First living *Alexandrium minutum* resting cysts in Western Baltic

On a global scale, the frequency and intensity of toxic phytoplankton blooms seem to be on the rise, and there is also some evidence of geographical spreading of nuisance species\(^1\). In Europe, this has been suggested for the toxic dinoflagellate *Gymnodinium catenatum* (p. 1, HAN No. 7). The PSP-producing *Alexandrium minutum*, first described from Alexandria Harbour, Egypt\(^2\), has since been reported from South Australia\(^3\), the Atlantic coast of North America\(^4\), Spain and Portugal\(^5\), Italy\(^6,7\), Turkey\(^8\), Ireland\(^9\), France\(^10\), and the Netherlands\(^11\).

In France, since 1985, toxic blooms of *A. minutum* have occurred along the Brittany coast within small embayments or shallow estuaries, especially in the northwestern area. Each time, shellfish harvesting had to be prohibited as a consequence of excess toxicity levels in bivalves\(^12\). At present, the region represents the main site for toxic blooms of this species in Europe.

Resting cysts of *A. minutum* were first described in 1991 from surface sediments of Port River near Adelaide, South Australia\(^13\) and has also been recorded since then along the Brittany coast of France\(^12\). It was suggested that blooms of *A. minutum* are primarily initiated from their benthic seed beds\(^14\). During a cyst survey in the Baltic Sea (Kiel Bight, Germany) in April 1993, several living cysts of *A. minutum* were found in the topmost centimeter of sediments. The cyst is circular in apical view (21-25 mm in diameter) and reniform in lateral view. The clear cyst wall is lightly covered with mucilage and a prominent orange-red accumulation body is present. This resting cyst is similar to the descriptions and figures of specimens from Australia\(^13\) and France\(^12\).

As specified for *G. catenatum* (p. 1, HAN No. 7), the recent occurrence of

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**Is the European *Alexandrium tamarense/ excavatum* toxic?**

There have been reports of PSP in Europe for several decades, and in many of them *Alexandrium tamarense* (= *Gonyaulax tamarensis*) was considered the causative species\(^12,13,14\). Some more recent cases have been attributed to other species such as *Gymnodinium catenatum* in Spain\(^9\) and Portugal\(^6\), or to *Alexandrium minutum* in Spain\(^7\) and France\(^6\).

Since the first reports, great progress has been made, both in the taxonomy of genus *Alexandrium*, and in the improvement of culture techniques and media. The older reports were all based on bioassays of suspected shellfish, but now the development of analytical tools like HPLC and the increased number of laboratories in which this kind of analysis is routine, provide frequent reports of the toxin composition of unialgal cultures of dinoflagellates.

Reports of the toxin composition of cultures of toxic dinoflagellates isolated from European waters are for *G. catenatum*\(^9\), *A. minutum* (= *Alexandrium lusitanicum*)\(^10\) and *A. ostenfeldtii*\(^11\). But HPLC analyses of strains of *A. tamarense*\(^11\) (the Plymouth and Vigo strains) indicate that this species is not toxic\(^12,13\).

It is surprising that the North Sea and surrounding waters are the areas where PSP reports attributable to *A. tamarense* are more frequent, yet there are no reports of HPLC analyses of cultures of *Alexandrium tamarense* isolated from those areas, in which this species appears to be toxic.

The hypothesis, that some of the PSP outbreaks reported in the North Sea area may be attributable to either *A. minutum* or *A. ostenfeldtii*, should be tested.

*A. minutum* on the north coast of Brittany has proved to be toxic, but the Plymouth strain of *A. tamarense* isolated not far from there is not toxic. As Brittany is the

(Cont'd on p. 2)
Annual mollusc toxicity variation on Morocco Mediterranean shore

An investigation into the agent responsible for food poisoning events associated with eating seafood has led to the incrimination of molluscs collected on the Mediterranean shoreline of Morocco. Their gathering and sale are now banned. The study was carried out on two species currently consumed in the region: the cockle, Cardium tuberculatum, and the hard-shelled clam, Catananche chroma. These species were sampled at regular intervals over a year, from May 1993 to May 1994, in the region of Tetouan at Kaa Stras and Oued Laou, in order to assess the toxicity of the bivalves there. Extracts of the molluscs were prepared according to the official method (Association of Official and Analytical Chemists, 1980).

The toxicity of the extracts was assayed on Swiss Albino mice. Cardium turbeculatum gave the highest toxicities at Kaa Stras, but in both cases toxicity levels remained high throughout the year and produced rapid death, preceded by PSP-type symptoms, while Catananche chroma gave lower levels, whatever the period of collection.

Cardium tuberculatum:
- Kaa Stras, toxicity 1207 Mouse Units (MU)/100g of meat.
- Oued Laou, 773 MU/100g
Catananche chroma: 190 MU/100g

Comparative investigation of the toxicity in different organs of the cockles showed that toxicity levels were clearly higher in the foot (5580 MU/100g) than in the mantle (1030 MU/100g).

The investigation carried out has shown that bioxotin presence is permanent in cockles in the region of Tetouan, but varies over the year.

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(Cont'd from p. 1, 'Western Baltic')

overlooked in the plankton of the region because of its scarce occurrences. This may even be the case since the genus Alexandrium now includes a rather long list of species, easy to confuse, which may lead to misclassification. Identification especially of the small, inconspicuous A. minutum requires careful study of the thecal plates. The variation of the first apical plate in A. minutum complicates the identification[3,4,12]. Recently, A. ibericum has been synonymized with A. minutum[5] and this may also be the case for A. lusitanicum[11]. Further investigations will be needed to ascertain whether its cysts can germinate and if vegetative cells can multiply under the specific conditions of this sea area, whether the Baltic A. minutum is toxic, and whether it is genetically distinct from other A. minutum populations.

In the study of harmful algae, there is an increasingly urgent need for correct identification of species. Cyst studies offer a valuable tool for an early warning on the presence and potential of toxic species in a given area and should be considered also in monitoring systems.

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(4) Badech, E. Personal communication in (3).
(11) Elbrächter, M. Personal communication.
(16) Ellegaard, M. Personal communication.
Dinophysis bloom in West Scotland

Reports of brown-red water discoulour-a-tion in Loch Long, a fjord-like sea loch on the west coast of Scotland were referred to the Marine Laboratory on 31 July 1992. The discoulouration extended over a 10 km strip along the eastern shore of the upper part of the loch. Water and mussel (Mytilus edulis) samples were collected from four sites along the shore of the loch. Water samples were examined and counts made of the dominant species and the shellfish were tested for the presence of DSP toxins.

At four sampling stations, the numbers of Dinophysis acuminata ranged from 8,000 cells l-1 (Portincaple) to 103,000 cells l-1 (Ardnay), 916,000 cells l-1 (Arrochar) and 942,000 cells l-1 (Finnart). D. acuta and D. norvegica were also present at all four stations sampled and at Finnart, Scripsiella trochoidea and Ceratium spp. were abundant. All shellfish sampled from the area tested positive for DSP toxins. This was the first reported incident of a Dinophysis bloom on the west coast of Scotland. There were no reports of any human intoxications as a result of this incident, probably as the area is not one which is regularly har-vested for commercial shellfish species.

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Comment

The involvement of Alexandrium tamarense/exuvatum in the UK toxic events has been enigmatic, as was pointed out by Fraga and Franco. However, over the last few years, the Ministry of Agriculture, Fisheries and Food has funded a detailed programme of work on the causes of the regular shellfish intoxications on the British coast. As part of this work, we have isolated a number of strains of Alexandrium from the west coast of Scotland. One of these cultures has been the subject of detailed study. Plate dissection shows this culture to be Alexandrium tamarense and it has also been shown to be toxic.

The suggestion that Alexandrium minutum may also be involved in UK events is an intriguing one, and we have been open to this possibility. Cysts reminiscent of Alexandrium minutum have been found in the Firth of Forth, and hatching trials are being carried out to investigate this further.

The hypotheses of Fraga and Franco concerning the identity of organisms involved in toxic events are interesting. However, it is always risky to formulate such hypotheses in the absence of adequate information.

Development of a national HAB programme in the USA

As the international Harmful Algal Bloom (HAB) Programme of the Intergovernmental Oceanographic Commission (IOC) started to take shape over the last several years, it became clear that this programme should, in part, be a coalescence of national programmes established independently in different countries. The USA has never had a 'national programme', or a plan to attack these problems, despite a long history of problems associated with harmful algae and a solid history of HAB research. No single federal agency had jurisdiction over the many different types of HAB problems, and in many cases, funding fluctuated with HAB events - rising when significant impacts occurred, and decreasing during the years when problems were less obvious.

Faced with growing HAB problems in the USA and limited financial resources to deal with those problems, a series of steps was initiated to create a national programme that could help guide USA HAB activities. The first of these steps was a workshop convened in 1992 at the National Marine Fisheries Laboratory in Charleston, South Carolina. This workshop produced the report Marine Biotoxins and Harmful Algae: A National Plan, which identified numerous impediments to progress in the HAB field, and made specific recommendations to address those impediments. Equally important, the National Plan tried to organize the different HAB activities in a way that helped agencies identify their role in the overall programme.

This report was widely distributed within the USA scientific community as well as throughout government agencies and Congressional offices. In order to implement the recommendations of the plan, it was deemed necessary to establish a committee of programme managers from all government agencies concerned with HABs and their impacts. Accordingly, an ad hoc Task Force on Marine Biotoxins and Harmful Algae was established and now meets several times a year to discuss ways to co-ordinate agency initiatives and to more efficiently target resources on HAB issues. (At present, this Task Force includes programme managers from the National Science Foundation, the Food and Drug Administration, the Department of Defense, the National Institute of Environmental Health Sciences, the Centers for Disease Control, and several National Oceanic and Atmospheric Administration agencies (Sea Grant, the Coastal Oceans Programme, and the National Marine Fisheries Service).

During the early deliberations of this group, it became clear that certain areas (such as those associated with toxin detection or analysis) were reasonably well supported by several funding programmes, whereas others, notably those dealing with the bloom dynamics of harmful algae, were not well supported. An effort was thus initiated between two of the agencies represented on the panel (NSF and NOAA) to establish a programme on the ecology and oceanography of HABs. Although the National Plan had identified this as an important area for research, a detailed scientific agenda for this specific topic needed to be developed. A workshop was then convened in August of this year (1994) at Snow Mountain Ranch, Colorado, to develop a science plan on the ecology and oceanography of HABs. Forty participants, including academic scientists and state and federal agency officials, met to discuss those issues related to the population dynamics of HAB species and the manner in which they affect ecosystems. Oral presentations and working group discussions were organized around three topic areas: the organisms; foodweb/community interactions; and environmental regulation of blooms. Within each of these theme areas, working groups identified major hypotheses or questions, and proposed approaches that might be used to address them.

Where relevant, new technologies were identified that would greatly facilitate the attack on these questions.

The proceedings of the Workshop will be printed and distributed within the next several months. It is expected that this science plan will be used to guide agency funding initiatives, as well as to help agency officials evaluate the relative importance of proposal submissions from individual investigators. To fully achieve the goals of this Ecology and Oceanography of Harmful Algal Bloom initiative (ECOHAB), it will be necessary to form agency partnerships to share in the programme implementation costs. As with many other countries, USA HAB phenomena affect many different areas of the country's coastline, involve numerous HAB species, impact many different marine organisms and human populations, and are associated with a variety of different hydrographic systems. A programme to address all of these environmental impacts and species is clearly ambitious, and thus requires co-operation between multiple agencies for full implementation. It is expected that the Interagency Task Force will play an important role in forming these partnerships.

Another important step towards the development of a national HAB programme was the establishment of the National HAB Office at the Woods Hole Oceanographic Institution through joint funding from NOAA and NSF. This office has many a role to play in the planning and implementation of a national HAB programme. The office assists in organizing and chairing ad hoc Interagency Task Force meetings, and it serves as a direct contact point for requests for information on HABs, both technical and general. It also provides a mechanism for USA participation in numerous working groups, workshops and related activities. This office is working to co-ordinate efforts to attract funding to the HAB programme. The first activity in the latter context was to organize and convene the ECOHAB workshop. The office will also be directly involved in establishing an electronic network linking USA HAB workers. This network will also be accessible to others throughout the world via the Internet. The USA HAB office can be considered one of the 'national science and communication centres' now being established in several countries to support the IOC-FAO HAB Programme. IOC HAB science and communication centres, directed towards assisting developing countries, are planned in Denmark and in Spain.

The outlook is thus quite bright for the HAB programme in the USA. The National Plan has helped to clarify the nature of the problems we face and the steps that are needed to address them, and these issues are being further refined through supplemental workshops such as the ECOHAB initiative. High level government officials meet regularly on the Interagency Task Force to guide the activities of the programme. In the USA, at least, this type of planning has brought together the community of researchers and regulatory officials involved with marine biotoxins and harmful algal blooms, and has provided a degree of visibility at the governmental level that should lead to sustained support for this programme, and thus significant progress in understanding and managing HAB phenomena.

Those wishing to receive copies of "Marine Biotoxins and Harmful Algae: a National Plan or the ECOHAB Science Plan (when published) should contact:

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IOC Training Course on Algal Toxins

The first IOC-WHO-FAO Training Course on Qualitative and Quantitative Determination on Algal Toxins was held from 18 to 28 October 1994, at the Friedrich-Schiller University of Jena. Twelve scientists from eleven countries participated in the course, organized by Prof. Bernd Luckas, Dr. Malte Elbächter (Biologische Anstalt Helgoland) and Dr. Helle Ravn (IOC). Invited lecturers were Prof. T. Yasumoto (Tohoku University) and Dr. A. Boenke (Commission of the European Communities). Dr. M.L. Fernandez (Vigo, Spain), a visiting researcher at the Friedrich-Schiller University of Jena, introduced the work of the European Community Reference Laboratories.

The course was geared to the participants' needs, with respect to the toxins found in their countries, so as to enable them to use the knowledge gained from the course to develop methods specifically relevant to their countries. Most of the scientists had brought samples from their countries, which samples were analysed during the course.

The participants were given an overview of state-of-the-art analytical methods for determination of algal toxins. The presence of algal blooms and the most important characteristic markers for identification of algae species were discussed.

Practical experience was gained in qualitative and quantitative determination of algal toxins based on two different HPLC-methods for analysis of DSP and PSP toxins, and on one method for analysis of ASP toxins. The AOAC Mouse Bioassay for PSP toxins and the ELISA bioassay, DSP-Check Test Kit (Japan) were demonstrated respectively by Dr. Schubert (Germany) and Dr. Tubaro (Italy). The practical aspect was emphasized to enable the participants to perform analytical experiments.

All participants presented reports on toxin chemistry as well as toxicology research and monitoring in their countries. The general impression of the course was very positive, and valuable contacts for future collaboration were established amongst the participants.

Red tide flagellate rediscovered in Himalayan valley

A red tide flagellate was observed in some parts (Fig. 1) of Dal Lake, Kashmir during summer-autumn of 1991. The organism responsible turned out to be a rarely recorded flagellate, *Euglena pedunculata Gojdic* (Khan, M. A.: 1993, Arch. Hydrobiol. 127, 101-103). The flagellate formed a red scum layer at the surface in the proximity of the Centaur Hotel Complex, Srinagar. No such algal bloom in the lake had been documented previously.

The cells were mostly contracted, 20-40 mm in diameter, some slightly elongated. Few cells had flagella. Paramylon granules were round, cup-like or small and oval-shaped. Cyst formation was the most diagnostic feature; the empty cyst wall was trumpet-shaped (Fig. 2).

The bloom was mono-specific with approximate numerical density of 15 x 10^9 individuals per litre obtained in September 1991, at the peak of the period. The bloom is attributed locally to accelerated eutrophication caused by sewage from adjacent hotels. The bloom lasted 7-8 weeks and caused no apparent fish mortality.

Further research should throw more light on the environmental implications of such algal blooms in the Kashmir Himalayan waters.

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Harmful algae and PSP toxicity along north Patagonia coast

There have been recurrent reports of PSP toxicity along the Atlantic coast of northern Patagonia, Chubut Province, Argentina. In 1980, values of 173,360 MU/100 g of mussel tissue were reported for sites on the Valdés Peninsula\(^1\); in 1985 values of 173,000 MU were reported from the Chubut River estuary\(^2\), and in 1988 values of 66,150 MU were reported for Nueva Bay\(^3\). These observations indicate that there has been an expansion in the distribution and increasing abundances of *Alexandrium excavatum*, a PSP-producing species in Argentine waters\(^4\). Since then, the Argentine Environmental Agency, in collaboration with the Department of Maritime Affairs, Chubut Province, has been monitoring shellfish toxicity by bioassay throughout these areas. High values of PSP toxins in spring and summer have resulted in closures of shellfish harvesting in these areas.

In the Nueva and San José Gulfs (Fig. 1), between November 1990 and April 1991, PSP toxicity increased, beginning in late spring, peaking in mid-summer and declining to undetectable levels in late summer (Fig 2). Maximum values were 7,900 and 22,300 MU/100 g of mussel tissue for the Nueva and San José Gulfs respectively, and the temporal pattern of toxicity in *Mytilus edulis platensis* D'Orb was similar in both locales. During this period, *A. excavatum* occurred at concentrations of \(2 \times 10^7\) cells \(^1\) and a maximum of \(16 \times 10^7\) cells \(^1\) of *Proorocentrum micans* were recorded during this period. Dormant cysts of *A. excavatum* were also detected in these plankton samples, but in very low numbers.

![Fig. 1: Monitoring area in North Patagonian coast.](image)

**Prynnesium parvum** blooms on the north German coast

Recurrent blooms of the potentially toxic phytoflagellate *Prynnesium parvum* Carter were observed in a shallow semi-enclosed brackish lagoon, formerly a tidal channel, located on the German North Sea coast. In 1992 maximum cell concentrations of \(6 \times 10^6\) cells per litre were reached at the end of May at a salinity around 5% and a water temperature of ca. 20°C. Despite persistent sunny and calm weather conditions, a decline of the bloom to \(2 \times 10^6\) cells per litre was associated with a rise in salinity and temperature at the end of July. In autumn, *P. parvum* disappeared from the plankton but in late November a locally restricted revival of the bloom, with cell numbers up to \(6 \times 10^6\) cells per litre, was observed in a small branch of the basin. Salinity was at 5% and water temperature about 5°C. In 1993 only a single bloom (2-3 \(10^6\) cells per litre) was recorded in July at ca. 10% and 15°C. This is the first ascertained record (by EM) of *P. parvum* on the German West coast.

Although no toxic effects have been observed yet in this area, further attention should be paid to stagnant and eutrophic brackish water bodies in view of their potential function as seedbeds, especially for nearshore eurythermal and euryhaline coastal blooms.

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Fig. 2: Toxicity levels in mussels during spring and summer, 1990-1991.

In addition to the above-mentioned dinoflagellates, *Dinophysis acuminata* was recorded in the plankton samples, although in relatively low densities (< 200 cells \(^1\)). This dinoflagellate is of interest as it is one of the species reported to produce DSP toxins. Furthermore, *Proorocentrum lima*, another known DSP-toxin producer, was found in concentrations of 45 cells \(^1\) at Bengoa Beach, San José Gulf. More recently this species was found in relative abundance in beach sediments in Nueva Bay at Puerto Madryn (J.L. McClellan & A. Woolaver, unpubl. results). These are the first reports of this DSP-producing species for the Argentine coast.

Our observations suggest that the northern coastal region of Patagonia is a potential danger area for shellfish toxicity, not only for PSP toxins but also for DSP toxins.

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HawaiianChemptec International Aquatic-Biotoxin Research

Current aquatic biotoxin research activities at HawaiianChemptec International (HCI), focuses on the development of rapid marketplace screening methods based on immunochemical technology for toxins associated with finfish and shellfish poisoning outbreaks, i.e., ciguatoxins (CTX), okadaic acid (OA) and related marine polyethers, tetrodotoxin, and domoic acid (amnesic shellfish poisoning).

Additional areas of focus include the development of a comprehensive harvesting area and marked place monitoring programme for these toxins, evaluation of biotransfer and accumulation of ciguatera-related toxins from toxigenic dinoflagellates to fish, and the development and evaluation of clinical diagnostic methods for ciguatoxins, okadaic acid and related polyether toxins in human serum.

For an analytical method to be of value in screening marketplace seafoods, it must meet the following criteria: (a) easy to use and interpret; (b) rapid, i.e., able to test many samples quickly; (c) accurately differentiate between toxic and non-toxic products; (d) low cost; (e) available in sufficient quantity to meet private, industrial and regulatory agency testing demands; and (f) where feasible, confirm the identity of toxic products. Our research programme has been guided by these principles.

The solid-phase immunobead assay (S-PIA, Ciguauect™), soon to be available from HCl, has high potential for application to marketplace screening of fish products for CTX and DSP toxicity. Testing fish or shellfish early in a screening plan is recommended since this will minimize the cost expended for the product, and reduce potential economic losses to industry.

The kit can be used on board fishing vessels, at receiving docks, processing plants, distribution organizations, retail outlets, consumers and regulatory agencies. The self-contained assay is available as a single analysis kit designed for non-laboratory use by untrained personnel. Organizations conducting large numbers of analyses would be more inclined to use the laboratory kit, which contains sufficient material for 60 tests.

Research on ciguateric fish in the Pacific and Caribbean regions is in progress with the Food and Drug Admin-

istration (Washington, D.C. and Dauphin Island, Alabama, USA), the Canadian Health Protection Branch (Ottawa), Hancock Institute of Marine Sciences (Los Angeles, California, USA), Institut Territorial de Recherches Medicale Louis Malarde (Papeete, Tahiti), Queensland Department of Primary Industries, (Deception Bay, Queensland, Australia), ORSTOM Centre de Noumea (Noumea, New Caledonia), Ecole Nationale Vétérinaire de Nantes (France), and the research studies in Guam of the Department of Geology and Geophysics, University of Minnesota (USA). These studies are demonstrating the utility of the test kit under specific applications and that the toxins are present in a significant number of fish at levels below the sensitivity of the mouse bioassay.

Earlier studies at the Laboratoire Central d’Hygiène Alimentaire (Paris, France) focused on the extraction and purification of toxins from barracuda implicated in ciguatera poisoning. These studies confirmed the existence of three separate toxic fractions and the presence of okadaic acid in the flesh of the fish.

The Ciguauect™ test kit and the high performance liquid chromatography (HPLC) methods have also been used to evaluate DSP potential in mussels collected from Denmark and France. Such study confirmed the presence of okadaic acid and related DSP toxins in mussels implicated in a DSP poisoning outbreak (Denmark) and mussel depuration operations (France). Hence, an excellent correlation was shown between the presence of okadaic acid as determined by HPLC and the Ciguauect™ test kit for mussel samples collected before, during, and after depuration operations.

The Ciguauect™ test kit is being developed specifically as a screening tool which will allow testing of a large number of samples, thus identifying high risk products. The test kit determines the presence or absence of toxins by binding the toxins to a membrane attached to a plastic strip and exposing the toxin-laden membrane to a monoclonal antibody-colored latex bead complex which has a high specificity for the toxins of interest. The intensity potential can be determined directly on edible tissue or following specific extraction procedures.

For those products where additional testing is desired, possibly for samples testing positive, a rapid extraction method (REM™) capable of extracting and of partial purification of polyether toxins in less than 30 minutes has been developed. Toxins are extracted with a chloroform : methanol mixture and partitioned into select phases by varying polarity. When the REM™ test kit is used to extract and purify toxic components, the limit of detection for the Ciguauect test kit is < 0.05 ng. Also, at this point chemical methods based on thin layer (TLC) and HPLC technology can be used to confirm the presence of individual toxins.

A TLC method for okadaic acid in fish tissue and dinoflagellate cultures has been developed. Specificity of this methodology is obtained following exhaustive purification of toxins extracted from fish tissue. These methods, although not suitable for routine screening programmes, could play an important role in confirming the presence of individual toxins in fish products for laboratories not equipped with HPLC capabilities.

Seafood safety monitoring programmes: An effective food safety monitoring programme comprises primarily these components: (1) monitoring fish and shellfish harvesting areas for toxin potential, (2) establishment of regulatory limits, and (3) a screening programme for testing fish products in commercial channels designed to test a large number of samples in a short period of time so that toxic or high risk products can be identified. Unacceptable products can then, if desired, be subjected to additional testing to confirm the presence and identity of the toxin(s).

Monitoring fish harvesting areas: A key aspect of an effective seafood safety monitoring programme, where feasible, is the establishment of a programme for predicting and identifying high risk harvesting locations. This is accomplished through the testing of marine specimens endemic to fish harvesting locations. This will vary whether for CTX, DSP, or other aquatic biotoxins. Since seafoods commonly associated with ciguatera poisoning outbreaks come from highly mobile fish, collecting and testing the fish alone could provide incorrect information with both false positive and false negative predictions. This possible problem can be corrected by identifying and testing a specimen or bio-marker of limited motility, i.e. invertebrates, endemic to the fishing area.

The Ciguauect™ test kit has been
used to screen 36 species of near-shore invertebrates off Hawaii for ciguatoxin and related polyether compounds (R. G. Kvetek, Moss Landing Marine Laboratories, Hawaii) when marine life. Specimens included snails, sea urchins, sea cucumbers, crabs, brittle stars, bivalves, and zoanthids. Invertebrates were collected at six ‘toxic’ locations having documented histories of ciguatera fish poisoning along the Kona coast and the three ‘non-toxic’ sites along the Hauanaua coast, where there had been only one reported case of ciguatera since 1980.

A significant positive correlation between assay results and site-specific ciguatera history was found for the cowry Cypraea maculifera. While assay results for most other species indicated very low or no ciguatoxin present, cone snails (Conus spp.), ophiuroids (Ophiocoma spp.) and sea cucumbers (Holothuria spp.) tested positive frequently. However, for these genera, there was no correlation between assay results and site history. These results suggest that invertebrates – particularly grazers and deposit feeders, and especially cowries – accumulate ciguatoxins and related polyether compounds at sites known for ciguatera fish poisoning outbreaks and have the potential utility of being bio-indicators of reef toxicity. Cypraea maculifera, or another invertebrate native to the area under study, could be an integral part of the ciguatera monitoring programme.

The Danish and French studies mentioned earlier, demonstrate that the assay could (i) identify DSP-contaminated shellfish and (ii) serve as a useful tool to monitor shellfish on-shore or during depuration operations to determine at what point shellfish can enter the market following a Dinophysis sp. bloom.

Establishment of regulatory limits: An important aspect of any food safety monitoring programme is the establishment of regulatory limits designed to assure wholesomeness of the food supply. Animal toxicological and human clinical data are crucial to the determination of these values. Since multiple toxins are involved with CTX and DSP poisoning outbreaks, it is not practical to use a single compound.

Historically, the establishment of a seafood safety monitoring programme for ciguatera has been hampered by the lack of reference standards. At present, okadaic acid is the only toxin associated with both CTX and DSP poisoning outbreaks in sufficient quantities to serve as a reference standard. Therefore, the term okadaic acid equivalents (OAЕ) could be used to establish regulatory limits, being aware of the relative potencies of other toxins involved with the CTX or DSP phenomenon. Again, the term OAЕ is used because multiple toxins are involved in the poisoning outbreaks. Recent studies in collaboration with Canadian and USA governments support the hypothesis that CTX-related toxins are present in a significant number of fish, but not necessarily at levels posing a risk to humans.

Our current studies on the detection of marine polyether toxins in human serum show a significant correlation between the presence of these toxins in the serum of patients clinically diagnosed with chronic fatigue syndrome.

Screen fish in the marketplace/commercial channels: Because of the etiology of toxin accumulation in fish and shellfish, screening in the marketplace is necessary. Our efforts in this area focus on evaluating the kit for on-shore testing and commercial retail outlets for fish products originating from Hawaii, Puerto Rico, U.S. Virgin Islands and Saint Barthélemy (French Territory).

Laboratories or individuals who wish to participate in the clinical diagnosis or interlaboratory method validation studies should contact:

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HARMFUL ALGAE NEWS

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