APPENDIX D

Workshop report on *Taxonomy and identification of HAB species and data management*

Introduction

The workshop was held over one and a half days prior to PICES X, at a venue provided by the Botany Department at UBC. After welcoming the 23 participants, the convenor, Dr. Max Taylor, stated the goals of the workshop, reminding them that it was not a training workshop but rather an opportunity for analysts to discuss problems related to the accurate identification of harmful species, uniformity of taxonomy and data reporting, management and usage. The agenda included presentations by Drs. F.J.R. "Max" Taylor, Yasuwo Fukuyo, Rita Horner, Laurie Connell and Ms. Michelle (Shelly) Tomlinson (in order of appearance), but provided as much time in the laboratory as possible to observe practical demonstrations and to microscopically examine material brought by the participants.

Presentations

Dr. Taylor used a brief summary of problems with fish- and shellfish-killing flagellate species to introduce taxonomic difficulties with HABs. These include misidentification (*Heterosigma* as *Olisthodiscus* in much earlier literature), taxonomic priority and usage (*H.carterae* vs. *H.akashiwo*), recent name changes (*Karenia, Karlodinium, Akashiwo*), species recognition (within *Chattonella*) and the need for infraspecific levels of discrimination. Problems arising from the complex putative life-cycle stages and modes of nutrition in *Pfiesteria*, plus difficulty in distinguishing it from "*Pfiesteria-like organisms"* which may not be closely related (e.g. *Karlodinium galatheanum*), as well as toxin type and source were briefly mentioned. It is suspected that some common (psammophilic) sand dinoflagellates having a similar mode of feeding, currently attributed to *Katodinium*, may be closely related (Taylor, unpubl.).

It was noted that almost any bloom-forming phytoplankter can kill marine fauna if locally over-concentrated, leading to plankton death and oxygen depletion. Members of *Gonyaulax* have been commonly involved in this type of HAB phenomenon. The special case of *Noctiluca*, a microzooplankter often included in HABs because of numerous kills of fish and shrimp, especially in China, was illustrated and discussed. Only one species, *N. scintillans* (syn. *N.miliaris*) has been morphologically discriminated but more may exist and there is a need for genetic studies. The mechanism(s) of death due to *Noctiluca* blooms is unclear although high ammonia levels may be involved. It usually occurs in confined bodies of water, such as shrimp ponds. In passing it can be noted that this common, cosmopolitan species is often treated in ecological studies as if it was a phytoplankter, with possible links to inorganic nutrients being sought, but such links can only be indirect since its blooms have to follow those of a prey species.

The HAB biogeographic picture shows extraordinary latitudinal cosmopolitanism, including bihemispherism and a general lack of true endemicism (except in polar regions) is the norm. This is generally not appreciated by non-phytoplanktological taxonomists and has an important bearing on the significance of supposed ballast water introductions. It is to be expected from general dinoflagellate biogeography that, for example, it is highly likely that species of *Pfiesteria* will be found in shallow estuaries in other countries with similar coastal temperature ranges such as Brazil or southern Africa or Australia. Given the present climate of interpretation, artifical introduction would almost certainly be invoked as an explanation.

Dr. Fukuyo began by illustrating the seven orders of dinoflagellates involved in HABS, with most HAB species being found in the Prorocentrales (*e.g.*, okadaic acid-producing *Prorocentrum* spp,), Dinophysiales (DSP-associated *Dinophysis* spp.), Gonyaulacales (several genera including *Alexandrium, Pyrodinium, Gambierdiscus, Ostreopsis*) and Gymnodiniales (*Karenia, Karlodinium* etc.). He provided plentiful excellent

identificatory aid material to the participants, including publications and a CD produced in Japan. In the Peridiniales, the recently described *Heterocapsa circularisquama* requires electron microscopy of its scales in order to identify it, but it has a characteristic movement when seen alive. It has killed oysters and other bivalves in Japan but fish in Hong Kong. The toxin of this economically important, recently described species is unknown.

Dr. Fukuyo then focussed on the PSP-producing genus *Alexandrium* with more than 20 species implicated in this widespread phenomenon as well as fish killers. He used it to illustrate the criteria employed in identification (tabulational features revealed by calcofluor or iodine staining) and visual aids to the identification of the species, including a manuscript by M. Yoshida prepared for a recent IOC-DANIDA training workshop. It was noted here, as seen earlier by Dr. Taylor, that the shape of cells and number of plates change in culture. In particular, chain formation is often reduced in culture, resulting in cells more rounded in shape.

This was followed, after laboratory material examination of dinoflagellates, by Dr. Horner who gave a talk on HAB diatoms, focussing on domoic acid-producing species of *Amphora, Nitzschia* (a recently-described benthic species from Vietnam) and *Pseudo-nitzschia* (six species so far). After a brief history of HAB diatom studies on the west coast of North America, noting that it is almost certain that records of *Pseudo-nitzschia seriata* (= *Nitzschia seriata*) before the late 1990's are actually of *P. australis,* focus turned to the problems of visually discriminating between various toxic and non-toxic species of *Pseudo-nitzschia.* Electron microscopy, SEM or TEM, is needed to observe the fine details of valve structure needed to discriminate the species. Examples of local representatives of well-known toxic species were illustrated, including *P. australis, P. multiseries* and *P. pseudodelicatissima. P. granii* has been isolated recently from the open North Pacific Ocean by researchers from UBC. Problems of overlap in descriptions and arising from different views (valve, girdle) were discussed. As toxicity varies with strains or physiological state within known toxic species their mere presence cannot be taken as evidence of the presence of toxins in shellfish. This is also found in culture. Even the type of chain formation can vary, including the formation of *Fragilariopsis*-like chains. A fungal parasite is commonly seen in wild coastal N.E. Pacific populations.

Another problem associated with diatoms in the PICES region is the death of farmed fish due to physical gill damage by *Chaetoceros concavicornis* and, possibly, *Ch. convolutes.* Earlier records referred only to the latter species but Taylor and co-workers have concluded that the former is the greater threat, having more developed spinulae on the setae. It is interesting that this species does not seem to be a problem, or is unrecognized, in other temperate fish farming areas.

In the laboratory, Dr. Connell gave a talk and demonstration of a commercially available LSU RNA sequence quantitative technique for HAB species identification (Saigene). It is almost fully automated and can handle large numbers of samples. It is in current use for identifying species of *Chattonella, Heterosigma, Alexandrium* and *Pseudo-nitzschia* (there are outstanding difficulties with *P. pseudodelicatissima*). In the future, based on complete sequences of rRNA, it is likely that microchip probes will be developed. A very recent presentation at the 7th International Phycological Congress by Linda Medlin and European colleagues showed excellent promise for discriminating species of *Alexandrium.*

Other fluorescent probes are used for toxins, using labelled antibodies. ELISA and other antibody methods for toxin detection in cells or shellfish were not discussed here since this workshop dealt with species recognition, but the need for a workshop on recent developments in these techniques was recognized as a need.

Ms. Tomlinson gave a talk and a web-based demonstration of an online HAB Data Management System (HAB-DMS), which is now available through the National Oceanographic Data Center (NODC, U.S.A.) at www.nodc.noaa.gov/cgi-bin/hab/hab.pl. A Pacific region website has been created and can be found

at www.nodc.noaa. gov/col/projects/habs/ pacindex.html. The FGDC record for the Washington State Department of Health PSP and Domoic Acid 1998-2000 (NODC #0000559) has been completed. The online linkage can be found at www.doh.wa.gov/ehp/sf/.

The Northwest Fisheries Science Center (NWFSC, NMFS, USA) has supplied harmful algal bloom datasets to the NODC. These include data from Washington State Department of Health, the Alaska Department of Fisheries. These data have been archived and documented using the FGDC format and are available in the originator's format through the NODC Direct system (www.nodc.noaa.gov/col/project/access/ nodcdir. html). The FGDC metadata descriptions will be provided to the Howard Diamond by NODC, as a part of a routine transfer of metadata. These HAB data sets have been re-formatted, and are in the process of being loaded into the HAB Data Management System (HAB-DMS). Currently, the HAB-DMS and web-based interface are being migrated to an operational mode. Therefore, sample data, which was loaded into the database for testing, are being removed and replaced by the current Washington State data sets archived at NODC.

In collaboration with Michelle Tomlinson, the NWFSC has developed a web-based form to facilitate the acquisition of information regarding Harmful Algal Bloom reports in Pacific Rim countries. These will be linked to the HAB database as another source of HAB data and information. A statement of work is being written to describe additional enhancements to the system, as well as requirements for linking these HAB reports, as well as other sources of coastal data sets which reside within NODC, to the system.

Conclusion and recommendations

There is a need for at least one training workshop in which inexperienced PICES phytoplankton analysts become familiar with a wide range of HAB species potentially harmful in their waters (both Dr. Fukuyo and Dr. Taylor have taught several of these before, mostly in S.E. Asia). A special workshop to be convened in Japan, next October, immediately prior to PICES XI, was recommended, and would focus on antibody-based toxin detection techniques.

Participation by more PICES countries at future workshops, especially countries not present at this workshop (e.g., China, Korea, Russia) has to be encouraged

The initial entry of HAB shellfish data from the western US into the NODC database has been successful. The goals for the upcoming year include entry of data from western Canada and Asian Pacific countries. It was recommended that Asian Pacific country representatives make available their historical shellfish toxin data for entry into the database. There is concern that these data may be of a sensitive nature, and not desirable for general release to the public. This concern can be circumvented by focusing on historical data that is at least two years old. Additional funding should be sought to continue collaboration of PICES with NODC.

There is a need to continue the development of possibly the most useful PICES HAB database. Much further discussion of HAB databases is required to deal with design and standardization issues. A further workshop on the latter seems to be essential.

Appendix D Endnote 1

Workshop agenda

October 5, 2001 (Friday):

0900 Gather at main entrance to Biosciences Bldg.
0915 Opening remarks, introductions, objectives, schedule
0930 *F.J.R. Taylor*. Fish-killing flagellates
0950 *Y. Fukuyo*. Dinoflagellate identification
1010 Coffee break
1030 Sample examination (lab.)
1200 Lunch break
1300 *R. Horner*. Diatom identification

1330 Sample examination/discussion (lab.)
1500 Coffee break
1520 *L. Connell.* Molecular identification aids demo
1700 Close Day 1

October 6, 2001 (Saturday):
0900 *M. Tomlinson.* Web access and HAB data handling
0930 Discussion
1030 Coffee break
1050 Sample examination/discussion (lab)
1200 Workshop conclusion

Appendix D Endnote 2

Participation list

Canada
Alexander Culley, Helen Drost, Nicky Haigh, Lawrence, Adrian Marchetti, F.J.R. (Max) Taylor (Convenor), and J.N.C. (Ian) Whyte

Colombia
Juan Saldarriaga

France
Pascale Loret

Indonesia
Gabriel Wagey

Japan
Yasuwo Fukuyo, Yuichi Kotami

U.S.A.
Brian D. Bill, William Cochlan, Laurie Connell, David Garrison, Julian Herndon, Rita Horner, Racheal Howard, James Postel, Michelle Tomlinson, Vera L. Trainer