PERGAMON
www.elsevier.com/locate/watres

PII: S0043-1354(99)00181-5

Wat. Res. Vol. 34, No. 2, pp. 550–556, 2000

Printed in Great Britain
0043-1354/99/$ - see front matter

TOXIC EFFECTS OF *GYMNODINIUM* CF. *MIKIMOTOI* UNSATURATED FATTY ACIDS TO GAMETES AND EMBRYOS OF THE SEA URCHIN *PARACENTROTUS LIVIDUS*

FÉRIEL SELLEM[1], DANIELLE PESANDO[2]*, GUY BODENNEC[3], AMOR EL ABED[1] and JEAN-PIERRE GIRARD[2]

[1]Institut National des Sciences et Technologies de la Mer, 28 rue du 2 Mars 1934, 2025, Salammbo, Tunisia; [2]LPTE, EA 2138, Faculté des Sciences, BP 71, 06108-Nice-cedex 02, France and [3]Département Ecologie Côtière, IFREMER Centre de Brest, BP 70, 29280-Plouzané, France

(First received 1 November 1998; accepted in revised form 1 April 1999)

Abstract—The toxicity of the main *Gymnodinium* cf. *mikimotoi* polyunsaturated fatty acid, has been investigated using the sea urchin gamete and embryo bioassays. The 18:5n3 fatty acid delays or inhibits first cleavage of *Paracentrotus lividus* eggs and provokes abnormalities in the embryonic development. These effects were compared with those of other polyunsaturated fatty acids, 18:4n3, 20:5n3 and 22:6n3, which are also present in this alga. A classification of the different fatty acids, based on their effects on sea urchin egg cleavage and the determination of the half inhibiting concentrations (IC_{50}) is proposed. © 1999 Elsevier Science Ltd. All rights reserved

Key words—*Gymnodinium* unsaturated fatty acids, sea urchin, embryo–larval development

INTRODUCTION

In the marine environment, microalgae represent a large part of primary production. Some microalgae are toxic and may interfere with the environment and with marine organisms at many stages of their life cycles. Blooms of the marine red-tide dinoflagellate *Gymnodinium* cf. *mikimotoi* (alias *Gymnodinium nagasakiense, Gyrodinium aureolum*) have frequently been reported during summer months along the western coast of France (Partensky *et al*., 1991; Gentien, 1998) and more recently on Tunisian coasts, inducing massive mortality in fish and invertebrates (Hamza and El Abed, 1994). Mortality or abnormalities in *Pecten maximus*, probably attributable to ingestion of algal cells are reported by Erard-Le Denn *et al*. (1990). Gentien *et al*. (1991) and Arzul *et al*. (1993) showed that a suspension in seawater of chloroform-methanol extract of the French isolate of *Gymnodinium* cells or of culture medium having contained algal cells was toxic to *Mytilus* sp. embryos and capable of stopping diatom growth. Yasumoto *et al*. (1990) associated the hemolytic activity of *Gyrodinium aureolum*, species closely related to *Gymnodinium mikimotoi*, to the presence of lipid compounds containing free or esterified polyunsaturated fatty acids 'PUFA' in a glycolipid structure.

Bodennec *et al*. (1995) showed that hexadecatetraenoic acid (16:4n3), octadecapentaenoic acid (18:5n3) and docosahexaenoic acid (22:6n3) were the main PUFA in the lipid extract of the French *Gymnodinium* strain. 18:5n3, a fatty acid rarely encountered in algal species has been shown to be more potent than saturated compounds and even more active than the other polyunsaturated fatty acids in hemolytic and diatom growth inhibition tests (Arzul *et al*., 1995). Fatty acid effects would concern mainly cell membrane structure where they may modify passive and active ionic transports, as recently described by Gamberucci *et al*. (1997) in relation to calcium conductance, with consequences for calcium-dependent cellular events. More recently Parrish *et al*. (1998) analysed glycolipids in *Gymnodinium* sp. They found that monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) which represent 35% of the total lipids, were hemolytic and that the major unsaturated fatty acid in these glycolipids was 18:5n3

Of various *in vitro* bioassays, the sea urchin egg test is one of the most extensively utilized (Kobayashi, 1980; Hose, 1985; Pagano *et al.,* 1986; Dinnel *et al*., 1988; Fusetani *et al*., 1989; Trieff *et al.,* 1995). The sea urchin egg has many advantages for screening developmental toxicity. This biological

*Author to whom all correspondence should be addressed.

model has been shown to be an accurate and rapid test for screening teratogenesis as a consequence of environmental pollution (Congiu *et al*., 1984; Pagano and Trieff, 1992; Graillet *et al.,* 1993) as well as in response to mycotoxins (Morrel and Adams, 1993) or marine toxins isolated from marine algae (Pesando *et al*., 1991, 1996), sponges (Fusetani *et al*., 1983; Ohta *et al*., 1996), starfish (Fusetani, 1987) tunicates (Pesando *et al*., 1995) and anemones (Malpezzi and Freitas, 1990).

We therefore decided to investigate the effects of 18:5n3, the major PUFA in *Gymnodinium* cells on *Paracentrotus lividus* gametes during fertilization, cleavage and subsequent embryonic development and to compare them with those obtained with three other PUFA: 18:4n3, 20:5n3 and 22:6n3.

MATERIAL AND METHODS

Chemicals

Different concentrations of 18:5n3 and 18:4n3, 20:5n3, 22:6n3, were used for carrying out toxicity tests on the first stage of sea urchin development. 18:5n3 is not commercially available. It has been synthesized according to the method described by Kuklev *et al*. (1992) by the γ-iodolactonization of 22:6n3. Its structure (all-*cis*-3,6,9,12,15-octadecapentanoic acid) was confirmed by GC–MS, FAB–MS, IR and H-NMR and by comparison with a sample isolated from *Gyrodinium aureolum* culture (Parrish *et al*., 1993). On account of the difficulties encountered in growing large volumes of this species and the lability of 18:5n3 fatty acid, its synthesis from 22:6n3 appeared to be a beneficial procedure to obtain this fatty acid in sufficient amount (*ca*. 100 mg) for biomedical tests. GC analyses on the corresponding fatty acid methyl esters (FAME) on polar (Supelcowax-10) and apolar (SE-54) columns confirmed a purity above 82%, the major impurities being 20:5n3 (5.4%), 18:4n3 (2%) and 22:n6 (1%) fatty acids. The stock solution of 18:4n3, 20:5n3 and 22:6n3 were obtained by dissolving standard fatty acids from Sigma chemicals in pure ethanol. All solutions were refrigerated until use.

Biological material

Paracentrotus lividus sea urchins were collected in the bay of Villefranche-sur-Mer (France) during the breeding season and maintained in captivity in well aerated water at 15°C in open circuit. The gonads were excised in seawater and the eggs maintained in 0.2 μm filtered natural seawater (NSW) and dejellied by five successive passages through a 90 μm mesh nylon filter. With this treatment, they could be kept in a well preserved state for 4–5 h. Sperm were collected dry and kept at 4°C. Shortly before use they were diluted 1:50 in NSW and 10 μl of this suspension were added per ml of an egg suspension adjusted to 20,000 eggs/ml. In order to obtain a perfect synchronization of cell division, eggs from a single female were used in each experiment which was done in triplicate.

Toxicity test procedure

The highest concentration of ethanol used in the experiments (0.5%) did not affect cell division.

Fertilization. To assay the fertilizing capacity of sperm, 18:5n3 was added to diluted sperm which was added 5 min later to an egg suspension. The effect of 18:5n3 on oocytes was tested by incubating oocyte suspension with the compound. After 5 or 10 min the gametes were rinsed twice with seawater and fertilization carried out. In both cases, samples of eggs were taken 5 min after fertilization and the extent of fertilization judged by the number of elevations of the fertilization envelope as seen under a light microscope. Rates of fertilization of treated eggs were compared with those obtained, in the same conditions, with control gametes to which only the solvent had been added.

Determination of cleavage rates. Cleavage rates were determined as described by Biyiti *et al*. (1990). Increasing concentrations of 18:5n3 diluted in ethanol were added to the egg suspension at various times after fertilization. Samples were taken at different times during cleavage and the percentage of divided cells recorded.

Following the same procedure, the toxicity of 18:5n3 was then compared with that of the other standard PUFA: 18:4n3, 20:5n3 and 22:6n3. The IC_{50} values were recorded for treated eggs at the time at which 90–95% of control eggs reached the two cell stage.

Embryotoxicity assay. The toxicity of 18:5n3 was studied on the developmental stages of sea urchin embryos up to the pluteus stage. The compound was added to egg suspensions 30 s after fertilization (20 000 eggs/ml), a delay presumed to be sufficient for ensuring total fertilization (Epel, 1978). Four h after fertilization, control and treated egg suspensions were diluted 40 times in NSW and maintained in suspension (500 eggs/ml) by stirring with propellers (37 rpm) for 98 h after fertilization. The first stages of cleavage and the embryonic, blastula, gastrula, prismatic and pluteus stages were observed under a light microscope in samples fixed with glutaraldehyde (final dilution 0.1%) at appropriate intervals after fertilization.

RESULTS

18:5n3 does not affect fertilization

When gametes were treated with 18:5n3 at a concentration of 2.5×10^{-4} M 5 or 10 min prior to insemination, fertilization was not affected: almost 98% of the eggs elevated a normal fertilization envelope.

Fatty acids inhibit sea urchin egg cleavage

We observed that the presence of 18:5n3 reduced the rate of progression of fertilized eggs through the first cycle of cleavage (Fig. 1(A)). When added 30 sec after fertilization, a delay presumed sufficient to permit total fertilization (Biyiti *et al*., 1990) the percentage of first cleavage decreased with the concentration of 18:5n3, a total inhibition being observed for concentrations above 1 mM.

Inhibition of the first cleavage was also dependent on the time at which 18:5n3 (1 mM) was added after fertilization. Fig. 1(B) shows that inhibition was total when 18:5n3 was added less than 40 min after fertilization. When eggs were exposed to 18:5n3 later in the cell cycle (50 and 60 min after fertilization), the inhibition declined progressively until almost 50% of the eggs were capable of division.

Fig. 2 showed that fatty acids with different chain lengths or unsaturation numbers inhibited egg cleavage to different extents (Fig. 2(A)). For each fatty acid 3 dose–response curves corresponding to 3 independent experiments were obtained by considering the percentage of cleavage shown in (A)

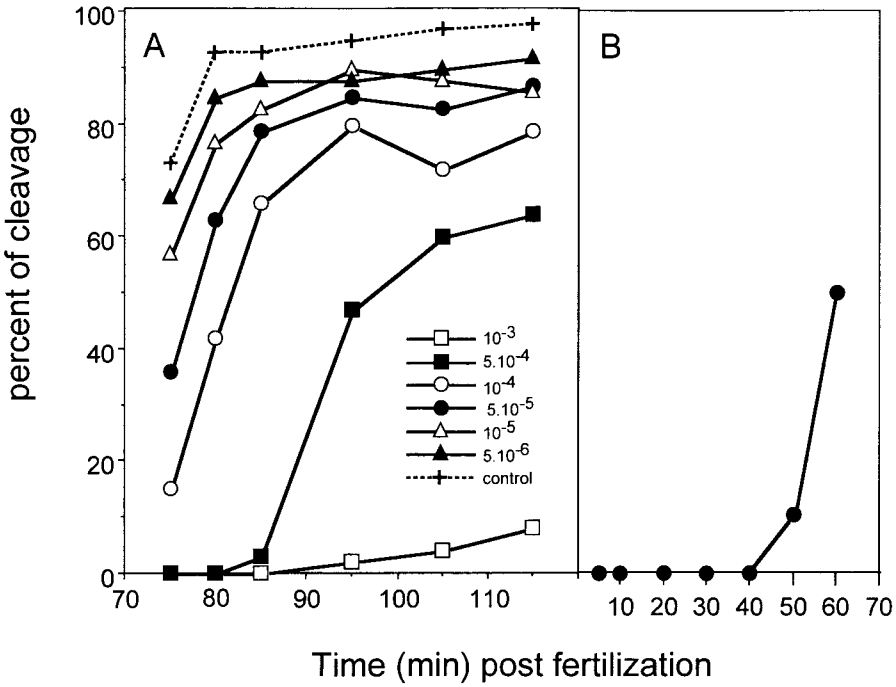

Fig. 1. Effects of 18:5n3 from the *Gymnodinium* cf. *mikimotoi* on the time course of *Paracentrotus lividus* egg cleavage. (A) Eggs were fertilized at time zero and increasing concentrations of 18:5n3 were added 30 s after insemination. (B) Eggs were fertilized at time zero and 10^{-3} M 18:5n3 was added at different times after sperm contact. Percent inhibition was evaluated at the time at which 90–95% of the control eggs had reached the two cell stage. One of three experiments giving similar results is shown.

80 min after the fertilization. According to the model given by the StatSoft Statistica 5.1 (1995) that can be used to estimate organism's responsiveness to a drug, the dose response follows a sigmoid function. An example of this dose response is given in Fig. 2(B) for the 18:5n3. The IC_{50} values which are the means of the IC_{50} values calculated from the dose–response curves obtained from the three experiments performed on each fatty acid, are respectively 0.156 μM $\pm$ 0.011 (18:4n3), 0.234 μM $\pm$ 0.031 (18:5n3), 0.337 μM $\pm$ 0.035 (20:5n3) and 1,06 μM $\pm$ 0;04 (22:6n3) (Fig. 2(C)).

Alteration of embryonic development induced by 18:5n3

The normal sequence of development of sea urchin embryos is shown in Fig. 3(A), (D) and (G). After the first cleavage (Fig. 3(A)), within 5 h of fertilization, the majority of embryos are at the morula stage (Fig. 3(D)). Gastrulation occurs within 24 h and after 72 h the pluteus stage characterized by an arrowhead shape with an endoskeletal structure is attained (Fig. 3(G)).

Fatty acid 18:5n3 was added to egg suspensions at concentrations of 10^{-6} and 5×10^{-6} M within 30 s. from insemination, thus allowing the proliferative phase to occur but with a very slight delay relative to the controls (see Fig. 1). At the time of the morula stage, 5 h after fertilization, embryos incubated with 18:5n3 were more or less retarded depending on the concentration of the added compound (Fig. 3(E) and (F)). In the control group 94% of the embryos were morulae. After treatment with 10^{-6} or 5×10^{-6} M 18:5n3, there were 45% or 17% respectively at the morula stage. In this last group, 26% of the embryos exhibited uneven numbers of blastomeres and inequalities of size suggesting that they would not continue developing (Fig. 3(F)). During the differentiation stages, the delay provoked by 10^{-6} M 18:5n3 during the proliferating stage was progressively recovered, thus the embryos reached the pluteus stage in a synchronous manner with the controls (Fig. 3(G) and (H)). We observed that in presence of 5×10^{-6} M 18:5n3, embryos reached partially the pluteus stage, almost 40% of them remaining under the prismatic shape. 5×10^{-6} M 18:5n3 greatly modify the appearance of the plutei, the arms were thicker, with intersecting rods and the gut wall was abnormally enlarged (Fig. 3(I)).

DISCUSSION

The results reported in this paper indicate that 18:5n3, the major constituent of *Gymnodinium* toxins (Bodennec *et al.*, 1995) delays cleavage and embryonic development and induces defects in sea urchin embryos. Our work emphasizes that 18:5n3

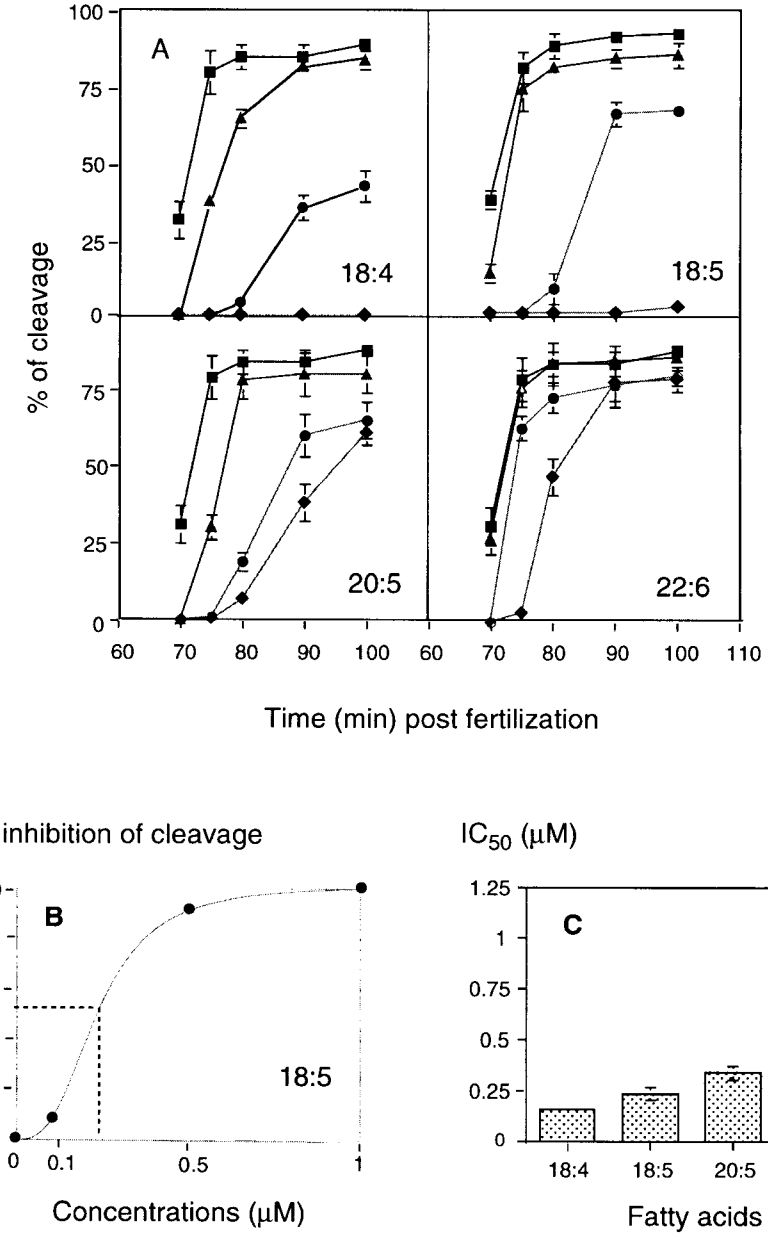

Fig. 2. Effects on cleavage of sea urchin eggs (*Paracentrotus lividus*) of 18:4n3, 18:5n3, 20:5n3 and 22:6n3 fatty acids. (A) Eggs were fertilized at time 0 and compounds added 30 s after sperm addition. (◆) 10^{-3} M, (●) 5×10^{-4} M, (▲) 10^{-4} M, (■) control. (B) Dose–response curves were obtained from the cleavage percentages in A at 80 min after fertilization and allowed the calculation of the IC_{50} values, one example is given for 18:5n3. (C) IC_{50} values of the four fatty acids. Values are the means ± S.E. of three independent experiments.

affects the first steps of embryogenesis, with a dose dependence, especially during the period between fertilization and the morula stage. Two ranges of concentrations can be considered: those above 5×10^{-4} M which inhibited cleavage and lead to cell cycle arrest and those from 1 to 5×10^{-6} M which slightly retarded cleavage and ultimately caused embryonic malformations. From the latter it would appear that developmental defects could be provoked without much noticeable cleavage delay and thus, embryonic abnormalities would ultimately be observed.

During sea urchin embryogenesis, the vegetal hemisphere gives rise to ectodermal, endodermal and mesodermal cells (Cameron *et al.*, 1987). In our experiments, development of calcified spicules is stopped or severely delayed in the presence of 5×10^{-6} M 18:5n3 and when the calcified skeleton is formed, we observed a gut enlargement that could be ascribed to an abnormal development of

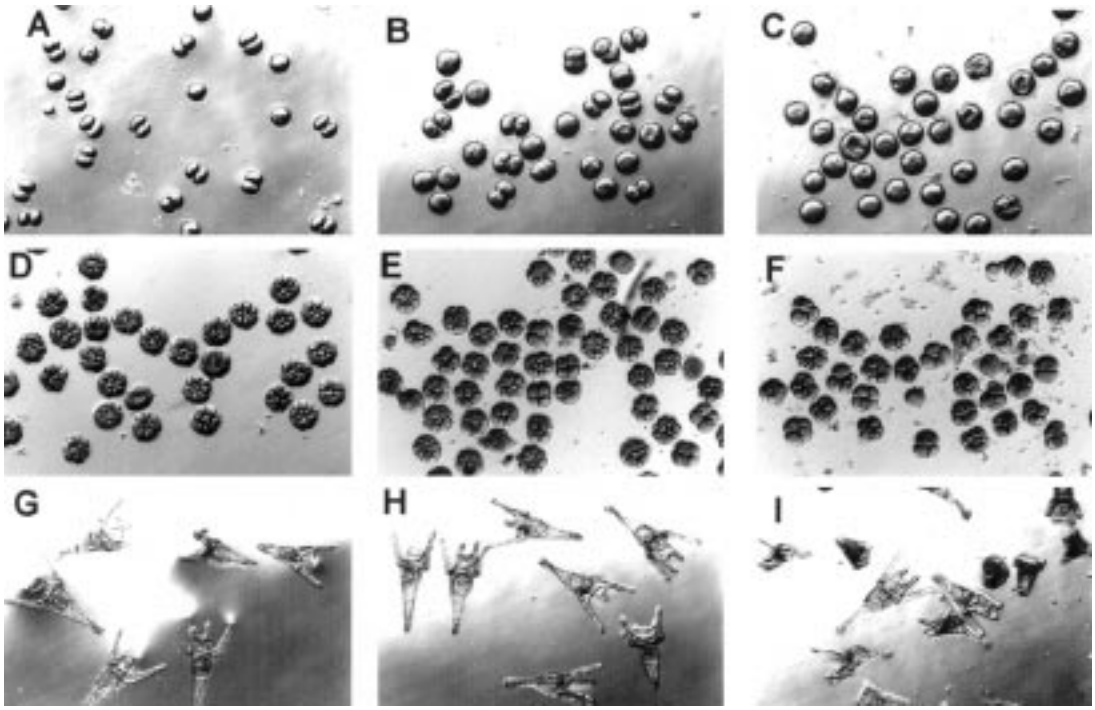

Fig. 3. The effects of 18:5n3 on the embryonic stages of proliferation and differentiation of *Paracentrotus lividus*. Photos on the left represent the two cell stage (A), the morula stage, 5 h after fertilization (D) and the pluteus, 72 h after fertilization (G) in control untreated embryos. Photos B, E and H show embryos treated with 10^{-6} M 18:5n at the same stages and on the right, C, F and I are embryos treated with 5×10^{-6} M 18:5n3. (A–C, E, F) ×50, (G–I) ×100.

the endodermal territory. These results could suggest that 18:5n3 affects the endodermal and mesodermal territories, resulting in dose-dependent malformations.

A comparison between the toxicity of the synthesized 18:5n3 and that of standard fatty acids, allow the establishment of a scale of toxicity for the various PUFA studied here and a classification of the different fatty acids, based on their effects on sea urchin egg cleavage and the estimation of the half inhibiting doses (IC_{50}) is proposed. The statistical analysis of the IC_{50} values (ANOVA followed by Tukey test) shows that the IC_{50} value of 20:5n3 is significantly different ($p < 0.01$) from that of the shorter chain PUFA 18:4n3 which shows the higher toxicity whereas the IC_{50} value of 18:5n3 is not significantly different from those of 18:4n3 and 20:5n3. The IC_{50} value of 22:6n3 is significantly different ($p < 0.01$) from those of the other fatty acids and 22:6n3 presents the lower embryotoxicitiy. From our results, 18:4n3 is the most toxic compound and 22:6n3 the least and it can be suggested that the toxicities of PUFA are inversely proportional to the length of the carbon chain and the number of insaturations. These conclusions can be compared with those of Arzul *et al.* (1995) who classified PUFA according to their effects on the growth of the diatom *Chaetoceros gracile*, hemolysis tests and *Photobacterium phosphoreum* bioluminescence (Microtox test). According to these authors, 18:5n3 is a harmful compound, except on bioluminescence and surprisingly 18:4n3 is far less toxic than the 20-carbon chain PUFA (all compounds were studied within the micromolar range). Such a classification suggest that the level of toxicity of PUFA could be hardly generalized as it varies according to the biological material used.

Using the sea urchin bioassay we observed that 18:5n3 affects rate of cleavage and also the first stages of embryonic development when used at lower concentrations, resulting in some larval abnormalities. A similar effect has already been reported by Gentien *et al.* (1991) who found that 90% of *Mytilus* sp. embryos showed abnormalities in a medium containing *Gymnodinium* cells at a density exceeding 2×10^6 cells/l. According to Bodennec *et al.* (1995) who measured the intracellular concentration of 18:5 n3 in *Gymnodinium* species, a fatty acid concentration of 10^{-5} M might be expected of this red tide density.

From our results and those of Arzul *et al.* (1995) it appears that the toxicity of PUFA on the various biological models occurs over the same range of concentration and 18:5n3 is generally one of the most toxic followed by 20:5n3 and 22:6n3. However, when compared with other toxins of mar-

Table 1. Toxic doses of various toxins of marine origin reported in the literature dealing with *Paracentrotus lividus* egg cleavage

Toxins	Origin	μM used	References
Steroidal saponins	starfish	6–15	Fusetani (1987)
Polyacetylene alcohol	sponge	1	Fusetani *et al.* (1983)
Quinone and diterpene	alga and sponge	3–6	Jacobs *et al.* (1981)
Caulerpenyne	alga (*Caulerpa* sp.)	45	Pesando *et al.* (1996)
Astrogorgiadiol and astrogorgin	gorgonia (*Astrogorgia* sp.)	2.5	Fusetani *et al.* (1989)
Pseudopterosin	coral	40	Ettouati and Jacobs (1987)

ine origin, also tested on sea urchin embryos, 18:5n3 exhibited a lower toxicity. We found that toxic doses for the first stage of embryonic development were around 10^{-4} M while previous studies recorded toxic doses varying between 10^{-6} M and 4×10^{-5} M depending on the origin of the toxins (Table 1).

The present study on the sensitivities of eggs and larvae of the sea urchin to these toxins confirms the results of previous work on the toxicities of *Gymnodinium* fatty acids on other marine organisms. Thus the presence of algal metabolites in the marine environment must also play a potential role in modifying the ecosystem. Further knowledge of the mode of action of these toxins on reproduction and cell proliferation will allow the assessment of the ecological impact of this alga.

Acknowledgements—The authors are indebted to Dr HabibLangar for revising the manuscript, to Dr Jacqueline Porthe for her helpful discussion and criticism and Dr P. Francour and Dr R. Lemée for their helpful in statistical analyses. The authors wish to thank B. Maetz for revising the manuscript.

REFERENCES

Arzul G., Erard-Le Denn E., Videau C., Jegou A. M. and Gentien P. (1993) Diatom growth repressing factors during an offshore bloom of *Gyrodinium* cf. *aureolum*. In *Toxic Phytoplankton Blooms in the Sea*, eds T. J. Smayda and Y. Shimizu, pp. 719–724. Elsevier Science Publishers B.V, Amsterdam.

Arzul G., Gentien P., Bodennec G., Toularastel F., Youenou A. and Crassous M. P. (1995) Comparison of toxic effects in *Gymnodinium* cf. *nagasakiense* polyunsaturated fatty acids. In *Harmful Marine Algal Blooms*, eds P. Lassus, G. Arzul, E. Erard, P. Gentien and C. Marcaillou, pp. 395–400. Technique et Documentation: Lavoisier, Intercept Ltd, Paris.

Biyiti L., Pesando D., Christen R., Puiseux-Dao S., Girard J. P. and Payan P. (1990) Effect of antibacterial plant flavanones on the intracellular calcium compartment involved in the first cleavage. *Toxicon* **28**, 275–283.

Bodennec G., Gentien P., Parrish C. C., Arzul G., Youenou A. and Crassous M. P. (1995) Production of suspected lipid phycotoxins by *Gymnodinium* cf. *nagasakiense* in batch cultures. In *Harmful Marine Algal Blooms*, eds P. Lassus, G. Arzul, E. Erard, P. Gentien and C. Marcaillou, pp. 407–412. Technique et Documentation: Lavoisier, Intercept Ltd, Paris.

Cameron R. A., Hough-Evans W., Britten R. J. and Davidson E. H. (1987) Lineage and fate of each blastomere of the eight cell sea urchin embryo. *Genes Development* **1**, 75–84.

Congiu A. M., Calendi E. and Ugazio G. (1984) Effects of metal ions and CCl4 on sea urchin embryos. *Research Communication in Chemistry Pathology and Pharmacology* **43**, 317–325.

Dinnel P. A., Pagano G. G. and Oshida P. (1988) A sea urchin test system for marine environmental monitoring. In *Echinoderm Biology*, eds W. Burke, *et al.*, pp. 611–619. Balkema Publisher, Rotterdam.

Epel D. (1978) Mechanisms of activation of sperm and egg during fertilization of sea urchin gametes. *Curr. Topics Dev. Biol.* **12**, 186–246.

Erard-Le Denn E., Morlaix M. and Dao J. C. (1990) Effects of *Gyrodinium* cf. *aureolum* on *Pecten maximus* (post larvae, juveniles and adults). In *Toxic Marine Phytoplankton*, eds E. Graneli, *et al.*, pp. 132–136. Elsevier Publishers, New York.

Ettouati W. S. and Jacobs R. S. (1987) Effect of Pseudopterosin on cell division, cell cycle progression, DNA and protein synthesis in cultured sea urchin embryos. *Molecular Pharmacology* **31**, 500–505.

Fusetani N. (1987) Marine metabolites which inhibit development of echinoderm embryos. In ed. P. J. Scheuer, *Bioorganic Marine Chemistry*, Vol. 1. Springer-Verlag Publishers, Berlin, Heidelberg, pp. 61–92.

Fusetani N., Kato Y., Matsunaga S. and Hashimoto K. (1983) Bioactive marine metabolites III 1 a novel polyacetylene alcohol, inhibitor of cell division in fertilized sea urchin eggs, from the marine sponge *Tetrosia* sp. *Tetrahedron Letters* **24**, 2771–2774.

Fusetani N., Nagata H., Hirota H. and Tsuyuki T. (1989) Astrogorgiadiol and astrogorgin, inhibitors of cell division in fertilized starfish eggs, from a gorgonian *Astrogorgiata* sp. *Tetrahedron Letters* **30**, 7079–7082.

Gamberucci A., Fulceri R. and Benedetti A. (1997) Inhibition of store-dependent capacitative Ca^{2+} influx by unsaturated fatty acids. *Cell Calcium* **21**, 375–385.

Gentien P. (1998) Bloom dynamics and ecophysiology of the *Gyrodinium mikomotoi* species complex. In *Physiological Ecology of Harmful Algal Blooms*, eds D. M. Anderson, A. D. Cembella and D. M. Hallegraeff, *NATO ASI series*, Vol. G.41. Springer-Verlag Publishers, Berlin, Heidelberg, pp. 155–173.

Gentien P., Arzul G. and Toularastel F. (1991) Modes of action of the toxic principle of *Gyrodinium* cf. *aureolum*. In *Marine biotoxins*, ed. J. M. Fremy, pp. 83–86. CNEVA Publishers, Paris.

Graillet C., Pagano G. and Girard J. P. (1993) Stage specific effects of teratogens on sea urchin embryogenesis. *Teratogenesis Carcinogenesis and Mutagenesis* **13**, 1–14.

Hamza A. and El Abed A. (1994) Les eaux colorées dans le golfe de Gabès: bilan de sept ans de suveillance. *Bulletin de l'Institut National Technique et Ocenographique des Pêches de Salammbô* **21**, 66–67.

Hose J. E. (1985) Potential use of sea urchin embryos for identifying toxic chemicals: description of a bioassay incorporating cytologic, cytogenetic and embryologic endpoints. *Journal of Applied Toxicology* **5**, 245–253.

Jacobs R. S., White S. and Wilson L. (1981) Selective compounds derived from marine organisms: effects on cell division in fertilized sea urchin eggs. *Fed. Proc.* **39**, 26–29.

Kobayashi N. (1980) Comparative sensitivity of various developmental stages of sea urchin eggs. *Marine Biology* **58**, 163–171.

Kuklev D. V., Aizdaicher A. B., Imbs A. B., Bezuglov V. V. and Latyshev N. A. (1992) All-*cis*-3,6,9,12,15-octadecapentaenoic acid from the unicellular alga *Gymnodinium kowalevskii*. *Phytochemistry* **31**, 2401–2403.

Malpezzi E. and Freitas J. C. (1990) Antimitotic effects of an extract of the sea anemone *Budonosoma caissarum* on sea urchin egg development. *Brazilian Journal of Medical Biology Research* **23**, 811–814.

Morrel C. M. and Adams J. A. (1993) Toxicity of verrucarin a to gametes and embryos of the purple sea urchin (*Arbacia punctulata*). *Bulletin of Environmental Contamination and Toxicology* **51**, 889–894.

Ohta S., Mihoko U., Tokumasu M., Hiraga Y. and Ikegami S. (1996) Hippospongic acid A: an unusual triterpenoic acid from a marine sponge, *Hippospongia* sp., which inhibits gastrulation of starfish embryos. *Tetrahedron Letters* **37**, 7765–7766.

Pagano G. and Trieff N. M. (1992) Use of echinoids in *in vitro* embryotoxicity and teratogenicity studies. *Toxicology in Vitro* **6**, 177–179.

Pagano G., Cipollaro M., Corsale G., Esposito A., Ragucci E., Giordano G. G. and Trieff N.-M. (1986) The sea urchin: bioassay for the assessment of damage from environmental contaminants. In *Community Toxicity Testing*, ed. J. Cairns Jr., pp. 66–92. American Society for Testing and Material, Philadelphia.

Parrish C. C., Bodennec G., Sebedio J. L. and Gentien P. (1993) Intra- and extracellular lipids in batch cultures of the toxic dinoflagellate *Gyrodinium* cf. *aureolum*. *Phytochemistry* **32**, 291–295.

Parrish C. C., Bodennec G. and Gentien P. (1998) Haemolytic glycoglycerolipids from *Gymnodinium* species. *Phytochemistry* **47**, 783–787.

Partensky F., Gentien P. and Sournia A. (1991) *Gymnodinium* cf. *nagasakiense* = *Gyrodinium* cf. *aureolum* (Dinophycées). In *Le Phytoplancton Nuisible des Côtes de France: de la Biologie à la Prévention*, eds A. Sournia, *et al*., pp. 63–82. IFREMER-CNRS Publisher, Brest, France (ISBN-2-905434-30-90).

Pesando D., Girard J. P., Durand-Clément M., Payan P. and Puiseux-Dao S. (1991) Effect of maitotoxin on sea urchin egg fertilization and on Ca^{2+} permeabilities of eggs and intracellular stores. *Biology of the Cell* **72**, 269–273.

Pesando D., Dominice C., Dufour M. N., Guillon G., Jouin P. and Ciapa B. (1995) Effect of nordidemnin on the cell cycle of sea urchin embryos. Role in synthesis and phosphorylation of proteins and in phosphoinositide turnover in mitosis progression. *Experimental Cell Research* **220**, 18–28.

Pesando D., Lemée R., Ferrua C., Amade P. and Girard J. P. (1996) Toxic effects of caulerpenyne extracted from *Caulerpa taxifolia* on mechanisms related to sea urchin egg cleavage. *Aquatic Toxicology* **35**, 139–155.

Trieff N. M., Romana L. A., Esposito A., Quiniou F., Iaccarino M., Alcock N., Ramanujam V. M. S. and Pagano G. (1995) Effluent from bauxite factory induces developmental and reproductive damage in sea urchins. *Archives of Environmental Contamination and Toxicology* **28**, 173–177.

Yasumoto T., Underdal B., Aune T., Hormazabal V., Skulberg O. M. and Oshima Y. (1990) Screening for hemolytic ichthyotoxic components of *Chrysochromulina polylepis* and *Gyrodinium* cf. *aureolum* from Norwegian coastal waters. In *Toxic Marine Phytoplankton*, eds E. Granéli, B. Sundström, L. Edler and D. M. Anderson, pp. 436–440. Elsevier Science Publishers, Amsterdam.