

## 2006 Harmful Algal Bloom Section annual workshop

By Charles G. Trick and Yasunori Watanabe

The Section on Harmful Algal Blooms (HAB-S) was established under the Marine Environmental Quality Committee (MEQ) at PICES XII in 2003, in Seoul (Korea). At PICES XIII in 2004, in Honolulu (U.S.A.), HAB-S recommended to MEQ to convene an annual series of workshops that “review selected harmful algae that impact all or most countries in the PICES region”. The first workshop of this series on *Pseudo-nitzschia* and *Alexandrium* spp., preceded by a laboratory demonstration on detection techniques for algal toxins, was held at PICES XIV in 2005, in Vladivostok (Russia). This year, at PICES XV in Yokohama (Japan), we had our second workshop on *Dinophysis* and *Cochlodinium* spp., also accompanied by a half-day laboratory demonstration on detection techniques for algal toxins.

The laboratory demonstration was organized by Drs. Yasunori Watanabe and Ichiro Imai and held on October 13 at the National Research Institute of Fisheries Science (NRIFS) of the Fisheries Research Agency (FRA). In total, 28 scientists from 7 nations (Canada-3, China-6, France-1, Japan-10, Russia-2, Spain-2, and U.S.A.-4) were in attendance. They gathered at the Red Brick Warehouse (venue of the Annual Meeting) and took lunch together at a Chinese restaurant after the registration for the meeting. Then they went to NRIFS by bus chartered by FRA. Two demonstrations were carried out. The first demonstration “Protein Phosphate 2A (PP2A) inhibition assay for okadaic acid and its analogs in shellfish” was conducted by Dr. Reiji Sekiguchi (sekiguchir@jfrl.or.jp), Ms. Natsuki Takahashi and Dr. Toshiyuki Suzuki (tsuzuki@affrc.go.jp) (**Photo 1**). They introduced a new toxin determination kit, based on the principle that okadaic acid and its analogs (OAs) strongly and specifically bind to PP2A and inhibit its dephosphorylation activity. If OAs is absent, PP2A hydrolyze a colorless substrate pNPP and produce a yellow product. But in the presence of OAs, the substrate remains colorless. Therefore, OAs concentration is determined by

measuring the intensity of the yellow color. This newly developed determination kit is expected to be used for on-site monitoring of OAs in shellfish.

The second demonstration on “Simple, rapid, specific and cost effective method for identifying *Alexandrium tamarense* and *A. catenella* using the LAMP method” was conducted by Drs. Shigeru Itakura (itakura@affrc.go.jp) and Satoshi Nagai (snagai@affrc.go.jp) (**Photo 2**). LAMP, which stands for **L**oop-mediated **I**sothermal **A**mplification, is a simple, rapid, specific and cost-effective nucleic acid amplification method solely developed by Eiken Chemical Co., Ltd., in Japan. Advantages of LAMP are:

- There is no need for a step to denature double stranded into a single stranded form;
- The whole amplification reaction takes place continuously under isothermal conditions (~65°C);
- The amplification efficiency is extremely high, with DNA being amplified  $10^9$ - $10^{10}$  times in 15-60 minutes;
- By designing 4 primers to recognize 6 distinct regions, the LAMP method is able to specifically amplify the target gene;
- The total cost can be reduced, as LAMP does not require special reagents or sophisticated equipment such as a thermo-cycler and other basic apparatus for molecular biological experiments; and
- The amplification can be checked by the naked eye through the presence of an amplified product (the turbidity of magnesium pyrophosphate, a by-product of the amplification reaction).

Drs. Itakura and Nagai, together with Dr. Yukihiko Matsuyama, have developed LAMP primers for detecting *A. tamarense* and *A. catenella*, and it is now possible to identify each species from a single cell within 1 hour (starting from isolation of the cell to the detection of the amplification). This method can be performed using standard equipment needed in other molecular biological



Photo 1 Dr. Reiji Sekiguchi pointing at a graph monitoring the PP2A reaction.



Photo 2 Dr. Shigeru Itakura sampling *Alexandrium tamarense* using a micropipette during the LAMP method demonstration.

experiments. Though the time schedule was tight (from 14:00–18:00 hours), the laboratory demonstration finished smoothly because of the help of Dr. Hiroshi Oikawa (NRIFS) and cooperation of all attendees. After the demonstration, participants returned to the Minato Mirai District and enjoyed a communal Japanese dinner.

The workshop entitled “Review of selected harmful algae in the PICES region: II. *Dinophysis* and *Cochlodinium*” was held on October 14 at the Red Brick Warehouse, and co-convened by Drs. Charles Trick (Canada) and Yasunori Watanabe (Japan). There were 22 scientists in attendance from 9 nations (Canada-2, China-1, France-1, Japan-8, Korea-1, Norway-1, Russia-1, Spain-2, and U.S.A.-5). Three experts were invited: Drs. Kazumi Matsuoka (University of Nagasaki, Japan), Patrick Gentien (IFREMER, France) and Beatriz Reguera (Instituto Español de Oceanografía, Spain). Travel expenses for these invited speakers were covered by PICES and FRA.

At the workshop, the ecology, physiology, taxonomy and toxicity of two exceptionally important harmful algal species, *Cochlodinium* and *Dinophysis* spp., were discussed and compared. Ideas regarding these common themes were summarized in the three invited talks on “Recent progress of the study on a harmful dinoflagellate – *Cochlodinium polykrikoides*” (by Dr. Matsuoka), “The rare marine protist *Dinophysis acuminata*” (by Dr. Gentien) and “What we know and what we do not know about *Dinophysis*” (by Dr. Reguera). These presentations resulted in considerable discussion among the participants. As PICES had hoped, much of the discussion focused on two major themes:

- (1) Why does the distribution and toxicity of these two genera reside in the PICES countries of the western North Pacific, and are not (as yet) detrimental HABs of the PICES countries in the eastern North Pacific (Canada and U.S.A.)?
- (2) What are the environmental situations that enhance or stimulate toxin production?

The invited lectures were complemented by a series of presentations concerning *Dinophysis* and *Cochlodinium* in

the individual PICES countries. Drs. Vera Trainer (U.S.A.) and Charles Trick reviewed the low levels of these two genera in waters adjacent to the North American shore. These levels are in stark contrast to the deep and profound negative impact of *Dinophysis* and *Cochlodinium* in waters of Korea (presented by Drs. Hak-Gyoon Kim and Chang-Kyu Lee), Japan (presented by Drs. Kazutaka Miyahara and Ichiro Imai) and China (presented by Dr. Jinhui Wang). More specific methodologies were also presented. Dr. Toshiyuki Suzuki described modern methods for the analysis of lipophilic toxins, and Dr. Takafumi Yoshida documented the activities of the Northwest Pacific Action Plan (NOWPAP) on developing a regional HABs database. The details of each topic were summarized in an “enthusiastic” hour-long summary discussion aimed at establishing a list of “what is known”, “what is unknown” and “what sort of information is required next”.

We, the conveners, would like to thank all of the participants for making our workshop a great success. Special thanks go to the PICES Secretariat, the Fisheries Agency of Japan and FRA, especially NRIFS, for their support and efforts in arranging the venue and logistics for the meeting. We also greatly appreciate suggestions given by the Co-Chairmen of HAB-S, Drs. Vera Trainer and Hak-Gyoon Kim, in the course of our preparation of the workshop.

The third workshop of this series will focus on *Heterosigma akashiwo* and other harmful raphidophytes, and will be held at PICES XVI in 2007, in Victoria (Canada), and co-convened by Drs. Charles Trick and Ichiro Imai (Japan), and will include a half-day laboratory demonstration on *Heterosigma* cell and toxin detection to be led by Drs. V. Trainer, C. Trick and Robin Brown.

It is expected that the results of the annual HAB workshops will be published as a PICES Scientific Report or other publication. A product from this series will also be a list of recommendations to help guide collaborative HAB research priorities in PICES member countries over the next five years.



Dr. Charles G. Trick ([trick@uwo.ca](mailto:trick@uwo.ca)) is the Beryl Ivey Chair for Ecosystem Health at the Schulich School of Medicine and Dentistry at the University of Winnipeg, Canada. Dr. Trick researches issues of human health and environmental conditions, with a strong emphasis on harmful algal blooms. He is a member of the PICES Section on Harmful Algal Blooms.

Dr. Yasunori Watanabe ([ywat@affrc.go.jp](mailto:ywat@affrc.go.jp)) is the Director of the Harmful Algal Bloom Division at the National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency of Japan. Dr. Watanabe puts his focus on the fisheries ground protection issue including HAB problems, based on the experience of his study of chemical oceanography. He is a member of the PICES Section on Harmful Algal Blooms.