paris, 94 p.

Luthy J., V. Zweifel, C. Schlatter, G. Hunyady, S. Hasler, C. P. Hsu, Y. Shimizu, 1978. Vergiftungsfälle durch Miesmuscheln in der Schweiz 1976. Sanitätspolizeiliche Mapnalimen und Analyse der PSP toxine. *Mitt. Gebiete. Lebensen. Hyg.*, **69**, 467-476.

Maruyama J., T. Noguchi, Y. Onoue, Y. Ueda, K. Hashimoto, S. Kamimura, 1983. Anatomical distribution and profiles of the toxins in highly PSP infested scallops from Ofunato Bay during 1980-1981. *Bull. Jpn. Soc. Scient. Fish.*, **49**, 233-235.

Nezan E., M. Ledoux, 1989. Red tide by *Alexandrium minutum* in France. *Red Tide Newsl.*, **2**, 2-3.

Nezan E., G. Piclet, 1989. Alerte aux coquillages empoisonnés. *La Recherche*, **212**, 956-957.

Nezan E., G. Piclet, 1991. Ecotoxicological studies on some recent *Alexandrium* sp. outbreaks in France. *In*: Actes du Colloque Biotoxines marines. CNEVA Editions, Frémy J. M. ed., Paris, 195-202.

Noguchi T., Y. Ueda, K. Hashimoto, M. Seto, 1981. Isolation and characterization of Gonyautoxin-1 from the toxic digestive gland of scallop *Patinopecten yessoensis*. *Bull. Jpn. Soc. Scient. Fish.*, **47**, 1227-1231.

Noguchi T., J. Maruyama, Y. Onoue, K. Hashimoto, T. Ikeda, 1983. Toxins of mussels infested with *Protogonyaulax catenella* isolated from Senzaki bay, Yamaguchi Prefecture. *Bull. Jpn. Soc. Scient. Fish.*, **49**, 499.

Onoue Y., T. Noguchi, J. Maruyama, K. Hashimoto, T. Ikeda, 1981. New toxins separated from oysters and *Protogonyaulax catenella* from Senzaki Bay, Yamaguchi Prefecture. *Bull. Jpn. Soc. Scient. Fish.*, **47**, 1643.

Oshima Y., Y. Shimizu, S. Nishio, T. Okaichi, 1978. Identification of paralytic shellfish toxins in shellfish from Inland Sea. *Bull. Jpn. Soc. Scient. Fish.*, **44**, 395.

Shimizu Y., M. Yoshioka, 1980. Transformation of paralytic shellfish toxins as demonstrated in homogenates. *Science*, **212**, 547-549.

Sullivan J. J., M. M. Wekell, 1984. Determination of Paralytic Shellfish Poisoning Toxins by High Pressure Liquid Chromatography. *In*: Seafood Toxins. Ragelis E. P. ed., Washington D. C., *Am. Chem. Soc.*, 197-205.

Sullivan J. J., M. M. Wekell, 1986. The application of high performance liquid chromatography in a paralytic shellfish poisoning monitoring program. *In*: Seafood. Quality Determination. Kramer D. F., J. Liston eds., Anchorage, Univ. Alaska, 357-371.