—— *Revisiones* ——

Harmful algal blooms, red tides and human health: Diarrhetic shellfish poisoning and colorectal cancer

VICTORIA LOPEZ-RODAS [1], EMILIA MANEIRO [1],
JUAN MARTINEZ [2], MACARENA NAVARRO [1] &
EDUARDO COSTAS [1]

[1] *Genética, Facultad de Veterinaria, Universidad Complutense, 28040. Madrid. Spain.*
[2] *Servicio de Medicina Preventiva y Salud Pública. Hospital Carlos III. 28029. Madrid. Spain.*

ABSTRACT

Certain blooms of unicellular microscopic algae that change the colour of the seawater to a reddish tone are called red tides. Hundred kilometres of the sea seem blood during a red tide. In some cases the microalgal species of red tides produce toxins or/and anoxic conditions, causing massive mortalities of marine animals. The proliferation of toxic algae is denominated harmful algal blooms (HAB). The majority of the toxic and red tide species are dinoflagellates, which present fascinating nuclear features (permanently condensed chromosomes organized in stacked rows of parallel nested arches without histones). Considerable time and effort are required to identify a HABs species under light microscopy in monitoring programs. Nowadays, the use of alternative molecular probes (antibodies, lectins, DNA probes) that bind target harmful algae is an increasing procedure in monitoring programs.

[*] Corresponding author: Eduardo Costas, Genética, Facultad de Veterinaria. Universidad Complutense, 28040. Madrid. Spain. Ecostas@vet.ucm.es

Abbreviations: harmful algal blooms (HAB), diarrhetic shellfish poisoning (DSP), colorectal cancer incidence (CRC).

Toxins produced by harmful species are transferred to food chain and cause numerous human intoxications with different clinical profile such as ciguatera fish poisoning (CFP), paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP), and amnesic shellfish poisoning (ASP). DSP is perhaps the main public health (and economic) problem in Spain and Europe[1].

The principal measures to avoid DSP outbreaks are the monitoring of shellfish harvesting areas and toxin analysis. European legislation allows up to 0,16 µg of DSP toxins per gram of meat.

Nowadays, the debate raised by DSP is only as a toxin causing diarrhoea. However, we think that the residual levels of DSP toxins ingested through shellfish consumption could contribute to increase colorectal cancer incidence (CRC). An epidemiological study to correlate dietary customs and tumour incidence shows a statistically significant correlation ($p < 0.001$) between consumption of molluscs and the incidence of colorectal cancer (coefficient determination = 0.50). An increase of 7 times in shellfish consumption produced duplication in the risk ratio of CRC in the Spanish population. Further analysis is necessary to conclusive association between shellfish consumption and CRC. In a context of global change that favours blooms of toxic microalgae a good approach for public health would be to change legislation to reduce the presence of residual levels of DSP toxins OA in shellfish. This point of view produces a conflict between the economic interests of the sector and public health.

Keywords: HABs.—DSP.—Cancer risks.—Dinoflagellates.—Marine toxins.

RESUMEN

Proliferaciones algales tóxicas, mareas rojas y salud: envenenamiento diarreico por moluscos y cáncer colorrectal

Las grandes proliferaciones de microalgas unicelulares se denominan mareas rojas cuando cambian el color del agua del mar. Algunas de estas especies fitoplanctónicas producen potentes toxinas y/o condiciones ambientales anóxicas que son capaces de provocar mortandades masivas de animales marinos. Los episodios de proliferaciones de microalgas tóxicas se conocen en el mundo entero como «harmful algal bloom» (HAB).

La mayoría de las especies tóxicas y formadoras de mareas rojas forman parte del grupo de los dinoflagelados, los cuales presentan unas características nucleares fascinantes (cromosomas permanentemente condensados organizados en hileras amontonadas de arcos unidos en paralelo sin histonas). Se requiere un tiempo y un esfuerzo considerable para identificar las especies de algas peligrosas bajo el microscopio en los programas de monitorización. Por eso, en la actualidad, se está incrementando el uso de sondas moleculares (anticuerpos, lectinas, DNA) que marcan específicamente las células tóxicas diana de manera más eficaz.

Estos organismos son el inicio de la cadena trófica en el mar, por tanto las toxinas producidas por las especies tóxicas son transferidas a la cadena alimenticia y causan numerosas intoxicaciones en seres humanos con diferentes perfiles clínicos como el envenenamiento por ciguatera (CFP), envenenamiento paralizante (PSP), envenenamiento neurotóxico (NSP), envenenamiento diarreico y envenenamiento amnésico (ASP). El DSP constituye quizá el principal problema de salud pública (y económico) en España. Las principales medidas para evitar proliferaciones de DSP son la monitorización de las áreas de producción de moluscos y el análisis de la toxina. La legislación europea permite 0,16 µg de toxina DSP por gramo de carne.

Hoy en día el debate que se plantea es que el DSP es solamente una causa de diarrea. Sin embargo, nosotros pensamos que los niveles «legales» de toxinas DSP ingeridas al consumir moluscos pueden contribuir al incremento de la incidencia de cáncer colorectal (CRC). El estudio epidemiológico para correlacionar las costumbres dietéticas y la incidencia de tumores, muestra una correlación estadísticamente significativa ($p < 0,001$) entre el consumo de moluscos y la incidencia de cáncer colorrectal (coeficiente de determinación = 0,50). Un incremento de siete veces el consumo de moluscos duplicará el riesgo de sufrir CRC en la población española. Es necesario realizar más estudios para asegurar de forma concluyente la relación entre el consumo de moluscos y el CRC. En un contexto de cambio global que favorece las proliferaciones de microalgas tóxicas, deberíamos reducir a cero los niveles residuales de toxinas DSP en moluscos. Este punto de vista da lugar a un conflicto entre los intereses económicos del sector y la sanidad de los alimentos.

Palabras clave: HABs.—DSP.—Riesgo de cáncer.—Dinoflagelados.—Toxinas marinas.

A BIBLICAL PLAGUE

«...*all the waters that were in the river were turned to blood, and the fish that were in the river died...*» (Exodus 7:20)

This biblical reference is believed to be the first written mention to a harmful algal bloom (HAB), which occurred about 3300 years ago in the Nile estuary. Certain blooms of unicellular microscopic algae are called «red tides» when planktonic microalgae grow in such abundance (up to millions per liter) that they change the colour of the seawater to a reddish tone. Red tides are extraordinary events. As bible describes, hundred kilometers of the sea seem blood during a red tide.

In some cases the microalgal species of red tides produce toxins that are deleterious to fauna. In other cases algal cells become so dense during the red tides that they produce anoxic conditions. As result red tides usually cause the death of thousands of marine animals (invertebrates, fishes, birds...), including vertebrates as big as monk seals and humpback whales (1-3).

Ancient proverbs and oral traditions of seaside peoples prevent against red tides and HABs effects. As an example, a popular tradition in NW of Spain warns people against eating mussels towards the end of the summer. Thousands cases of human poisoning by microalgal toxins (usually from shellfish consumption) are reported each year in the first world (4-5). But only recently we commence to understand how HABs, red tides, the marine ecosystem integrity, global change and human health are intimately linked.

MICROALGAE, HABS, AND RED TIDES

Marine unicellular microscopic algae generate 40% of primary production on the Earth (6-7). They form part of marine food chain and make higher life possible in oceans and shores, and allow human fisheries and aquaculture. Of the thousands of marine microalgae species ($\approx$ 70000) that occurs in the sea, only a small number are harmful (8). Around a dozen produce red tides, and only 40 are known to be toxic (9). Not all red tides are toxic, and not all toxic microalgae produce red tides. When these species proliferate, they can cause massive mortality among marine organisms (Figure 1), damage marine cultures, fisheries, and human health (9-10). The proliferation of toxic algae is denominated harmful algal blooms (HAB). HAB may generate high densities of toxic population, but low dense population (200 cells/l or less) of some toxic species can be enough to cause poisoning (11).

DINOFLAGELLATES

The majority of the toxic and red tide species are dinoflagellates (9). Dinoflagellates comprise a widely diversified group of unicellular

protists (≈ 4000 species), which exhibit a great diversity in size (from a few µm to 2 mm), form and lifestyle (Figure 1). Ninety percent of all dinoflagellates are marine plankton, and the rest are benthic, freshwater or parasitic species. About half the species are free-life photosynthetic organisms, and the others are heterotrophic predators on bacteria, microalgae, other protists (including other dinoflagellates), fish eggs, saprophytes, take up residence within other organisms as symbiotic partners, and even parasites. Dinoflagellate biology is reviewed in detail in Lee (12) and Graham and Wilcox (7).
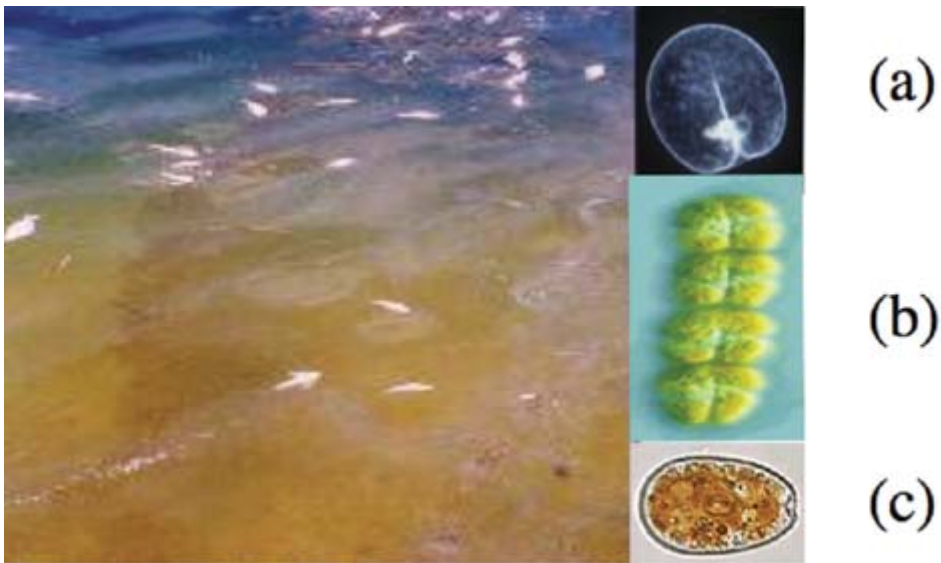

FIGURE 1. **Red tide and red tide-producing species.** *a) red tide producing dinoflagellate (Noctiluca); b) PSP tide producing dinoflagellate (Gymnodinium); c) DSP producing dinoflagellae (Prorocentrum).*

Dinoflagellates also present fascinating nuclear features that have intrigued researchers for many years (13-14). As examples, a dense nuclear matrix accommodates permanently condensed chromosomes that are composed by fibbers organized without histones and nucleosomes in stacked rows of parallel nested arches (Figure 2). The macromolecular chromosome structure corresponds to cholesteric liquid crystals with a constant left-handed twist. RNA is determinant to maintain the chromosome structure. Whole mounted

395

chromosomes have a left-handed screw-like configuration, with coils, which progressively increase their pitch. Dividing chromosomes remain highly condensed. The nuclear envelope remains through the cell cycle showing spindle fibbers, which penetrate intranuclear cytoplasmic channels during mitosis constituting an extra nuclear spindle.
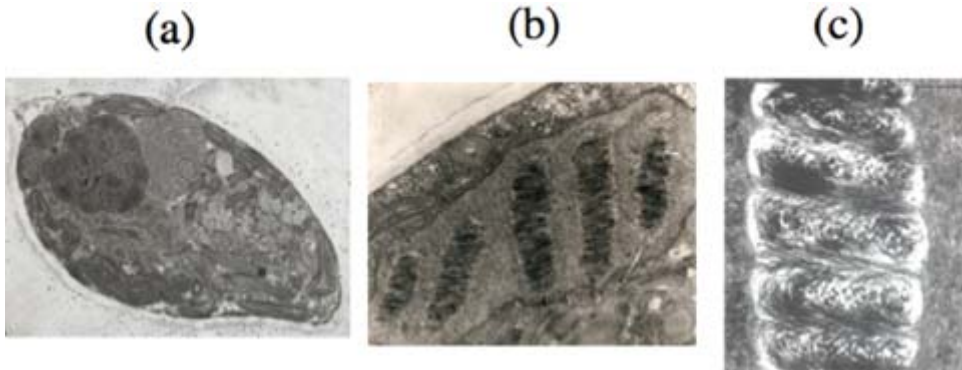

FIGURE 2. *Electron microscopy of dinoflagellate Prorocentrum lima.*
a) Section of Prorocentrum: nucleus with cromosomes; b) permanently condensed chromosomes anchored in nuclear membrane; c) chromatin organization in arcs without histones.

These and other cytogenetic features suggest that dinoflagellates are a group of enigmatic protists, unique and different from the usual eukaryotes. In contrast, DNA sequence studies propose that dinoflagellates are true eukaryotes, closely related to apicomplexa, and ciliates (Alveolata) (15), suggesting that the unusual features of chromosome and nuclear organization are not primitive but derived characters. Nevertheless, dinoflagellates have reached enigmatic specific nuclear and chromosome solutions, extremely far from those of other living beings.

TOXINS

There are human intoxications with different clinical profile caused by marine species (fish and shellfish) consuming toxic algae: ciguatera fish poisoning (CFP), paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning

(DSP), and amnesic shellfish poisoning (ASP). Toxins produced by harmful species are transferred to food chain throughout filtering organism and can remain active within them for long time. Briefly, the causative toxin responsible of PSP is saxitoxins (SXTs) and is produced by dinoflagellates *Alexandrium spp.*, *Gymnodinium catenatum*, and *Pyrodinium bahamense*. These toxins can be divided in two groups: SXTs 5-18 and neosaxitoxins 19-27. STXs are specific and potent agents that block the sodium channels in neuronal and muscular tissue, which prevents propagation of the action potential. The clinical profile is mainly neurological and their onset is rapid (within 30 minutes), there is a tingling or numbness around the lips, in the face and neck, a prickly sensation in the fingertips, headache, fever, nausea, vomiting and diarrhoea. The most severe cases result in respiratory arrest within 24 h of consumption of the toxic shellfish (mussels, oysters, scallops, clams). There is no antidote at the moment, only supportive therapy.

The intoxication caused by domoic acid and its congeners denominated ASP is produced by diatoms *Pseudonitzschia pungens f. multiseries*, *P. australis*, *P. pseudodelicatissima*, *P. seriata*, *Nitzschia actydrophila*, *Amphora coffeiformis*. The toxin, an excitatory amino acid, acts as an agonist to glutamate receptor (16), which conducts Na+ ion channels inducing depolarisation increasing the Ca+ ion permeability, which leads to cell death. Symptoms include gastroenteritis (within 24 h of the consumption of toxic shellfish) and in severe cases, neurological symptoms also appear, usually within 48 h. Dizziness, headache, disorientation, short-term memory loss, respiratory difficulty and coma are also observed (17).

Ciguatoxins (CTXs) and Maitotoxin (MTX) are the agents responsible for the CFP. The main causative organism is *Gambierdiscus toxicus*, an epiphytic dinoflagellate that produce a group of lipid-soluble toxins (polycyclic ethers) CTXs 28-37 and gambierol 38 (18). CFP affects more than 50,000 people annually, caused by ingestion of coral reef fishes. Ciguatoxins are potent neurotoxins, which act by binding quasi-irreversibly to site 5 on sodium channels, a site overlapping the brevetoxin binding site (19), causing them to open and modifying the sodium current. Several effects are consequently observed at motor nerve terminals (20). Ciguatera fish poisoning (CFP) produces gastrointestinal,

neurological and cardiovascular disorders. The gastrointestinal symptoms are diarrhoea, vomiting and abdominal pain occur, followed by neurological dysfunction including muscular aches, reversal of temperature sensation, dizziness, anxiety and a numbness and tingling of the mouth and digits. In the most severe cases, paralysis and death have been reported. The symptoms appear within 2 to 6 h, and the complete syndrome develops within 24 h and lasts around one week, but it may persist for weeks or months. Recovery time is variable, weeks, months or years. Rapid treatment (within 24 h) with mannitol and calcium is reported to relieve some symptoms (21). Prevention of intoxication depends on complete abstinence from eating any tropical reef fish.

NSP toxins are polycyclic ethers denominated brevetoxins (BTXs). The primary producer is a dinoflagellate *Gymnodinidium breve*, which causes mass mortality of fish. Pharmacological studies have revealed that BTXs produce their damaging effects by acting on site 5 of voltage-sensitive sodium channels (22). NSP produces an intoxication syndrome nearly identical with that of ciguatera. In this syndrome, gastrointestinal and neurological symptoms predominate. The recovery is generally complete in a few days. In addition, formation of toxic aerosols by wave action during an algal bloom can produce respiratory asthma-like symptoms. No deaths have been reported and the syndrome is less severe than ciguatera.

However, in Spain Diarrethic Shellfish Poisoning (DSP) is perhaps the main problem at both public health and economic levels.

HABS AND DIARRHETIC SHELLFISH POISONING (DSP) SYNDROME

Diarrethic Shellfish Poisoning (DSP) is a human syndrome that is produced by the consumption of shellfish that feed DSP toxic-producing dinoflagellates (Dinophysis spp., Prorocentrum lima, P. maculosum, Protoceratium reticulatum). The first reported cases of DSP were in the Netherlands in the 1960s. In Spain the first toxic outbreak of DSP was registered in August 1978 in the Ría of Ares (La Coruña, NW Spain) (4). Repeated outbreaks of DSP are detected worldwide (23-25). During the last decades, it has been observed a

high percentage of DSP toxins present in some seafood products (25-26). Ingestion of contaminated shellfish or fish results in a wide variety of human symptoms, depending upon the toxins present, their concentrations and the amount of contaminated shellfish consumed. Diagnosis is based on observed symptomatology and recent dietary history. The principal target for the effects of the DSP toxins is the gastro-intestinal tract and the clinical profile of DSP is characterised by nausea, vomiting, diarrhoea, abdominal pain, shivering, headaches and fever. Depending on the dose of toxin ingested, the onset may vary from 30 minutes to 12 hours after ingestion. Complete recovery is even in severe cases 2-3 days with or without medical treatment (23, 27).

The DSP toxins comprise a group of high molecular weight polyethers (Okadaic acid and dinophysistoxins, pectenotoxins, yessotoxins, and azaspiracids) (28). The main toxin responsible of DSP is okadaic acid (OA), a cyclic C38 polyether fatty that is concentrated by shellfish in their digestive glands (29-30). Detection of DSP toxins is performed by HPLC or mouse bioassay, concentration is expressed in okadaic acid equivalents (μg OA/g meat).

MONITORING, REGULATION AND PREVENTION OF DSP TOXINS

Shellfish is one of the main commercial productions in aquaculture industry. Shellfish associated with human outbreaks of DSP are mainly mussels, oysters, clams and scallops (26, 31). European legislation allows up to 0,16 μg Okadaic acid equivalent per gram of meat (Decision 2002/225/CE) (28).

The principal measures to avoid DSP outbreaks are the monitoring of shellfish harvesting areas and analysis for toxins. A major constraint to monitoring programs for HABs and marine biotoxins is the need to identify the microalgal species responsible for toxicity. The identification and enumeration of cells of potentially dangerous species within a mixed plankton assemblage is a serious problem. If the toxic alga is unknown, a lot of work is necessary to see whether toxin presence and algal occurrence are related. In

addition, some species are so toxic that they can produce serious problems occurring at such a low density that they are virtually invisible to cell counts in monitoring programs. Considerable time and effort are required to identify a particular species especially when its distinguishing characteristics are difficult to discern under light microscopy (5).

Invariably, microalgae maintain a fixed morphology, whilst accumulating genetic variability within them. Hence, while great effort is required to identify and counting toxin-producing microalgae using microscopy, misidentification may appear if morphology is the unique criterion for the identification of toxin producing species. A working alternative is the use of molecular probes that can bind species-specific sites on target harmful algae, to be visualized using flow-cytometry, spectrofluorometry, or epifluorescence microscopy. The use of immunological (32-33), lectin-based (34-35) and DNA based probes (36) for harmful algal species identification is increasing in monitoring programs. These cellular and molecular probes allow a precise identification that is impossible using classic microscopic identification. As an example of misidentification, the red tide non-toxic dinoflagellate *Gyrodinium impudicum* was continuously confused with the PSP toxin-producing dinoflagellate *Gymnodinium catenatum*. *G. impudicum* is so morphologically similar to *G. catenatum* that both species cannot be distinguished in formalin fixed samples. However, both species can be easily differentiated using the WGA lectin, which specifically binds to *G. catenatum*. Misidentification during decades between these species originated enormous economic looses when shellfish yield was forbidden after detect the innocuous species *G. impudicum* in water samples (5).

Shellfish yield is forbidden when the toxins exceed the prescribed limits providing a margin of safety to prevent contaminated shellfish reach the market. In spite of regulation on DSP toxins are usually taking into account, repeatedly outbreaks of DSP provoked by ingestion of mussels have been reported in European countries (24, 37). Even recently, shellfish contained more than 0,16 µg/g of okadaic acid have been detected in UE markets (25-26, 38). In addition, DSP outbreaks are probably undervalued because DSP is a self-limited and mild disease, and the most patients with DSP are detected when

suffering the symptoms and not through a screening (37). No human mortalities to date have been reported from any cases of DSP poisoning, although there has been considerable morbidity resulting in hospitalisation (39).

OA AND CANCER

Cancer is an important problem in public health worldwide. Colorectal cancer (CRC) is the second most frequent cause of death from cancer in the more developed regions of the world (40). While CRC mortality declined several years ago in most countries in the European (from 1950 to 1990's), mortality rates continues to increase for Spain (41). Spain has experienced a mean yearly increase higher in men (2.6%) than women (0,8%) since 1975, although there is a tendency towards stabilization (42).

A considerable number of environmental influences (mainly dietary factors) may play an important role in the development of CRC (41,43-46). In addition to that, hereditary factors have been observed in the aetiology of CRC (47).

OA can be a cancer-promoter. Numerous studies show that OK induces morphological transformation of cells (48-49), and micronucleus promoting (50). OA induce mutations and gene deregulation at low concentrations (51). OA also has growth-promoting effects on human cells (52-53). In addition, OA has been reported to be a tumor promoter of cancer in animal models, mainly related to skin, stomach and colon cancer (23, 54).

However, scarce works notice on real health-risk of consuming DSP toxins (39). The DSP syndrome is most prevalent in Europe, Japan and Chile, where aquaculture are extensively carried out (55). The regular shellfish consumers are exposed to continuous uptake of sub-acute levels of DSP toxins.

A HYPOTHESIS: OA AND COLORECTAL CANCER?

Several epidemiological studies associate CRC and dietary practices (47, 56-57). We propose that the residual levels of DSP

401

toxins ingested through shellfish consumption could contribute to increase CRC incidence.

To support our hypothesis (a shellfish consumers have a statistically significant higher risk to suffer colorectal cancer than a no-shellfish consumers), we have performed an epidemiological study to correlate dietary customs and tumour incidence. The data of epidemiological study were obtained from the latest available survey (1991) on habitual diet reflected into National Survey on Nutrition and Food (*Encuesta Nacional de Nutrición y Alimentación-*ENNA-91). The tumours incidence (rate of incidence in hospital admissions in cases per 100,000 population) was obtained from the Hospital Morbidity Survey (*Encuesta de Morbilidad Hospitalaria)* of 2003 (EMH - 03). The selected exposure variables were the consumption of fresh bivalve molluscs (mussels, clams, cockles) as the problem variable, and other food (bread, meat and meat products, fish, fresh fruit, olive oil, milk and dairy products) as control variables. The minimum of total admissions (CMBD) was coded in accordance with the International Classification of Diseases. We calculated regression, determination coefficients, confidence interval and others using Epi-Info, version 6.04 (58).

The output on epidemiological study (Figure 3) has established a statistically significant correlation ($p < 0.001$) between consumption of molluscs and the incidence of colorectal cancer (coefficient determination = 0.50). A correlation between the consumption of molluscs and the total incidence of tumours was also observed (Table 1). The consumption of other food was not associated with colorectal cancer (with the exception of total meat consumption, which is a known risk factor for this disease). An association between shellfish consumption and meat consumption was also observed ($r^2 = 0.39$; $p < 0.01$). In the Spanish population, an increase of 7 times in shellfish consumption produces duplication in the risk ratio of CRC (Table 2).
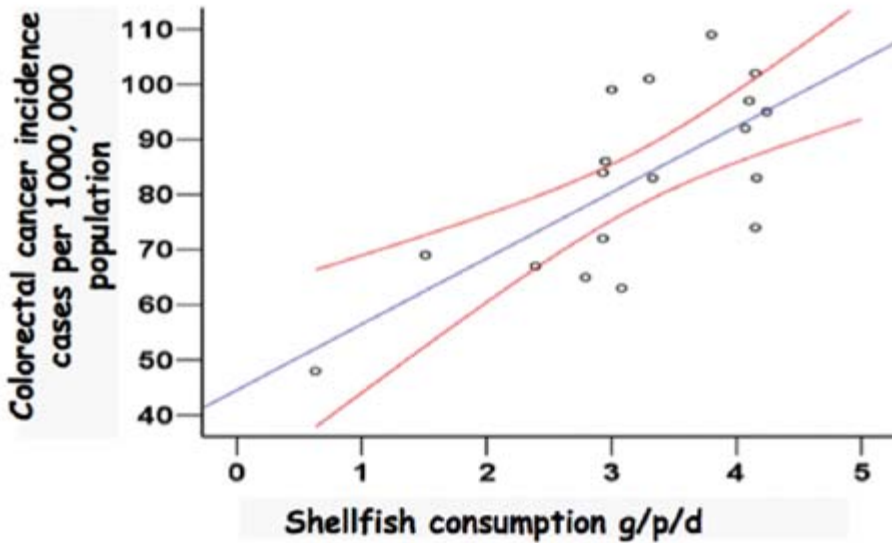
FIGURE 3. ***Correlation (p < 0.001) between comsumption of molluscs and the incidence of colorectal cancer.***

TABLE 1. *Values of determination coefficients and confidence interval for each of the food items*

	Total tumours				*Colorectal cancer*			
	Upper limits	R²	Upper limits	P	Upper limits	R²	Upper limits	P
Shellfish	–0,02	0,45	0,76	**<0,01**	0,04	0,50	0,78	**<0,01**
Bread	–0,68	0	0,68	>0,05	–0,46	0,01	0,47	>0,05
Fish	–0,67	0,02	0,69	>0,05	–0,68	0,01	0,69	>0,05
Fruit	–0,38	0,11	0,55	>0,05	–0,28	0,22	0,62	>0,05
Oil Olive	–0,45	0,02	0,48	>0,05	–0,44	0,03	0,49	>0,05
Milk	–0,38	0,1	0,54	>0,05	–0,36	0,13	0,56	>0,05
Meat	–0,12	0,36	0,71	>0,05	0,05	0,51	0,79	**<0,01**

TABLE 2. *Relative risk of colorectal cancer with increase of relative shellfish consumption in Spain*

Spanish Comunidad Autonoma	Relative Risk Consumption	Relative Risk colorectal cancer
CANARIAS	1	1
ASTURIAS	6,7	2
MADRID	6,6	1,8

Further analysis is necessary to conclusive association between shellfish consumption and CRC. In this sense, we are working in animal models and other epidemiological models. Nowadays, the debate raised by OA is only as a toxin causing diarrhoea. This study provides evidence that the hypothesis of the relation between OA and colon cancer should begin to be taken into consideration. Unfortunately, this point of view produces a conflict between the economic interests of the sector and public health. In a context of global change and coastal eutrophication that favours blooms of toxic microalgae a good approach for public health would be to change legislation to reduce the presence of OA in shellfish to zero. Although cost-benefit studies may throw light on the problem in the future, we believe that in a controversial situation such as this it would be well to remember the Hippocratic dictum of «primum non nocere».

BIBLIOGRAPHY

(1) ANDERSON, D. M. (1994): Red Tides. *Sci. Amer.* 271: 52-59.
(2) HERNÁNDEZ, M.; ROBINSON, I.; AGUILAR, A.; GONZÁLEZ, M. L.; LÓPEZ L. F.; REYERO, I.; CACHO, E.; FRANCO, J.; LÓPEZ-RODAS, V. y COSTAS, E. (1998): Did algal toxins cause monk seal mortality? *Nature.* 339: 28-29.
(3) COSTAS, E. y LÓPEZ-RODAS, V. (1998): Paralytic phycotoxins in monk seal mass mortality. *Vet. Rec.* 142: 643-464.
(4) FRAGA, S.; MARIÑO, J.; BRAVO, I.; MIRANDA, A.; CAMPOS, M. J.; SÁNCHEZ, F. J.; COSTAS, E.; CABANAS, J. M. y BLANCO, J. (1984): Red tides and shellfish poisoning in Galicia (NW Spain). ICES 5: 1-12.
(5) COSTAS, E. (1997): Red tides and toxic algal blooms: who's to blame? *Lagascalia.* 19: 165-178.

(6)   Falkowski, P. G. y Raven, J. A. (1997): Aquatic Photosynthesis. Malden, MA, USA. Blackwell Science.

(7)   Graham, L. E. y Wilcox, L. W. (2000): Algae. Upper Saddle River NJ, USA. Prentice Hall.

(8)   Peperzak, L. (2005): Future increase in harmful algal blooms in the North Sea due to climate change. *Water Sci. Technol.* 51: 31-36.

(9)   Sournia, A. (1995): Red tide and toxic marine phytoplankton of the world ocean: an inquirie into biodiversity en: Harmful Marine Algal Blooms (L. Patrick Ed.) 103-112.

(10) Yan, T. and Zhou, M. J. (2004): Environmental and health effects associated with Harmful Algal Bloom and marine algal toxins in China. *Biomed. Environ. Sci.* 17: 165-176.

(11) Keafer, B. A.; Churchill, J. A. and Anderson D. A. (2005): Blooms of the toxic dinoflagellate, Alexandrium fundyense in the Casco Bay region of the western Gulf of Maine: Advection from offshore source population and interactions with the Kennebec River plume. *Deep-Sea Research II:* 2631-2655.

(12) Lee, R. E. (1999): Phyvology. Cambridge University Press, UK.

(13) Costas, E. y Goyanes, V. J (1987): Ultrastructure and división behaviour of dinoflagellate chromosomes. *Chromosoma.* 95: 435-441.

(14) Costas, E. y Goyanes, V. (2005): Architecture and evolution of dinoflagellate chromosomes: an enigmatic origin. *Cytogenet. Genome Res.* 109: 268-275.

(15) Zardoya, R.; Costas, E.; López-Rodas, V.; Garrido, A. y Bautista, J. M. (1995): Revised dinoflagellate phylogeny inferred molecular analysis of large-subunit ribosomal RNA gene sequences. *J. Mol. Evol.* 41: 637-645.

(16) Hampson, D. R.; Huang, X. P.; Wells, J. W.; Walter, J. A. and Wright, J. L. (1992): Interaction of domoic acid and several derivatives with kainic acid and AMPA binding sites in rat brain. *Eur. J. Pharmacol.* 218: 1-8.

(17) Perl, T. M.; Dedard, L.; Kotsatsky, T.; Hockin, J. C.; Todd, E. C. D. and Remis, M. D. (1990): An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *N. Eng. J. Med.* 322: 1775-1780.

(18) Yasumoto, T. and Murata, M. (1993): Marine toxins. *Chem. Rev.* 93: 1897-1909.

(19) Poli, M. A.; Lewis, R. J.; Dickey, R. W.; Musser, S. M.; Buckner, C. A. and Carpenter, L. G. (1997): Identification of Caribbean ciguatoxins as the cause of an outbreak of fish poisoning among US soldiers in Haiti. *Toxicon.* 35: 733-741.

(20) Molgó, J.; Dechraoui, M. Y.; Juzans, P.; Morot-Gaudry, Y.; Mattei, C. and Legrand, A. M. (1998): Sodium-dependent alterations of synaptic transmission mechanisms by brevetoxins and ciguatoxins. In: Harmful Algae (Reguera, B., Blanco, J., Fernández, M. L., Wyatt, T., Eds.), pp. 594-597. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO.

(21) Palafox, N. A., Jain, L. G., Pinano, A. Z., Gulick, T. M., Williams, R. K., Schatz, I. J. (1988): Successful treatment of ciguatera fish poisoning with intravenous mannitol. *JAMA.* 259: 2740-2742.

(22) CESTELE, S.; SAMPIERI, F.; ROCHAT, H. and GORDON, D. (1996): Tetrodotoxin reverses brevetoxin allosteric inhibition of scorpion alphatoxin binding on rat brain sodium channels. *J. Biol. Chem.* 271: 18329-18332.

(23) AUNE, T. and YNDESTAD, M. (1993): Diarrhetic shellfish poisoning. *In* Algal Toxins in Seafood and Drinking Water (I. Falconer, Ed.), pp. 87-104. Academic Press, New York.

(24) VALE, P. and SAMPAYO, M. A. (2002): First confirmation of human diarrhoeic poisonings by okadaic acid esters after ingestion of razor clams (*Solen marginatus*) and green crabs (*Carcinus maenas*) in Aveiro lagoon, Portugal and detection of okadaic acid esters in phytoplankton. *Toxicon.* 40: 989-996.

(25) VALE, P. and SAMPAYO, M. A. (2003): Seasonality of diarrhetic shellfish poisoning at a coastal lagoon in Portugal: rainfall patterns and folk wisdom. *Toxicon.* 41: 187-197.

(26) JORGENSEN, K.; SCANLON, S. and JENSEN, L. B. (2005): Diarrhetic shellfish poisoning toxin esters in Danish blue mussels and surf clams. *Food Addit. Contam.* 22: 743-751.

(27) YASUMOTO, T. and SATAKE, M. (1998): New toxins and their toxicological evaluations. *In* Reguera, B., Blanco, J., Fernández, M. & T. Wyatt, eds. 1997. *Harmful Algae, Proceedings of the VIII International Conference on Harmful Algae* (June 1999, Vigo, Spain), pp. 461-464. Xunta de Galicia and IOC of UNESCO.

(28) COMMISSION DECISION OF 15 MARCH (2002): Laying down rules for the implementation of Council Directive 91/492/EEC as regards the maximum levels and the methods of analysis of certain marine biotoxins in bivalve molluscs, echinoderms, tunicates and marine gastropods. *Off. J. Eur. Communities* (2002/225/EC) L 75/62-63.

(29) SVENSSON, S.; SARNGREN, A., FORLIN, L. (2003): Mussel blood cells, resistant to the cytotoxic effects of okadaic acid, do not express cell membrane p-glycoprotein activity. *Aquat. Toxicol.* 65: 27-37.

(30) TORGERSEN, T.; AASEN, J. and AUNE, T. (2005): Diarrhetic shellfish poisoning by okadaic acid esters from Brown crabs (*Cancer pagurus*) in Norway. *Toxicon.* 46: 572-578.

(31) RAMSTAD, H.; HOVGAARD, P.; YASUMOTO, T.; LARSEN, S. and AUNE, T. (2001): Monthly variations in diarrhetic toxins and yessotoxin in shellfish from coast to the inner part of the Sognefjord, Norway. *Toxicon.* 39: 1035-1043.

(32) MENDOZA, H.; LÓPEZ-RODAS, V.; GONZÁLEZ, S.; AGUILERA, A. y COSTAS, E. (1995): The use of polyclonal antisera and blocking of the antibodies in the identification of marine dinoflagellates: specie-specific and clone-specific antisera against Gymnodinium and Alexandrium. *J. Exp. Mar. Biol. Ecol.* 186: 103-115.

(33) COSTAS, E. y LÓPEZ-RODAS, V. (1996): Enumeration and separation of the toxic dinoflagellate Alexandrium minutum from natural samples using immunological procedures with blocking antibodies. *J. Exp. Mar. Biol. Ecol.* 198: 81-87.

(34) Costas, E.; González de Chávarri, E.; Aguilera, A.; González, S. y López-Rodas, V. (1993): Use of lectins to recognize and differentiate unicellular algae. *Bot. Mar.* 36: 1-4.

(35) Costas, E. y López-Rodas, V. (1994): Identification of marine dinoflagellates using fluorescent lectins. *J. Phycol.* 30: 987-990.

(36) Costas, E.; Zardoya, R.; Bautista, J. M.; Garrido, A.; Rojo, C. y López-Rodas, V. (1995): Morphospecies vs. Genospecies in toxic marine dinoflagellates: an análisis of gymnodinium catenatum/Gyrodinium impudicum and Alexandrium minutum/ A. lusitanicum using antibodies, lectins and gene sequences. *J. Phycol.* 31: 801-807.

(37) De Schrijver, K.; Maes, I.; De Man, L. and Michelet, J. (2002): An outbreak of diarrhoeic shellfish poisoning in Antwerp, Belgium. *Euro Surveill.* 7: 138-141.

(38) Aasen, J. A.; Hardstaff, W.; Aune, T. and Quilliam, M. A. (2006): Discovery of fatty acid ester metabolites of spirolide toxins in mussels from Norway using liquid chromatography/tandem mass spectrometry. *Rapid Commum. Mass Spectrom.* 20: 1531-1537.

(39) Burgessa, V. and Shawb, G. (2001): Pectenotoxins, an issue for public health. A review of their comparative toxicology and metabolism. *Environment International* 27: 275-283.

(40) Sant, M.; Aareleid, T.; Berrino, F.; Bielska Lasota, M.; Carli, P. M.; Faivre, J.; Grosclaude, P.; Hédelin, G.; Matsuda, T.; Möller, H.; Möller, T.; Verdecchia, A.; Capocaccia, R.; Gatta, G.; Micheli, A.; Santaquilani, M.; Roazzi, P.; Lisi, D. and The EUROCARE Working Group (2003) EUROCARE-3: survival of cancer patients diagnosed 1990-94 —results and commentary. *Ann. Oncol.* 14 (Supplement 5), v61-v118.

(41) Boyle, P.; D'Onofrio, A.; Maisonneuve, P.; Severi, G.; Robertson, C.; Tubiana, M. and Veronesi, U. (2003): Measuring progress against cancer in Europe: has the 15% decline targeted for 2000 come about? *Ann. Oncol* 14: 1312-1325.

(42) López-Abente, G.; Pollan, M.; Aragones, N. and Pérez-Gomez, B. (2003): Informe sobre la salud de los españoles. Cáncer. Centro Nacional de Epidemiología Ambiental y Cáncer. Instituto Carlos III. Noviembre de 2002.

(43) Berrino, F.; Krogh, V. and Riboli, E. (2003): Epidemiology studies on diet and cancer. *Tumori.* 89: 581-585.

(44) Benito, E. and Cabeza, E. (1993): Diet and cancer risk: an overview of Spanish studies. *Eur. J. Cancer Prev.* 2: 215-219.

(45) Bingham, S.; Day, N.; Luben, R.; Ferrari, P.; Slimani, N.; Norat, T.; Clavel-Chapelon, F.; Kesse, E.; Nieters, A. and Boeing, H. (2003): Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. *The Lancet* 361: 1496-1501.

(46) Willett, C. (1999): Technical advances in the treatment of patients with rectal cancer. *Int. J. Radiation.* 45: 1107-1108.

(47) De la Chapelle, A. (2004): Genetic predisposition to colorectal cancer. *Nat. Rev. Cancer.* 4: 769-780.

(48) SHEU, C. W.; RODRÍGUEZ, I.; DOBRAS, S. N. and LEE, J. K. (1995): Induction of morphological transformation in BALB/3T3 mouse embryo cells by okadaic acid. *Food Chem. Toxicol.* 33: 883-885.

(49) FUJITA, M.; GOTO, K.; YOSHIDA, K.; OKAMURA, H.; MORIMOTO, H.; S. KITO, S. and FUKUDA, T. HANEJI (2004): Okadaic acid stimulates expression of Fas receptor and Fas ligand by activation of nuclear factor kappa-B in human oral squamous carcinoma cells. *Oral Oncology.* 40: 199-206.

(50) LE HEGARAT, L.; FESSARD, V.; POUL, J. M.; DRAGACCI, S. and SANDERS, P. (2004): Marine toxin okadaic acid induces aneuploidy in CHO-K1 cells in presence of rat liver postmitochondrial fraction, reveled by cytokinesis-block micronucleus assay coupled to FISH. *Environ. Toxicol.* 19: 123-128.

(51) CREPPY, E. F.; TRAORE, A.; BAUDRIMONT. I.; CASCANTE, M. and CARRATU, M. R. (2002): Recent advances in the study of epigenetic effects induced by the phycotoxin okadaic acid. *Toxicology.* 181-182: 433-439.

(52) JAHAN, I.; IIJIMA. M.; KONDO, T. and NAMBA, M. (1996): Effects of okadaic acid on cell growth, anchorage-independent growth, and co-cultures of normal (KMS-6), immortalized (KMST-6), and neoplastically transformed (KMST-6T and KMST-6/RAS) human fibroblasts. *J. Cancer Res. Clin. Oncol.* 122: 249-253.

(53) FUJIKI, H. AND SUGANUMA, M. (1993): Tumor promotion by inhibitors of protein phosphatases 1 and 2A: the okadaic acid class of compounds. *Adv. Cancer Res.* 61: 143-194.

(54) SUGANUMA, M.; TATEMATSU, M.; YATSUNAMI, J.; YOSHIZAWA, S.; OKABE, S.; UEMURA, D. and FUJIKI, H. (1992): An alternative theory of tissue specificity by tumor promotion of okadaic acid in glandular stomach of SD rats. *Carcinogenesis.* 13: 1841-1845.

(55) ZHAO, J.; LEMBEYE, G.; CENCI, G.; WALL, B. and YASUMOTO, T. (1993): Determination of okadaic acid and dinophysistoxin-1 in mussels from Chile, Italy and Ireland. In: Toxic Phytoplankton Blooms in the Sea, Smayda, J. and Shimizu, Y. (eds) Amsterdam, Netherlands Elsevier Science Publishers, pp. 587-92.

(56) MONROE, K. R.; HANKIN, J. K.; PIKE, M. C.; HENDERSON, B. E.; STRAM, D. O.; PARK, S.; NOMURA, A. M.; WILKENS, L. R. and KOLONEL, L. N. (2003): Correlation of Dietary Intake and Colorectal Cancer Incidence Among Mexican-American migrants: The Multiethnic Cohort Study. *Nutrition and Cancer* 45: 133-147.

(57) ALMENDINGEN, K.; HOFSTAD, B. and VATN, M. H. (2004): Dietary habits and growth and recurrence of colorectal adenomas: results from a three-year endoscopic follow-up study. *Nutr. Cancer* 49: 131-138.

(58) DEAN, A. G.; DEAN, J. A.; COULOMBIER, D.; BRENDEL, K. A. and BURTON, A. H. (1997): Epi Info, Version 6.04: A word-processing database and statistics program for public health on IBM compatible microcomputers. Atlanta, Georgia: Centers for Disease Control and Prevention.